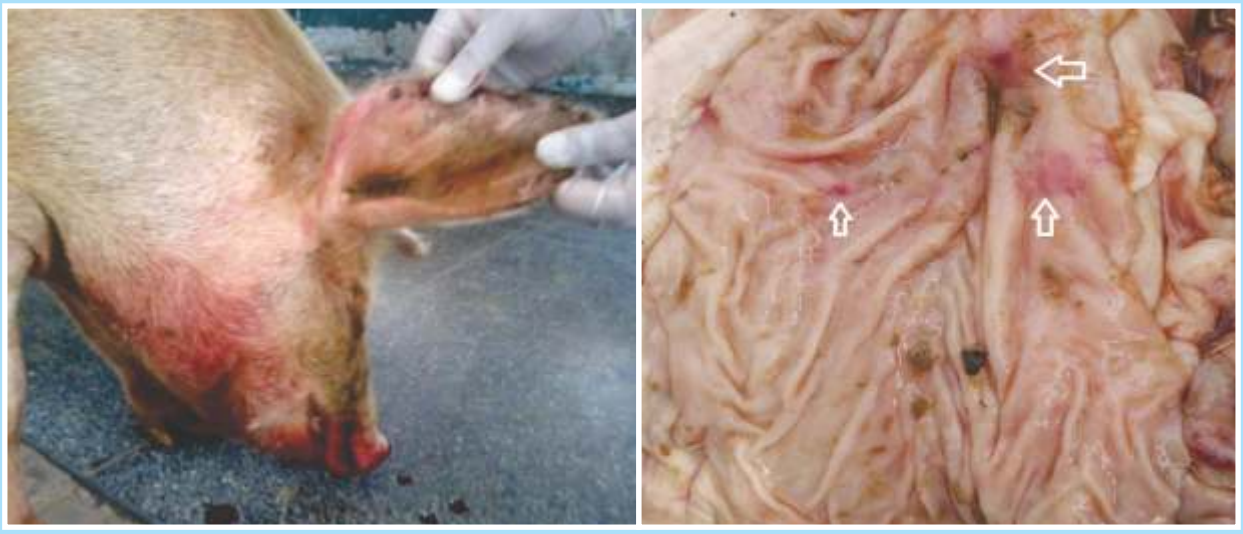
ISSN No. 0970-051X

Indian Journal of VETERINARY MEDICINE

Vol. 38 No. 1&2 June & December 2018



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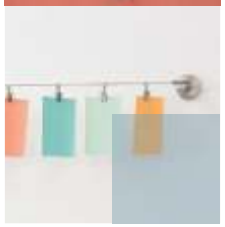

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Indian Journal of Veterinary Medicine

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 1-5

Invited Review Articles

Application of Contrast enhanced Ultrasound (CEUS) in the diagnosis of Chronic Kidney Disease (CKD) in dogs

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Chronic kidney dysfunction in dogs is a growing health concern, in India, because of its increasing prevalence and incidence rate. Assessment of tissue perfusion is an important component of CKD since it primarily involves perfusion changes in the renal cortex. The kidney function and its viability can be assessed by early and detailed visualisation of perfusion changes of the renal cortex. Different non-invasive imaging modalities such as multi detector CT, Positron emission tomography ,MRI and single photon remission CT with Tc dithylentriamine penta acetic acid are used in the tissue quantification in human medicine .But the high costs, reduced availability , long examination periods ,patient's exposure to radiation or nuclear traces limited their clinical application in veterinary practice(Winearls 2009). Grey scale renal ultrasound combine with colour Doppler flow imaging (CDFI) had become the main non-invasive imaging method for evaluating the renal anatomy and blood flow (Le Dorze 2012). But they were of limited diagnostic use in CKD as the CDFI parameters such as the resistance index (RI) and peak systolic velocity (PSV) provides only indirect microvascular parameters which could not directly assess renal cortex perfusion.

In recent years of contrast enhanced ultrasound (CEUS) has been proposed as an alternative imaging technique in this area. The microbubbles which are used as contrast enhancing agents are blood pool agents, when injected intra venously they remain entirely intra vascular, mix uniformly with blood in the circulation and possess the same intravascular rheology as red blood cells. The benefits of CEUS are a) absence of ionising radiation or nephro toxicity b) widespread availability and c) a short time only is needed to arrive at a final diagnosis, after a non-conclusive ultrasound study (Wilson 2009). The large blood supply of the kidney is a good base for contrast studies, these micro bubbles of Kidney remains strictly inside the vessels, enabling functional imaging of kidney.

Contrast agents: Contrast agents have been integral in all imaging modalities except US. The reasons for this limitation in ultrasound imaging include the following 1.cost of the media and need for intravenous pool access .Unlike MR and CT contrast media, ultrasound contrast agents are pool agents. These contrast agents are microscopic bubbles made from an outer shell and central core of gas. There are limited numbers of manufacturers of ultrasound contrast media. Older first generation contrast agents use air in the core (Lenovist).Air is not a potent medium for emission of ultrasound signal and results in poor S:N images. Second generation contrast media utilize inert gases in the core,which provide more non-linear effects. The first second generation contrast media (Optison) used human albumin in the shell. This had obvious limitations in veterinary medicine for immunological concerns.

Second generation contrast agents utilize an immunologically inert lipoprotein shell and more efficient inert gas in the lumen. The range of bubble sizes are predominantly small (most less than 10 micro millimetre, pass through the pulmonary circulation unaffected and can imaged in the small vessels of all organs currently being imaged in the diagnostic veterinary imaging. Contrast media currently recommended for the veterinary clinical applications are definity (USA) and sonovue (Canada). Both are very safe in dogs and no side effects have been reported. The bubbles of definity are more concentrated and rigorous than sonovue providing an advantage for clinical characterisation or detection of lesions, such as liver nodules. For experimental studies, where sonovue is advantageous.

New contrast agents are available like Targestar is a blood pool agent that deminstartes great promise because of high resonant frequency and improved stability of the reconstituted bubbles. Reconstituted Targestar is stable with refrigeration for weeks and months. This agent has the ability conjucate to an active coupling molecule. This is a very exciting new area of research called molecular imaging. Conjucation of ligands to a blood pool US contrast agent may provide an opportunity to couple monoclonal antibodies,various mediator proteins or possible therapeutics.

Practical advantage of using contrast enhanced ultrasound includes the relatively low cost of contrast ultrasound examinations: comparable sensitivity to computed tomography and magnetic resonance imaging for detecting the metastatic disease and the absence of ionising radiation. (O'Brien et.al 2007)

Contrast agent administration: Low MI contrast specific techniques allow dynamic imaging with evaluation of the different vascular phases using a low solubility for ultrasound contrast agents . The steps recommended are as follows.

1. Base line investigation of the target lesions, the transducer is kept in a stable position, while the imaging mode is changed to low MI contrast specific imaging. For comparison both normal and suspected abnormal renal tissue should be included in the scan plane.
2. The MI setting should be adjusted to provide sufficient tissue cancellation with maintenance of adequate depth penetration. Major vascular structures and some anatomical land marks should remain barely visible.

Sonovue the contrast medium consists of sulphahexa fluoride gas encapsulated in a phosphor lipid cell. The contrast medium was prepared by shaking the vials with mixing device according to the manufacturer's instructions .For dogs 0.04-0.06 ml/Kg of contrast medium is injected followed by a bolus of 5-10 ml saline flush. The needle diameter should not be smaller than 20 gauge to avoid the loss of bubbles due to mechanical impact during injection. A stop clock should be started at the time of UCA injection. Real time scanning is recommended to continuously assess the wash in and washout phase. (Rajarajan 2018)

In some contrast specific US modes display of tissue and contrast signals has been implemented. This modality is particularly useful for small lesions to ensure that the target lesion is kept within the scanning field, during CEUS. Because of the dynamic nature of real time CEUS the investigation has to be documented on video or digital media .In patients suspected with vascular diseases (mainly small vessel diseases) or trauma ,long and short axis views is to be obtained during both cortical and medullary phases.

The increase in echo signal intensity after micro bubble injection may be quantified by dedicated software packages to produce time intensity curves (TICs). Enhancement based representations had been used to assess unilateral kidney dysfunction , such as in renal artery stenosis by a simple analysis of tracer concentration curve (Ciccone et.al 2011).These features make low MI ,CEUS a promising technique in evaluation of renal cortex perfusion.

Renal CEUS : After contrast injection enhancement can be detected in real time for upto 5-7 minutes in the liver and spleen. Kidneys enhance for a shorter period of time. The arterial pedicle and main branches pick up the agent first .After a few seconds, the cortex enhances, followed by medullar perfusion.The outer medulla fills in earlier, while the pyramids fill i gradually later. Satisfactory uptake usually lasts for 2 min in the kidneys and subsequently contrast concentration in circulation decreases and enhancement fades .In chronic renal diseases enhancement is poorer and shorter, fading earlier (Dong et.al 2014). European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) Guidelines and Recommendations, updated in 2011, had documented the potential indications of CEUS in kidney diseases. Besides their echogenicity, renal tumors exhibit altered vascularity compared to the normal parenchyma, and CEUS can be helpful in their differential diagnosis. For detection of cyst-like lesions, CEUS is even more sensitive than contrast-enhanced CT. Owing to the excellent spatial resolution of CEUS, cortical necrosis is shown as a non-enhancing area with vascularity. CEUS is also superior to color Doppler ultrasound in diagnosing renal infarction, because CEUS can detect slower flow in smaller blood vessels. Kidney abscesses can also be confirmed and followed up using CEUS. Since contrast agents are not concentrated in the collecting system, CEUS assessment of kidney trauma cannot rule out pelvi-calyceal and ureteric injuries; therefore, CEUS may be better suited for limited injuries, or multiple solid organ traumas. (Demosthenes et.al 2013)

Renal microvascualr perfusion evaluation

The application of CEUS for studying renal microvascular perfusion has been examined in many animal models. Histological assessment in porcine models have shown that the ultrasound contrast agent used (sulfur hexafluoride) did not inflict any tissue damage to the kidneys. These findings suggest that CEUS could be safe for regular use in humans, with minimal risk of tissue damage. .Kogan placed transit-time flow probes in renal arteries of rats to measure the volume of RBF, and compared these readouts to those reported by CEUS. They reported that CEUS-derived

parameters were comparable to absolute measurements of blood flow in rat kidneys (R>0.9). Their results demonstrated that CEUS could indeed reflect RBF accurately and noninvasively. (Piscaglia et.al 2013)

CEUS-based renal perfusion parameters have been documented in healthy animals. Time-dependent intensity curves can readily be generated based on selected regions of interest (ROI) in the renal cortex and medulla, as exemplified in. Peak intensity (PI), time to peak intensity (TTP), mean transit time (MTT), and area under the curve (AUC) are some of the key observational parameters that are typically used in renal perfusion studies, . Different animals exhibited similar renal perfusion enhancement patterns under CEUS: the renal cortex enhanced initially followed by medullary enhancement moreover, another study revealed that the location and size of ROIs used for this imaging approach did not make significant differences to the renal perfusion metrics captured using CEUS. This result augurs well for the routine analysis of CEUS data based on ROIs in clinical practice.

Indications of Renal CEUS

As in most medical fields, when a new technique emerges, it is initially used in a wide variety of indications. Following the publication of clinical studies results, more appropriate indications for correct usage are identified. The same has happened in the last years with CEUS. The 2011 updated European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) Guidelines and Recommendations on the Clinical Practice of CEUS have identified the current indications for the administration of US contrast agents for studying different parts of the body, including the kidneys. According to these guidelines, CEUS should be used to answer specific clinical questions in the kidneys. In our practice, we have accumulated experience on most of the fields outlined by the EFSUMB Guidelines, which will be reviewed in detail.

Renal Ischemia

Many studies in animals and humans have concluded that CEUS has very good diagnostic performance in the detection of kidney parenchymal ischaemia, comparable to that of CECT In comparison to Colour Doppler US, CEUS is superior, detecting smaller blood vessels with slower flow, and is considered a recommended imaging technique in patients with suspected infarction. As in other organs, infarcts appear as triangular or wedge-shaped areas with no contrast uptake, while the rest of the parenchyma enhances normally. Due to its excellent spatial resolution, CEUS allows differentiation of infarction from cortical necrosis, the latter appearing as a nonenhancing cortical area with preservation of hilar vascularity. In addition, CEUS can differentiate infarcts from parenchymal areas with diminished perfusion. Although both appear as areas with no flow on Doppler ultrasound, only infarcts show complete lack of contrast uptake after injection.

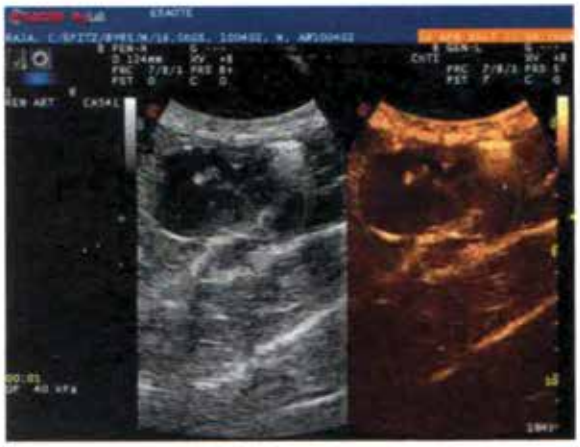
Renal artery stenosis

Doppler examination of the renal arteries is still in many institutions the first imaging examination to be performed for assessing renal artery stenosis. There are published studies advocating the injection of US contrast agents in order to improve sensitivity of conventional Colour Doppler examination for the identification of the main renal arteries, with a 10% improvement for correct location of the sample volume for detecting Doppler spectral tracings. However, it is debatable if this slight amelioration is worth the extra time and cost, since patients may eventually be referred to CT/MR angiography of the renal arteries. For this reason, it may be concluded that routine use of CEUS offers no significant advantage for the evaluation of renal artery stenosis.

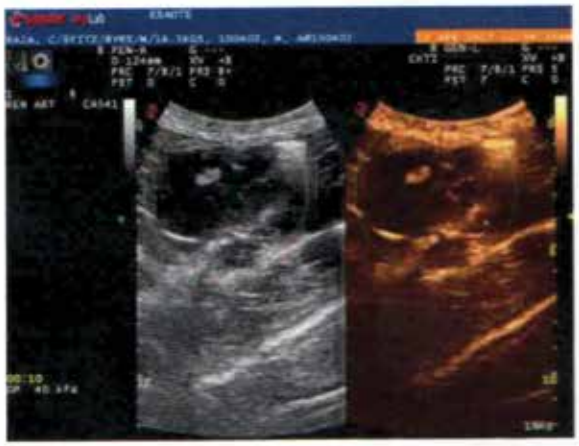
CEUS, by characterising the microvasculature with perfusion analysis during the course of interventions, provides a lot of possibilities for modified therapeutic strategies. Percutaneous ablation is increasingly being used effectively for the management of patients with kidney tumours. These cases are often imaged with CECT and/or CEMR both for pretreatment evaluation in specific time points during follow up after therapy. Although baseline, nonenhanced US may be useful for guiding the ablation procedure, it is not as effective for the assessment of ablation results. Studies have shown that CEUS improves imaging of patients referred for renal tumour ablation, with similar accuracy to that of CT/MR for confirmation of treatment results. It offers detailed important information on tumour vascularity, thus improving orientation and guiding of the ablation needle. Furthermore, CEUS ameliorates evaluation of treatment therapeutic results. A delay of 5–10 min after the ablation is concluded allows the heat-generated gas and related artefacts to dissolve. Areas still showing

Representative Serial Contrast enhanced Ultrasound images in Early stage kidney disease groups animals

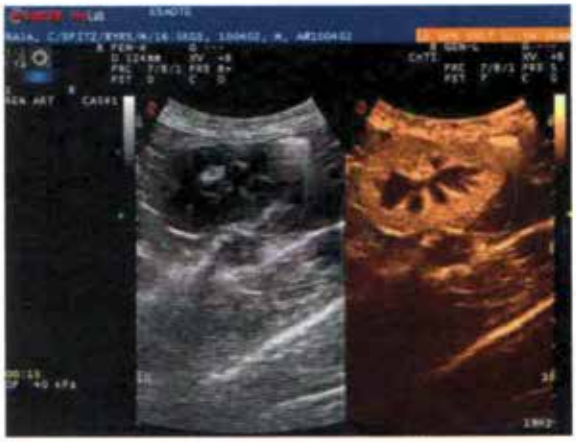
Before Contrast (0 second)



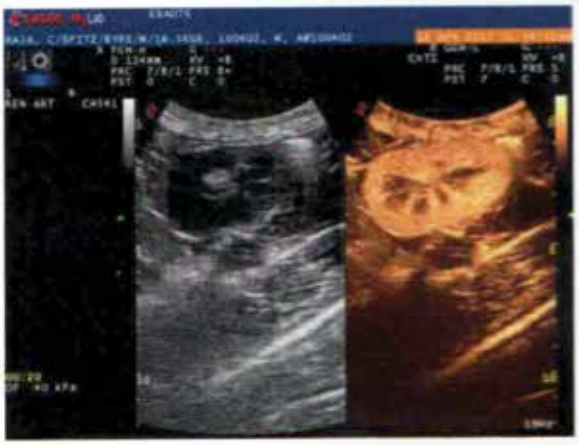
Early arterial phase (0 second)



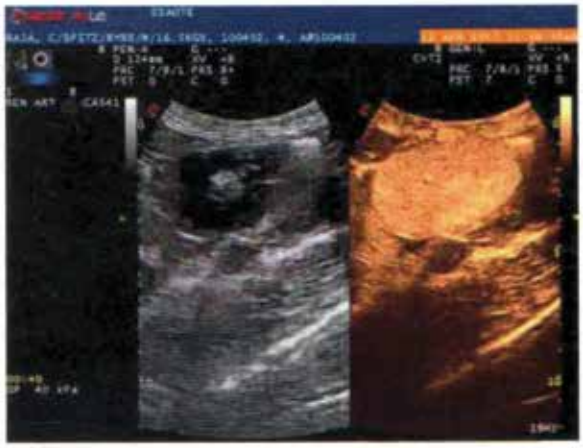
Cortical Enhancement (15 second)



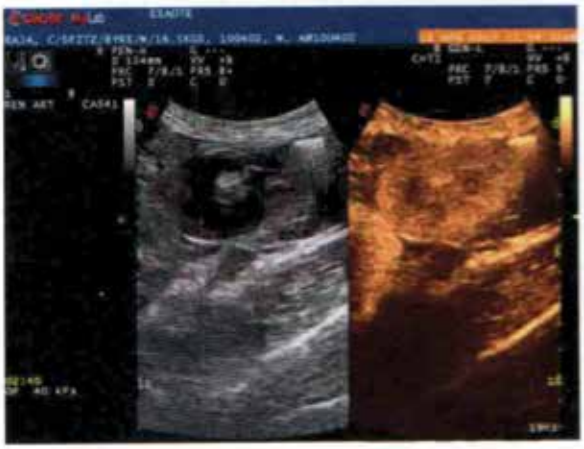
Cortical peak (20 second)



Cortical medullary peak (35 second)



Late phase / fading (160 second)



contrast enhancement after ablation are considered as residual tumour. The examiner should be cautious not to misinterpret larger blood vessels surrounding the ablated region as a residual lesion. For this reason, imaging results after therapy should be compared to pre treatment studies. Residual tumour is shown as a nodular or crescent-like area with contrast uptake, with close resemblance to pre treatment imaging findings. (Bertolotto 2008)

Limitations

The limitations of CEUS in the kidneys can be categorised into 3 groups.

The first group includes the known deficiencies of ultrasonography as a modality due to lesion location (obese patients, bowel gas interposition, etc.) that contrast agents cannot overcome. If a lesion is not seen on baseline examination, it will not be detected after post contrast injection either. Secondly, limitations exist for CEUS as a practice worldwide. Most US machines are not capable of imaging this technique, which is not included in structured Radiology training. Additional time is needed in order to place an intravenous catheter, while the drug's added cost should also be considered. Although, as already mentioned, patients with serious cardiopulmonary disease should not be scanned with CEUS, these cases are smaller in number in comparison to those with contraindication for contrast enhanced CT or MR because of anaphylactic history or renal failure. Finally, limitations exist for scanning the kidneys in particular: US contrast agents are not excreted to the pelvicalyceal system, while it is impossible to image the enhancement of both kidneys simultaneously, as in CT, MR or intravenous urography. Despite these limitations, however, CEUS is used extensively worldwide for imaging a variety of diseases of renal pathologic entities with excellent results.(Leen,et.al 2004)

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 6-17

Homeopathy in Veterinary Medicine- A Review

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Abstract

Homeopathy, a brain child of German physician Dr. Samuel Hahnemann, is an integral part of holistic medicine. It's birth took place in early 18th centuary.Its application in veterinary practice is as old as human homeopathy. Homeopathy is based on Hahnemann's doctrine of "Like cures Like", a claim that a substance that cures symptoms of the disease in healthy subjects would cure similar symptoms in diseased subjects. This system of medicine gained popularity due to its low doses, no toxicity, no residual effect, compassionate nature and low cost even when it was not possible to explain the mechanism of action of homeopathic drugs. Researches employing modern techniques have now provided sufficient evidence that homeopathic drugs are nano medicine and work by identification of work targets and therefore their effect is not a placebo effect. Homeopathic drugs are being used in the management of animal diseases in many countries including India. Despite use of homeopathy in animals by farmers and veterinarians, scientific approach is lacking . This necessitates more and more placebo controlled randomized large scale clinical trials using homeopathic drugs in animal diseases.

Keywords: Animal, clinical trial, homeopathy, nano medicine, placebo, veterinary medicine,

Veterinary medicine has followed in the footsteps of human medicine. High cost of the modern medicines, their inherited side effects and problem of antimicrobial residues in animal products have caused an apparent discomfort to animal owners invoking their interest in alternative approaches of animal health care. Further unorganized animal sector, particularly in the hands of poor marginal farmers or landless labourers below poverty line, urges for cheaper, ecofriendly, safe and effective alternative animal health care approach as a first line therapy. Amongst alternative approaches, Homeopathy is widely accepted as a complementary and alternative approach and is probably in vogue for more than 200 years. It is being practiced in the countries of the European Union and in USA. European Union committee has also urged recently to cut the use of antimicrobials in human as well as in veterinary practice to an essential level for maintaining the effectiveness of antimicrobials and to deal with the problems of emergence of resistance among microbes. Recently Americans are also inclined to encourage the use of homeopathic drugs in the management of animal diseases. The Indian scenario is also no way different. In this country many veterinarians are using homeopathic drugs in the management of animal diseases, though lacking in proper planning and execution that makes the claim of miracle cure unacceptable.

What is Homeopathy

Homeopathy is an integral part of alternative medicine**.** The word Homeopathy is derived from Greek words "Homeos" and "Pathos" meaning "similar" and "suffering" respectively (Mukherjee and Wahile, 2006). This system of Medicine is based on the principle of Similars propounded by Dr. Samuel Hahnemann. Homeopathy is a practice that came into being and gained wide spread popularity too early in the history of medicine at a time when it was impossible to provide any kind of explanation for its clinical efficacy (Bellavite and Signorini,1995). World Health organization has recognized the value of homeopathy as one of the system of traditional medicine that could be integrated with conventional medicine to provide health care. Clinical anecdotal evidence exists to indicate that homeopathy is beneficial in veterinary practice. The remedy stimulates the body to heal itself. This is the system of medicine in which practice came first and science is being discovered later.

Developments in Human and Veterinary Homeopathy

Homeopathy is a brain child of German physician Dr Samuel Hahnemann, who first experimented on himself to test the drug- China in early 1800's. He then propounded the law of "Similia

Similibus Curentus " (Hahnemann,1901). He noticed that the drugs in pure form in healthy persons produce an adverse reaction resulting in symptoms of illness but the same drug in potentized form had a tremendous ability to cure the symptoms caused by the drug in pure form. Homeopathy discovered the fact that the dilution followed by vigorous shaking (potentization) made a substance more powerful in terms of clinical response. In 1813, in Leipzig, Hahnemann lectured on the use of homeopathy in animals and stated that the principles and application in animals were broadly similar to those in humans. Boenninghausen and Lux were early proponents of homeopathy in animals. Boenninghausen, the German baron, lawyer and agriculturalist, used homoeopathy on animals and established the principles of Veterinary Homeopathy. An early advocate of high potencies, he conducted a successful prospective trial of 200C potency in domestic animals, reasoning that veterinary homeopathy was a good way to demonstrate that it was not a placebo. The veterinary practice of homoeopathy has existed since Hahnemann, in recent years it has emerged from the shadows and appears to be growing exponentially, expanding in as many directions. Veterinary Homeopathy has strongest modern tradition in Europe particularly in Germany, France and Great Britain. Macleod practiced veterinary homeopathy from World War II until his death in 1995. In the UK there are many vets practicing homoeopathy and over one third of these have qualified to the Faculty Homeopathy's MFHomVet level. Their interest is maintained by the British Association of Homoeopathic Veterinary Surgeons (BAHVS). By 1906, Humphrey's company was producing a range of homeopathic remedies for veterinary use

Presently many courses are offered in veterinary homeopathy in Great Britain, Holland and Germany. In USA, the Academy Of Veterinary Homeopathy offers courses in veterinary classical homeopathy (Voker,1999). In 1986 veterinarians from Belgium, Germany, Great Britain, Italy, France, Luxembourg and the Netherlands founded the International Association for Veterinary Homeopathy (IAVH) in Luxembourg.

Tradition of veterinary homeopathy is as old as humans. Initially homeopathic drugs viz. Aconitum napellus, Camphora, Nux vomica and Opium were being used for the treatment of certain diseases of horses and cattle as early as 1833 by German Practitioner – Guillaume Lux. Since then veterinary homeopathy has progressed considerably despite the apparent difficulty in adopting the patient questioning technique, lack of expression of subjective feelings and lack of experimental data

Homeopathy in the Treatment of Animal Diseases in India

In India there are many scattered case reports and claims on the use of homeopathic remedies in the treatment of certain ailments in animals based on the personal experience of the practitioners, but large scale scientific clinical trials are entirely lacking. Presently animals are being treated with homeopathic drugs by farmers and veterinarians on the basis of information available in humans. There is no doubt on professional acumen; nevertheless, their practice of homeopathy is not flawless owing to lack of planning and proper execution. The practices seem casual and erratic lacking scientific fervor owing to unconfirmed diagnoses, no laboratory substantiation, improper follow up observations and lack of documentary evidences. More research is certainly needed to help optimize use of homeopathic medicines in veterinary practice. Scientific validation of homeopathic drugs in the treatment of animal diseases in modern perspective will not only make veterinarians far more confident in adopting homeopathy as an efficacious cost effective therapeutic and/ or preventive modality of animal health care but also diffuse the apprehension of its folksy and wired system of esoteric medicine . Before 2001 no planned research project was undertaken to evaluate homeopathic drugs in the treatment of animal diseases anywhere in the country because of lack of confidence of veterinarians, trained in modern medicine, on the efficacy of homeopathic drugs .Recognizing the need of reducing the use of antibiotics in animal treatment and having a cheap and effective alternative medicine (as a first line therapy that may be used by farmers in remote places) a project entitled "Evaluation of Homeopathic Drugs in the Management of Animal Diseases" was conceived and initiated in December. 2001 in the Division of Medicine with collaboration of Division of Surgery and Division of Animal Reproduction, Indian Veterinary Research Institute, Izatnagar, Bareilly. Both concepts of individual drug and homeopathic combination remedy were put to trial in diseases of large animals and companion animals with confirmed diagnoses based on modern diagnostic techniques.

There is neither any association of veterinarians practicing homeopathy nor any specialized courses are offered. Whatever is being practiced has been borrowed from human homeopathy. Recently, Kerala Veterinary and Animal Sciences University has initiated a Post Graduate certificate course in Veterinary Homeopathy in order to develop a scientific pre-designed course in Homeopathy for Veterinary practitioners (Directorate of Academics and Research No. KVASU/DAR/Acad(B 1)/ 1 397 7 12014(15.4's) Dated,Pookode,05/03/20 1 5).

Despite absence of veterinary homeopathy forum in India , reluctance of veterinarians trained in modern system of medicine to accept findings of homeopathic trials in animals and general apathy, clinical research findings of homeopathic remedies in animals have been disseminated to sensitize practicing veterinarians through National and International forums of Human Homeopathy (Varshney and Kumar,2003, Banyopadhyay and Varshney,2004 , Bandyopadhyay and Varshney,2005, Varshney and Saghar,2005, Varshney,2005 a ,Chinkija and Varshney,2005 b , Swaminarayan and Varshney,2010, Varshney and Swaminarayan 2010 a and b, Varshney and Swaminarayan 2011 a and b , Varshney, 2011 a and b, Varshney and Swaminarayan,2014, Varshney, 2015, Varshney, 2016 a , Varshnety and Swaminarayan,2018) and other national and international scientific forums (Ram Naresh *et al*.,2002, Varshney, 2003, Kumar *et al*.,2003, Varshney and Ram Naresh, 2003, Kumar *et al*.2004, Varshney and Kumar,2004, Swaminarayan and Varshney,2005, Kumar and Varshney2005, Varshney,2005 b, Varshney,2005 c,, Chinkija and Varshney,2005 a, Varshney *et al*.,2007, Varshney,2013 a). Proper scientific proving will be the only route to consistent success and ethical practice of homeopathy in veterinary medicine. It is hoped that more and more scientific approach will help to optimize the use homeo drugs in veterinary practice. It is indeed a happy situation that Central Council for Research on Homeopathy (CCRH), an organization of Government of India, New Delhi has recognized the prospects of homeopathy in animal treatment and is encouraging research on veterinary homeopathy in India.

Homeopathic Drugs

Major sources of homeopathic drugs are plant, animals and minerals and the drug is prepared by dilution and vigorous shaking. Other class of homeopathic medicines are Nosode, Sarcode and Imponderablilia.

Nosode-are prepared from disease causing infectious agents (bacteria, virus, or disease parts) in specialized manner following standard protocol. These medicines are not antibiotics and do not possess bactericidal or bacteriostatic activity (Banerjee, 2006)

Sarcode- are prepared from endocrine glands (adrenaline, pancreas, pituitary, thyroid, ovaries or testicle) and their secretions.

Imponderablilia- These are the medicines made from energy, either from natural or artificial source (Wadhwani, 2011). Even many imponderable (immaterial) substances can produce most violent medicinal effects on human beings. The medicines from this source include: Luna (full moon), magnetis polus Australia (South Pole of the magnet), magnetis polus Arcticus (North Pole of magnet), magnetis poli ambo (magnet), sol (sun rays), radium, *Magnetis artificialis*, electricitus, X-ray (Banerjee,2006).

Preparation of Homeopathic medicines -Homeopathic medicines are prepared according to the guidelines of Homeopathic Pharmacopoeia. The Preparation of Homeopathic medicines consisted of preparation of mother tinctures and preparation of potencies

Decimal scale of Potentization- In this scale of potentization, first potency is made by adding one part of the original drug substance to the 9 parts of vehicle with vigorous shaking. Vehicles may be Saccharum lactis for insoluble substances and alcohol or water for soluble substances. Further potencies are prepared by taking one part of previous potency and 9 parts of vehicle. The decimal potency scale has been discovered by Constantine Hering in 1830 (Robert, 1853) It is also known as D or X scale dilution and is represented as X or D. Hahnemann does not preferred this scale, but it is commonly used in Europe during the 19th century and still present (Banerjee,2006).

Centesimal scale of Potentization -Hahnemann created and used the centesimal or "C scale" for preparation of Homeopathic medicines, dilution of a substance in a ratio of 1:100 at each step. The centesimal scale was preferred by Hahnemann mostly. Next dilutions are prepared by dilution of the previous potency and one part of previous potency and 99 parts of alcohol are mixed and so on. Each step is associated with vigorous shaking. Similarly, further potencies are prepared.

The potencies prepared according to this scale are represented as 30 C, 200 C etc. Potencies of 1000 C and above are usually labeled with Roman numeral 'M' and with the centesimal 'C'. 1M is used for 1000c, 10M is used for 10, 000 C; CM is used for 100,000 C, LM (showed 50,000 C) is typically not used because it is also used for LM potency scale [Banerjee,2006)].

50 Millesimal scale of Potentization- LM scale or 50 Millesimal scale of Potentization was developed by Dr. Hahnemann in last years of life. In this, dilutions are made by taking 1 part of medicinal substance is mixed in in 50,000 parts of vehicle (Banerjee, 2006).

Dosage of the homeopathic drug -Dosage of homeopathic drug is not related to body size of the animal but more to observable dynamics of the body and the disease. Therefore, amount of the drug does not matter much. The number of pilules taken in a single dose is relatively less important than the potency.

Potency of the homeopathic drug -Potency of the homeopathic drug is an important factor in the treatment. Potencies above than 30 centesimal are characterized as higher potency and below as lower potency. Potencies are selected on the basis of susceptibility of the patient, nature of the disease and nature of the drug, and symptoms of the drug and the patient. Lower potencies are used if focus of symptoms is physical/ organic, in the beginning of treatment, in acute cases, and in conjunction with patients on conventional medicines. While higher potencies are used if emphasis of symptom is psychological, repeated at less frequency and are generally considered for chronic ailments.

Frequency of drug administration -Frequency is determined according to disease dynamic and patient response. Acute conditions require more frequent dosing than chronic ailments. In general, the drug is repeated only when the effect of the first dose has worn off and symptoms persist. As long as the patients feel comfort, there is no need to repeat the drug. Single most error in prescribing homeopathic drug is over medication.

Duration of Treatment -Duration of therapy is governed by the response of the patient. Treatment should not be given longer than necessary. It should be stopped as soon as symptoms cease to exist.

Selection of Homeopathic Drug/ Remedy -Selection of homeopathic drug/remedy is based on matching of symptoms of the drug in the patient. The drug can be selected at various level.

a) At a pathologic level – example Arnica for injury
b) At a local level- example Arsenic allbum or mercurius solibulus for gastroenteritis, Colycynth for colic.
c) At organotropic level- Drug selected according to organ invoved.Example Chelidonium for Liver, Euphrasia for eye, Rhus Tox for muscle.
d) At a historical level- when any particular condition might have contributed to symptom. Example Injury- Arnica, Birth problem- Caulophyllum
e) Regulatory level- Homeopathic potencies of metabolites, dietary factors or poisons to facilitate metabolism, absorption or excretion of a particular substance. Example ferrum aids iron absorption, Calcarea aids calcium metabolism.
f) At a specific level- drug made from infetious material (Nosode). It is used to treat similar infections.Nosodes for Mastitis.
g) At a desensitizing level- Homeopathic potency of allergen is used to desensitize.
h) At a constitutional level-It is most important. Constitutional remedies take into account nature and individuality of the patient as a whole including his mental and physical characteristics.

Advantages of homeopathic treatment

The basic advantage of opting for homeopathy is its micro doses, more compassionate nature, eco friendly nature and more comprehensive approach.

Types of homeopathic remedies

There are many types of homeopathic remedies viz. Singular remedy, Specific remedies, Polycrest remedy, Constitutional remedy and .Combination remedy. Single remedies such as Arnica for injury, Aconite for fever. Specific remedies used for specific purpose. Constitutional remedies are those that take the entire picture of the patient into account. Polycrest remedies are deep acting and extensively applicable having a wide action on all parts of the body. Homeopathic combination remedies are mixture of different medicines, each of that is known to be effective for treating slightly different variations of a certain ailment.

Although the method of manufacturing homeopathic remedies has changed since Hahnemann's days, his principles are still utilized today. Many reputed companies have produced over- the –counter homeopathic single and composite remedies as Nux

vomica, Rhustox,Arsenic album and Calc. carb. As these remedies are treating a wide range of common ailments they are also known as polycrest remedies.

Despite the emphasis on individualizing a homeopathic medicine to an ailing patient by traditional homeopaths, homeopathic combinations remedies - a mixture of synergistic homeo drugs, are gaining popularity during recent days owing to their broad spectrum and their ability to cover many manifestations of the particular disease. Homeopathic drugs have been tried in many diseases of farm and companion animals.

Is Homeopathy a Placebo Effect?

For long Homeopathy has been regarded as a 'Placebo' therapy resulting from long interactions between patient and doctor (Brien *et al*., 2010). A placebo is a medical intervention that has a non-specific psychological or psycho physiological therapeutic effect and is thus lacking any known specific effect for the condition treated (McMillan, 1999), but products with specific efficacy can also produce placebo effects. The basis of the placebo effect in people is experiencing a beneficial effect, arising from belief in the treatment, and based partly on confidence derived from consultations, leading to expectations on the part of the patient. Mechanisms underlying the placebo effect are still poorly understood. Sumegi *et a*l. (2014) have demonstrated a conditioned placebo effect suppressing the signs of separation anxiety in dogs. It seems that placebo effect does operate for both homeopathic and other drug therapies. In Bavaria, it was reported that 88 per cent of GPs sent patients home with prescriptions for placebo drugs, the corresponding figure for the whole of Germany being 50 per cent (Jutte *et al*., 2011, Kupferschmidt 2011). It is unconceivable that an animal can distinguish mentally between a homeopathic and drug-based product, if both are identical in presentation and administered in same manner. Therefore placebo effect of homeopathic remedies in animals seems unlikely. The placebo component of the effect of a homeopathic veterinary product is presumably limited normally to the judgment of outcome, based on the subjective evaluation of the caregiver (veterinarian or animal owner) (Conzemius and Evans 2012, Talbot *et al*., 2013, Gruen *et al*., 2017).

Anology between Homeopathy and Immunology

The birth of Immunology and homeopathy took place at the end of the eighteenth century at the same time when Jenner gave first smallpox vaccination and German physician Samuel Hahnemann was conducting his first homeopathic 'provings'. The profound analogies between homeopathic principles and immunology are due to the fact that the both are based on the principle of regulating endogenous systems of healing and its neuroendocrine integrations. Jenner's discovery of anti small pox vaccination remained isolated episode in medicine until Pasteur connected its origin with a principle that can not be better characterized than by Hahnemanns word: Homeopathic (Behring,1915).Around the end of 19th century Schulz and Arndt developed a principle that described that weak stimuli slightly increase biological responses, medium and strong stimuli markedly raise them, strongones suppress them and very strong ones arrest them. Similar observations are also seen in modern medicine. It is apparent that immunology and modern biology can offer a considerable contribution to the understanding of Homeopathy in a frame work that is not very different from the conventional one.

Mechanism of Action of Homeopathic drugs.

Because of high dilutions, the mechanism of the action of homeopathic drugs could not be understood properly. Nevertheless, Chaos theory (Gleck, 1987) where it was assumed that minute changes can lead to huge difference ; and Resonance theory where it was considered that the water, in which most of the homeopathic medicines are made, not only store frequencies (Endler,1994) but also some form of memory, were suggested. These theories have not been substantiated experimentally. However, with advancement in molecular technology, many explanations are being postulated and it has been convincingly proved that homeopathic medicines are now nano medicines. Following are the few studies which have contributed significantly in revealing mechanism of action of homeopathic remedies.

MRI study on various serial homeopathic dilutions revealed that the hydroxyl groups in the solvent of solution continuously alter as dilutions become higher (Smith and Boericke,1965). The specific effects of homeopathic medicines are of a non-molecular origin, yet provide powerful clinical effect. It has been assumed that highly dilute substances transfer biological activity to cells by electromagnetic

fields. Another working hypothesis about homeopathic ultrahigh dilutions is interactions between the radiation fields of a charged molecule and the electric dipoles of water generate a permanent polarization of water which becomes coherent and has the capacity to transmit specific information to cell receptors, somewhat like a laser (Del Guidice *et al.,*1988) A recent Research study revealed the presence of Nano bubbles (NBs) in Homeopathic dilutions by using NMR spectroscopy. These NBs are associated with superstructures that seemed to increase in size with increasing dilutions well into the 'ultra molecular' range. These nano bubbles create superstructures related to specific solute (Demangeat ,2015).

Mechanism of action of potentized homeopathic drugs, particularly those diluted beyond Avagadro's limit, is still inconclusive. A team of scientist led by Dr. A.R. Khuda Bukhsh has provided convincing evidence of potentized homeopathic drugs' ability to trigger favourable regulatory changes in gene expression, possibly through epigenetic modifications. The conclusion was based on *in vivo* (mice, rats model, human subjects, bacteria, yeast, bacteriophage) and *in vitro* (normal and cancer cells) studies employing modern cytogenetic (CA,MN,SHA,MI,Comet assay etc) , molecular biology techniques (Rt-PCR, microarray analysis, signal pathway, DNA methylation and histone acelation), compound and electron microscopies (scanning, transmission,atomic force and confocal) and immunochemical assays(Khuda bukhsh ,2015).

Bellavite (2015) in his lecture highlighted the research conducted on hypothesis and findings on the action mechanism(s) of homeopathic drugs at university of Verona. Studies have revealed peculiar features of diluted/ succussed solutions. The current evidences strongly support the notion that the structuring of the water and its solute sat the nano scale can play a key role. The hypothesis invoked sensitivity to bio-electromagnetic information , participation of water chains in signaling and regulation of bifurcation points of systemic networks.It has been postulated that homeopathic medicines work by identification of biological targets, the means of drug-receptor interactions, the mechanism of signal transmission and amplification, and the models of inversion of effects according to the traditional "smile" rule (Bellavite,2015).

Researches on homeopathic ultrahigh dilutions

Many homeopathic medicines are used in which the dilutions exceed Avogadro's number (6.023×10^{-23}). When homeopathic medicines are diluted 1:10, with repeated succussion and dilutions by this process at least 24 times, potency is made that is so dilute that the chances of a single molecule of the original drug substance remaining in the volume are less than 1×10^{-10}.

High dilutions of homeopathic medicines have been effective in treating many conditions. Homeopathic potencies of 100C to 1M of typhoidinum, nux vomica, tuberculinum, hydrophobinum and malandrinum completely inhibited chicken embryo DNA virus induced pock-like lesions on the chorioallantoic membrane compared to controls. This study showed antiviral effects of Homeopathic ultrahigh dilutions *in vivo (*Singh and Gupta, 1985*)*.

A bibliometric (statistical analysis of books, articles, or other publications) analysis on experimental models in basic research on utradilution has shown reproducible results (Endler *et al.*, 2010).

In many countries and universities, research in Homoeopathy and its ultrahigh dilutions is a topic of interest. Treatment with Homoeopathic Medicines is assumed as the placebo effect. But Homeopathy medicines have proven their effect as anti-inflammatory, antipyretic, antimicrobial, antioxidant, anti-diabetic and many other diseases in several animal studies as well as randomized clinical trials (Neto *et al*.,2004; Ahmed *et al*.2017).

Upadhyay and Nayak (2011) conducted research on high dilutions of homeopathic belladonna, colchicum and pulsatilla using scanning electron microscopic (SEM) studies, transmission electron microscopic studies (TEM) and trace element analyses for silicon. These dilutions exhibited high nanoparticle contents. Such nanoparticles were rich in silicon and were crystalline in nature. They were of the opinion that during potentization, the nanoparticles might acquire the information of the diluted away starting-source encrypted on them by means of epitaxy.

Using new electron microscopy methods(like cryo-TEM, atomic spectroscopy) it has been shown for the first time that nanoparticles and aggregates of starting materials are present in the final product despite the extremely high dilutions(Bellare,2015).

This has put to rest a long standing controversy. In homeopathy a controversy has existed regarding high dilutions which goes against the tenets of Avagadro's number and molecular basis of the matter.Work done under the leadership of Dr. Bellare has established that the traditional and alternative medicinal products (including homeopathic drugs) also have nanoparticles in them.

Studies conducted in Brazil have contributed about physical and biological features of homeopathic phenomenon with some indication of cell pathways and mechanism involved.(Bonamin,2015).

Animal Models in Homeopathic Research

Bonamin *et al*.(2015) reviewed 53 articles on the use of animal models in homeopathic research (indexed in Pub Med data base) to elucidate the biological features and phenomenology of the effects of high dilutions on the living systems and found further demonstrations of biological effects of homeopathy using more refined animal models and *in vitro* techniques.

Evidence of Clinical efficacy of Homeopathy- A Meta analysis

There is some evidence that homeopathic treatments are more effective than placebo; however, the strength of this evidence is low because of the low methodological quality of the trials. Studies of high methodological quality were more likely to be negative than the lower quality studies. Further high quality studies are needed to confirm these results (Cucherat *et al.*, 2000)

Despite the resistance to change in general and to homeopathy specifically, it is getting increasingly difficult for physicians and scientists to doubt the benefits that homeopathic medicines offer. Italian hematologist Paolo Bellavite and Italian homeopath Andrea Signorini's *Homeopathy: A Frontier in Medical Science* is presently the most comprehensive resource of controlled studies on homeopathy. They concluded "The sum of the clinical observations and experimental findings is beginning to prove so extensive and intrinsically consistent that it is no longer possible to dodge the issue by acting as if this body of evidence simply did not exist."(Bellavite and Signorini,1995). They said that to reject everything en bloc, as many are tempted to do, means throwing out the observations along with the interpretations, an operation which may be the line of least resistance, but which is not scientific because unexplained observations have always been the main hive of ideas for research.

Doehring and Sundrum (2016) conducted the analysis of the research publications on the efficacy of homeopathy in animals in peer reviewed journals from1981 to 2014.They observed that a significant higher efficacy was recorded for homeopathic remedies than control groups. However this does not imply that homeopathic remedies are effective under different conditions. Due to a lack of prognostic validity, replacing or reducing antibiotics with homeopathy currently cannot be recommended unless evidence of efficacy is reproduced by RCTs and proven in various farm practice condition.

Another meta analysis of randomized placebo-controlled trials in veterinary homeopathy conducted by Mathie and Clausen (2015) provided some very little evidence that clinical intervention in animals using homeopathic medicines is distinguishable from corresponding intervention using placebo. Definite conclusion needs large number of quality trial using homeopathic remedies.

Homeopathy in the Treatment of Animal Diseases.

(a) Foreign scenario

Homeopathic medicines are being increasing used in animal treatment in Armenia, Belgium, Bosnia and Herzegovina, Bulgaria, Czech Republic, Finland, Germany, Greece, Ireland, Israel, Norway, Serbia, Spain, Sweden, Switzerland, United Kingdom – member countries of European council of Classical Homeopathy . Animals are treated using homeopathic drugs for both acute and chronic conditions such as eczema, eye inflammation, allergies, cough; diseases of gastrointestinal tract, urinary tract, liver , thyroid, loco motor system, nervous system; diabetes; hormonal disturbances; injuries and behavioral problems. A legislation restriction has been imposed on the practice of homeopathy in animals in Ireland, Sweden and the United Kingdom. In Ireland and in the United Kingdom treatment of animals using homeopathy is allowed to veterinarians only. Veterinarians in Sweden are not allowed to prescribe homeopathic medicines. In Germany veterinarians are permitted to

use only specifically registered homeopathic remedies in food producing animals. In Armenia and Serbia only homeopaths prescribe homeopathic remedies. In Finland and Sweden, homeopathic drugs are in the doping list (The Homeopathic Treatment of Animals in Europe, 2007). Many research studies on bovine mastitis (Day,1986), Kennel cough (Day,1987), rectal prolapse in pig (Searcy and Gujardo,1994), still birth in swine (Day,1984), post partum anoestrus (Williamson and Others, 1995), effect of Cheliodonium on reducing cholesterol in rabbits(Baumans and Others, 1987), anti-inflammatory effect of Hypericum perforatum (Varma and Others, 1988) and of Arnica montana in rat model(Desai and Others,1992), labor facilitating effect of Caulophyllum in cows and pigs (Day,1985), arsenic album in neonatal diarrhoea ((Kayne and Rafferty,1994) have appeared in the literature.

(b) Indian Scenario

It appears that both single homeopathic drugs and homeopathic combination remedies have been used in different trials. Some trials on the use of homeopathic combination remedies/ single homeopathic drug in the management of mammary affection/ infections in buffaloes(Varshney and Ram Naresh, 2004) and in cows (Varshney and RamNaresh,2005, RamNaresh and Varshney2005, Sharma *et al.*,2006, Varshney,2007, Chandel *et al*.,2009), anoestrus in dairy animals (Kumar *et al*.,2004, Kumar *et al*.,2006)), non specific diarrhoea in calves (Ram Naresh and Varshney,2004),diarrhoea in pups (Varshney,2006 a), canine viral gastroenteritis (Varshney,2006 b), canine babesiosis (Chaudhuri and Varshney,2007a), anaemia management in dogs with babesiosis (Chaudhuri and Varshney,2007 b), hypolipidaemic properties of homeo-complex (Bandyophadhyay *et al*.,2007), cardiac arrhythmias in dogs (Varshney and Chaudhuri, 2007), haematuria in dogs (Varshney,2013 b), epilepsy in dogs (Varshney,2006), gall bladder diseases in canine (Bandyopadhyay *et al.,* 2010) and osteoarthritis in dogs (Varshney ,2016 b)have been published in International and National journals. Studies on evaluation of homeopathic drugs in hepatopathies (in rats model and clinical cases in dogs) ; pyrexia syndrome (Brewer's yeast induced pyrexia in albino mice , and clinical cases in dogs);.haematuria in dogs; seborrhea in dogs; stye in dogs; wounds in animals, and arrhythmias in dogs have also been conducted at Indian Veterinary Research Institute, Izatnagar (Annual Reports IVRI Izatnagar, Varshney and Swaminarayan, 2007).Calcarea carb 30c/200c has shown efficacy in relieving symptoms associated with transitional cell carcinoma in a dog (Varshney and Swaminarayan,2018).

Most of the researches on the use of homeopathic drugs in animal diseases suffer from small number of patients in the trial and lack of substantiation of research findings by other researchers. Curiously published reports on the use of homeopathic drugs in animal health care has done little to convince veterinarians trained in modern medicine possibly due to inappropriate research procedures to satisfy a modern veterinarian. This necessitates further research satisfying both the basic tenets of homeopathy as well as of allopathy. Animal experimentation is a well accepted research tool in modern medicine. Randomized controlled clinical trials, which are lacking in most of the Indian works on homeopathy, are also bastion of modern scientific clinical trial. Unfortunately neither of these methodologies is appropriate to homeopathy. What is needed is a large scale placebo controlled clinical trial under field conditions. Wherein at least disease diagnosis is based on sound scientific footings including history, clinical manifestations, laboratory investigations, electrocardiography/ echocardiography, ultrasonography, radiography and / or endoscopy as the case may be.

In homeopathy, uniqueness of the individual is the key factor for selecting the drug and its potency. Actually this factor hinders the controlled randomized large scale clinical trials. In one study, Rhus Tox was used in the randomized group of osteoarthritis in humans and found not to have impact greater than placebo as remedy was not matched with the individuals symptoms (Shipley *et al*.1983) while Rhus tox when used in patients of fibromyalgia whose entire picture matched with Rhus Tox showed significantly better results than placebo (Fischer *et al*.,1989).

Homeopathy and Organic Farming

There is an increasing concern about the use of antibiotics, other antimicrobial products, and growth promoters in farming industry. Growing resistance to antimicrobials and emergence of strains of microbes to multiple antibiotics has led to increasing difficulties in the treatment of diseases such as pneumonia, mastitis, diarrhea and salmonella infections. European Union

committee has stressed that drugs used as growth promoters in animal production as well as in humans and antimicrobials should be phased out. It seems that the use of homeopathy is of great benefit in increasing the health and well being of animals; and its use in food producing animals reduces the risk of medicinal traces in meat and milk.

Problems To Be Addressed To Promote Scientific Practice Of Homeopathy In Veterinary Medicine

1. Legal Issues- Using homeopathic drugs in veterinary practice by the Veterinarians trained in modern medicine has to be legalized to avoid litigations.
2. Veterinarians willing to practice homeopathy need to be trained in basics of homeopathy through short courses.
3. Faculty is to be created for training veterinary practioners.
4. There is a need to creat scientific data base through research in veterinary homeopathy.
5. Since homeopathy is individualized medicine where symptoms of the patients are matched with the symptoms of the drug and modern medicine is based on double blind, randomized and control trials, a compromising approach will facilitate scientific approach.
6. There is a need to develop proper research methodology to evaluate homeopathic drugs in animal diseases.
7. To achieve these goals more funding is needed. Therefore funding agencies should come forward to support research in veterinary homeopathy.

Benefits of integrating Homeopathy in Animal Health care

1. To some extent use of antibiotics can be reduced
2. Cost of treatment can be reduced
3. Can be used as 1st aid treatment in remote places
4. Can be used as low cost supportive therapy in some diseases.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 18-27

An overview and review of the clinical diagnosis of Chronic Kidney Disease (CKD) in dogs

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Overview

Kidneys are valuable as they remove waste substances from the blood, and maintain fluid, minerals and acid-base balance within the body. It's a major filtration organ of the body as about 1/5 of the total blood volume passes through the kidneys per minute (Robert et al 2007). Dog kidneys are composed of about half a million nephrons (Eisenbrandt and Phemister 1979). Any condition which damages the kidneys is referred to as kidney or renal disease. Kidney disease has traditionally been classified as acute and chronic, but more recently this classification is rather considered obsolete as there is spiraling of acute and chronic kidney disease(CKD) and acute kidney disease may end up in CKD.

CKD is also equally important in humans(Jha et al 2013) as an estimated 10-14 % people suffer from it in the western world and majority of them unaware due to early onset. Therefore the naturally occurring CKD in dogs is also a strong comparative disease model for devising new diagnostic and prognostic tests.

Chronic Kidney Disease: CKD is a long-term, slow, progressive but irreversible kidney damage with markedly decreased glomerular filtration rate (Gupta et al 2015), usually indolent during its early stage. Since the kidney is always under filtration work-load and the damaged nephrons unreplaceable, therefore the early signs of CKD remain elusive till about 75-80 % of the nephrons are damaged.

Risk Factors

The kidneys can be damaged by a wide range of conditions including injury, infection, toxins, and cancer (Brown 2013; Polzin 2013). Factors that can make dogs more prone to kidney disease include the following:

Age: Due to its slow progressive nature the risk of developing CKD in dogs increases after 7 years of age.

Food: Phosphorus and protein rich diets particularly in commercial pet foods increase the chances of CKD.

Breed: There is a reported breed predilection of CKD. Some breeds of dogs viz. English Cocker spaniels, Bull Terriers, Labradors and German Shepherds, are more likely to develop CKD due to specific kidney damaging conditions.

Environmental factors: Some chemicals, including certain disinfectants, lead paint, fungal metabolites, pesticides and heavy metals and long term medications can damage the kidneys.

Diabetes mellitus: The diabetic dogs, especially females are considered more vulnerable to CKD due to diabetic nephropathy.

Hypertension: Heart ailments resulting in congestive heart failure and hypertension also expedite the progression of CKD.

Causes of CKD in dogs

Major causes of CKD include following renal and post-renal insults:

- Glomerulonephritis
- Pyelonephritis
- Nephroliths
- Urinary obstruction and hydronephrosis
- Tubulointerstitial nephritis
- Heamoprotozoa- *Erhlichia canis* and *Hepatozoon canis*
- Leptospirosis
- Amyloidosis
- Immune mediated/auto-immune diseases
- Hereditary nephropathies
- Cancer

In addition, any severe acute renal injury or recurrent renal infections may later progress to CKD.

Clinical Signs

- Polydipsia and polyuria during initial stages and oliguria later on.
- Urinary urgency, incontinence and nocturia
- Dehydration and dryness of lips and gums
- Weight loss and anemia
- Decreased appetite
- Weakness- particularly hind limb
- Back or flank pain
- Lethargy and increased sleepiness
- Oral and gastric ulcers
- Bad breath and retching
- Vomiting and diarrhea
- Poor coat appearance
- Depression, delirium and coma

Diagnosis

- Signalment and history
- Physical examination
- Blood pressure measurement
- Urinalysis- specific gravity, urine chemistry, urine protein:creatinine ratio
- Urine culture
- Serum chemistry- blood urea nitrogen, creatinine, sodium, potassium, calcium, phosphorus and glucose
- Complete blood count
- Radiography and ultrasonography
- Renal biopsy

According to recent International Renal Interest Society (IRIS, modified 2017) classification, the CKD in dogs based on serum creatinine levels is classified as follows:

- Stage I: Nonazotemic
- Stage II: Mild azotemia (1.4–2.0 mg/dL)
- Stage III: Moderate azotemia (2.1–5.0 mg/dL)
- Stage IV: Severe azotemia (>5.0 mg/dL)

Recognizing CKD requires evidence from multiple sources, including renal function tests, serum electrolyte concentration and acid base status, urinalysis and renal imaging studies. The CKD is usually suspected on the basis of reduced kidney function or markers of kidney disease. Markers of CKD may be recognized from haematological or serum biochemical evaluations, urinalysis, imaging or pathological studies. Findings suggestive of CKD may also be found by physical examination or from the medical history. Brown *et al* (2007) reported that the diagnostic tests that are routinely used to establish a diagnosis in a patient with CKD include urinalysis, urine culture, and urine protein to creatinine ratio, serum chemistry profile, complete blood count and sequential evaluation of serum creatinine concentration.

The potential use of new biomarkers for kidney function, such as Symmetric dimethylarginine (SDMA) and asymmetric dimethylarginine (ADMA), provides for early recognition of kidney disease before conventional biomarkers like creatinine and BUN increase offers the prospect of early diagnosis of CKD.

Review

Clinical manifestations of CKD in dogs

Eriksen and Grondalen (2008) reported that the clinical signs of CKD in dogs were in appetence, weight loss, vomiting, depression, polydipsia and polyuria. Robinson *et al* (1989) studied CKD in Bull Terrier dogs and observed signs like lethargy, anorexia, weight loss polydipsia and polyuria. Brown *et al* (1990) recorded signs like vomiting, weight loss, polydipsia and polyuria in dogs affected with CKD. Hoppe *et al* (1990) stated the common clinical signs of CKD to be depression, polydipsia and vomiting. Polzin *et al* (2000) reported that up to 67% loss of renal function occurs as clinically asymptomatic condition and that with a 67-75 % of loss of renal function, polyuria and polydipsia may be manifested. They further stated that loss of 75-95 % of renal function would be manifested as vomiting, diarrhoea, apathy and when less than 10 % of renal function is present, the signs of uremic encephalopathy appear indicating terminal stages of illness. Raskin (2001) mentioned that uremic encephalopathy might accompany CKD. Muralikrishna (2003) observed clinical signs like vomiting, melena and oral ulcers in dogs suffering from CKD. Pugliese *et al* (2005) stated that with a loss of 67-75% of the GFR, severe polydipsia and polyuria occurred and the accumulation of blood nitrogen catabolic products determined the occurrence of systemic signs such as anorexia, weight loss and specific signs such as vomiting and diarrhoea. When the residual renal function was found to be less than 10

%, uraemia was associated with the neurological signs i.e. uremic encephalopathy that indicated terminal stage of illness. Reine and Langston (2005) opined that the presence of isosthenuria might be suggestive of primary renal failure. Isosthenuria occurs with greater than 66 % damage to the kidney, whereas azotemia occurs after more than 75 % of renal damage has accrued. However, Robertson and Seguin (2006) opined that many cases of CKD were asymptomatic. Grauer (2007) stated that decreased production of erythropoietin contributed to the non-regenerative anaemia of CKD and decreased metabolism and excretion of parathyroid hormone and gastrin contributed to osteodystrophy and gastritis, respectively. Lucre *et al* (2008) observed CKD in 13 dogs with advanced kidney disease and the clinical signs in those dogs were anorexia, lethargy and weight loss. McGrotty (2008) reported that the dogs with CKD had polyuria, polydipsia, anorexia, lethargy, weight loss, vomiting, oral ulcers, halitosis and acute blindness as common clinical signs. Ross (2008) stated that a history of signs such as polyuria, polydipsia, weight loss, selective appetite, deteriorating hair coat occurring over several months was a strong evidence of CKD. Pradhan and Roy (2012), in their study on CKD in dogs found that, in advanced stage of renal disease, nervous signs like ataxia, tremors, incoordination, seizures, syncope and progressive deterioration of health were common. Shaw and Ihle (2013) noticed polyuria, polydipsia, anorexia, lethargy, weight loss, vomiting, oral ulcers and dehydration as clinical signs of CKD in dogs.

Physical, chemical and microscopic examination of urine of the dogs with CKD

Larkin *et al* (1972) recorded proteinuria with low urine specific gravity (1.018) in a dog with nephritic syndrome. English (1973) reported that measurement of urine specific gravity and osmolality were used to determine the kidney's ability to concentrate urine. The ability to concentrate urine in the CKD depends upon adequate secretion of anti-diuretic hormone and the presence of enough nephrons. Schepper *et al* (1974) observed that proteinuria and low urine specific gravity in a dog with nephritic syndrome and glomerulonephritis. Brobst (1989) reported that gross examination of urine was an integral part of urinalysis, and blood and bile pigments were common causes of its abnormal coloration. The dipstick test for pH, blood, glucose, ketone bodies and bilirubin in urine could be used. Microscopic examination of urine sediment must be interpreted in combination with the physical and chemical composition of urine as excessive number of cells, casts, crystals and bacteria may provide evidence of disease. Robinson *et al* (1989) documented proteinuria and low urine specific gravity ranging from 1.01 to 1.017 in dogs affected with CKD. Booth (1990) observed proteinuria and low urine specific gravity of 1.012 in a dog with nephropathy. Carl *et al* (1995) reported that in the dogs with primary renal failure, azotaemia usually followed loss of ability to concentrate urine to a specific gravity of at least 1.030. Osborne *et al* (1995) opined that the specific gravity could not be taken as early indicator of renal damage as the kidneys had a tremendous reserve capacity. Impairment of the kidneys ability to concentrate or dilute urine may not be detected until at least two third of the total population of nephrons was damaged. Haller (2002) opined that determination of USG was very important in assessment of CKD. It should always be measured before any treatment is initiated because fluids, glucocorticoids or diuretics may result in artificially diluted urine. Hyposthenuric urine (1.001- 1.007) indicated active dilution, isosthenuria (1.007-1.015) indicated unchanged excretion and hypersthenuric urine indicated active concentration of the glomerular filtrate. Sato *et al* (2002) observed USG in a range of 1.006-1.025 in all dogs suffering from CKD. Albasan *et al* (2003) reported that urine sample should be analyzed within 60 minutes of collection to minimize temperature and time dependent effects on in vitro crystal formation. Presence of crystals observed in stored samples should be validated by re-evaluation of fresh urine. Lees (2004) concluded that detection and treatment of animals with persistent renal proteinuria was one of many possible manifestations of CKD in dogs that were important to evaluate and treat appropriately. Reine and Langston (2005) stated that urinalysis included evaluation of physical characteristics, biochemical parameters and microscopic sediment evaluation. Camacho *et al* (2005) stated that USG below threshold of 1.025 in all dogs exhibited azotemia of renal origin. Reine and Langston (2005) reported that the presence of isosthenuria (USG 1.007 to 1.012) might be suggestive of CKD. Isosthenuria occured with greater than 66 % damage to the kidneys whereas azotemia did not progressed until more than 75 % damage was sustained. Shaw and Ihle (2013) suggested that the urine was frequently

isosthenuric (USG < 1.030) and USG ranged from 1.007 to 1.015 in man with CKD.

Biochemical profile of dogs with CKD

Schepper *et al* (1974) observed BUN value as 73 mg/dl, serum creatinine as 2.36 mg/dl, serum calcium concentration as 8.2 mg/dl and noticed proteinuria, hypoproteinemia, edema and ascites in a three and half year-old German Shepherd dog suffering from CKD. Finco (1976) recorded the mean serum creatinine as 1.22 ± 0.6 mg/dl in dogs with familial renal disease. Watson and Canfield (1979) recorded increased BUN, serum phosphorus and decreased serum calcium in a dog affected with CKD and hyperparathyroidism. Lucre *et al* (1980) reported hyperphosphatemia in 13 dogs with CKD. Eriksen and Grondalen (1984) reported extremely high serum BUN and creatinine values in the dogs with CKD. Brown *et al* (1985) observed that hyperphosphatemia was the common clinical finding in all cases of CKD in dogs. Weller *et al* (1985) documented BUN value in a dog with CKD as 450 mg/dl. Dash (1987) reported reduced serum protein level reduced (<5g) in various renal dysfunctions. Taboada and Palmer (1989) studied four cases of dogs with CKD associated with bacterial endocarditis and observed increased serum creatinine in all cases. Booth (1990) reported the BUN in a dog with CKD as 48.6 mg/dl against the normal level of 2.8 to 8.3 mg/dl. King *et al* (1992) recorded serum calcium in dogs with CKD and these levels ranged from 6.9 to 11.2 mg/dl. Srinivasan *et al* (1993) mentioned that the mean creatinine in dogs with CKD was 4.56± 0.72 mg/dl and mean BUN in dogs affected with CKD was 78.18± 7.06 mg/dl. Hurley and Vaden (1995) reported that hypoalbuminemia occurred in patients affected with glomerular disease. DeMorais *et al* (1996) reported hyperphosphatemia, hypoproteinaemia and hypoalbuminemia in dogs suffering with CKD. Brown (1998) reported that azotaemia was the presence of elevated serum concentrations of creatinine and BUN and that for CKD; the presence of azotaemia of renal origin for a minimum duration of two weeks should be present. Cowgill *et al* (1998) calculated mean serum creatinine as 6.7± 2.5 mg/dl, mean BUN as 108.3 ± 31.3 mg/dl, mean serum phosphorus as 6.8 ± 2.2 mg/dl and mean serum calcium as 10.7 ± 0.08 mg/dl in dogs with CKD. Finco *et al* (1999) observed that the plasma creatinine concentration was commonly measured to assess renal function as the crude of GFR. Laurence *et al* (1999) reported that hypoalbuminemia occurred in CKD in dogs. Borku *et al* (2000) observed the mean BUN in dogs that were apparently healthy and suffering with nephritis as 16.93 ± 1.33 mg/dl and 263.94 ± 18.92 mg/dl, respectively. Creatinine is a substance that the body produces during normal metabolism. The body eliminates creatinine almost exclusively through the kidney's filtration process, so measurement of creatinine is an accurate estimation of how well the kidney filtration processes are working. Anything that alters the ability of the kidneys to filter efficiently can cause changes in the level of creatinine in the blood (Wyss and Kaddurah-Daouk, 2000). Muralikrishna (2003) observed the mean serum creatinine as 4.06± 0.65, mean serum phosphorus as 8.45 ± 1.3 mg/dl and mean serum calcium as 9.43 ± 0.55 mg/dl in dogs with CKD. Camacho *et al* (2005) stated that dogs presenting azotaemia of renal origin had significantly lower concentration of total serum proteins and albumins. Mrudula *et al* (2005) estimated the mean serum creatinine as 5.59± 0.45 mg/dl, the mean total serum protein as 6.08 ± 0.13 g/dl, serum albumin as 2.5 ± 0.10 and mean serum phosphorus 5.16 ± 0.2 mg/dl in dogs suffering with nephritis. Hasan *et al* (2007) reported that level of total serum protein in dogs with CKD ranged between 5.9 to 6.9 g/dl and serum albumin in dogs with CKD ranged between 2.9 to 3.8 g/dl. Kaneko *et al* (2008) reported that normal range of serum calcium in dogs as 9.0 ± 1.3 mg/dl. Lucre *et al* (2008) reported elevated levels of BUN in all dogs affected with CKD. McGrotty (2008) stated that plasma creatinin and BUN levels are typically used as biochemical marker of CKD. Arulmozhi *et al* (2010) observed systemic uraemia and encephalopathy in the dogs with progressive unresponsive renal failure. The serum chemistry revealed severe uraemia with 276 mg/dl urea nitrogen level. Kavitha (2010), in a study conducted on early diagnosis of CKD in dogs reported that the level of creatinine and BUN was studied to assess the kidney function, normal levels of creatinine and BUN in blood indicated the ability to eliminate nitrogenous waste products successfully. Girishkumar *et al* (2011) stated that hypoalbuminemia in dogs with CKD attributed to urinary loss of albumin and anorexia and also reported that there was a significant increase in serum phosphorus in dogs suffering from CKD. Lefebvre (2011) opined that serum creatinine concentration is currently considered as the best

indirect marker of GFR and is also used by the IRIS to stage canine CKD. Polzin (2011) reported the relationship between serum creatinine and GFR was such that every time GFR declined by half, the serum creatinine concentration doubled. Shaw and Ihle (2013) concluded that hypoalbuminemia and hypocalcaemia were common findings in dogs suffering from CKD associated glomerular disease.

Electrolyte and enzyme profile of the dogs with CKD

Schepper *et al* (1974) observed serum sodium concentration in a dog with nephritic syndrome as 144 m Eq/L and serum potassium concentration as 4.85 m Eq/L. Finco (1976) reported that the mean sodium concentration as 147 ± 1.74 m Eq/L and mean serum potassium concentration as 5.1 ± 0.6 m Eq/L in a dog with familial renal disease. In dogs with gentamicin-induced nephrotoxicity, GGT index increased prior to serum creatinine elevation (Rivers *et al* 1996). Pak (2000) reported serum sodium as144 m Eq/L and serum potassium as 5.7 m Eq/L in dog with CKD. Polzin *et al* (2000) reported that decreased renal function precedes the development of hypokalaemia in dogs. Adams (2004) reported hyperkalaemia as one of the clinical finding in dogs suffering with CKD. Mrudula *et al* (2005) stated that the dogs with renal insufficiency showed azotaemia, hypoproteinemia, hypoalbuminemia and hyperphosphatemia. Chandler *et al* (2007) observed 37 boxer dogs with clinicopathological findings of azotaemia, hyperphoaphatemia, anaemia, isosthenuria and proteinuria. Small amounts of GGT are excreted normally in the urine and tubular dysfunction greatly increases their excretion (Braun and Lefebvre 2008). Kaneko *et al* (2008) reported that normal range of serum potassium in dogs was 4.37 – 5.35 m Eq/ L and normal serum sodium concentration was 141.52 m Eq/L and reported that there was deficiency of sodium ions due to polyuria in generalized CKD. Detection of GGT may be best suited for detection of AKI rather than CKD as enzymuria may reflect acute tubular dysfunction instead of more chronic on-going damage (Brunker *et al* 2009). Girishkumar *et al* (2011) reported that there was a significant increase in serum potassium in dogs suffering from CKD.

Urinary chemistry of CKD in dogs

Finco *et al* (1997) suggested that the progressive increase in UPCR might be a marker of an accelerated rate of renal injury. Tanyel (2000) stated that a significantly higher UPCR (1.71 ± 0.19) occurred in dogs exhibiting signs of renal dysfunction than the healthy dogs. Jacob *et al* (2005) reported that initial high UPCR determination i.e. more than 1.0 in dogs with CKD was associated with greater risk of developing uremic crisis and death, which was then compared to dogs with UPCR less than 1.0 and thus concluded that UPC determination in dogs with CKD could be of prognostic value. Yathiraj (2006) concluded that UPC determination was one of the useful adjunctive procedures to evaluate the glomerular function in case of patients with CKD. Buranakarl *et al* (2007) stated that UPC was a good indicator of renal disease regardless of disease progression. Wehner *et al* (2008) observed that proteinuria and systemic hypertension were well recognized risk factors in CKD. Some of the dogs with CKD were proteinuric; almost all were hypertensive. Grauer (2007) reported that proteinuria in dogs could indicate the presence of CKD before onset of azotaemia or the presence of more severe CKD after the onset of azotaemia.

Haematological alterations in the dogs with CKD

Osborne (1970) stated that anaemia was a common clinical finding in dogs with CKD. Larkin *et al* (1972) observed WBC count as 17600 /µl in a dog with nephritic syndrome. Eschbach and Adamson (1991) reported that a major factor related to anaemia appeared to be decreased erythropoietin production by kidneys. King *et al* (1992) reported non-regenerative normocytic normochromic anaemia in 70.6 % dogs with CKD and were of the opinion that many factors, including decreased erythropoietin, haemolysis and blood loss might influence the development of anaemia and also reported that there was a decrease in PCV in dogs with CKD and mentioned that TLC of dogs with CKD ranged from 6000-17000/µl. Lulich *et al* (1992) stated that erythropoietin is produced primarily in the tubular interstitial cells of the inner renal cortex and outer medulla in the kidney, and as the CKD progresses, there are fewer erythropoietin-producing cells within the kidneys. Erslev and Besarab (1995), in their study on renal failure observed that uraemia was associated with decreased RBC survival, but the pathophysiology of that was unclear and was most likely multifactorial. Cowgill

et al (1998) stated that the severity and progression of the anaemia and the clinical signs correlated with the degree of CKD in dogs and also reported PCV as 27 ± 6.60 %, mean RBC count as 4.20 ± 0.96 × 10^6/µl and mean TLC as 10100 ± 696 /µl in dogs with protein losing glomerular disease. Cowgill *et al* (2000) estimated mean PCV as 17.6 ± 5.2 % and RBC count as 2.50 ± 0.7 × 10^6/µl in six dogs suffering from CKD. Mrudula *et al* (2005) observed that 90 % dogs with CKD were anaemic. Robertson and Seguin (2006) opined that CKD could be associated with lymphopenia, which reflected the effects of endogenous glucocorticoids or stress of chronic disease. Sastry and Rao (2007) stated that the average DLC was 20%, 70%, 4% and 5% lymphocytes, neutrophils, eosinophils and monocytes, respectively in healthy dogs and basophils were rare. King *et al* (2008) recorded that seven out of 17 dogs with CKD had mature neutrophilia. Rusenov *et al* (2009) observed severe erythropenia and hypochromasia in dogs with CKD and stated that the main cause of this was erythropoietin deficiency. Chalhoub *et al* (2011) stated that acute and chronic inflammation contributed to anaemia of renal disease by the production of inflammatory cytokines and substances such as hepcidin that decreased the erythropoietin function, red cell survival and available iron. Girishkumar *et al* (2011) reported that TLC and DLC were not much significant in dogs with CKD. Bradea *et al* (2013) concluded that Complete Blood Count (CBC) in CKD provided useful information about the progress of the disease as well as appreciation of type of anaemia offering additional information for therapeutic protocol adjustment for amending induced haematological consequences. Non-regenerative anaemia represented a common finding in the dogs with CKD.

Ultrasonographic and radiographic findings in the dogs with CKD

Ultrasound examination is an integral tool in the thorough evaluation of the urinary system. Ultrasound is a non-invasive, non-painful and economical procedure that provides valuable information concerning morphology, vascular status and luminal contents usually with little or no sedation. Cartee *et al* (1980) reported the use of ultrasonography in the diagnosis of renal diseases and found to be useful in diagnosis of hydronephrosis, renal calculi and renal neoplasia. Konde *et al* (1986) observed in his study of 14 dogs with renal lesions, that ultrasonography was more sensitive than radiography in differentiating the renal lesions. Walter *et al* (1987) observed ultrasonographic changes in 32 dogs with renal parenchymal disease and diagnosed 26 dogs with interpretation errors in six dogs. Wood and McCarthy (1990), in his study on 26 dogs with both ultrasonography and anatomical observations recorded precise correlation of anatomical studies with ultrasonographic images. Felkai *et al* (1996) in his study of eight young Cocker Spaniels evaluated the related ultrasonographic findings to histologically confirmed renal dysplasia. Based on the ultrasound findings alone, renal dysplasia was suspected when small kidneys with thin echogenic cortex were present in young dogs, but it could not differentiate chronic inflammatory disease from ESRD. Vaden *et al* (1997) stated that the ultrasonography could be used to characterize the renal shape and size. It provided information about renal parenchyma, increased overall renal echogenicity and decreased corticomedullary distinction and it may not be diagnostic for CKD. Churchill *et al* (1999) opined that, in normal sonographic anatomy of kidney, the cortex was outer rim of tissue and was normally hyperechoic to the more central hypoechoic medulla. The cortex was typically isoechoic to hypoechoic to the liver and hypoechoic to the spleen. Temizsoylu *et al* (2006) studied the use of radiography and ultrasonography in the diagnosis of renal diseases and concluded that ultrasonography was more sensitive than radiography and survey radiographs had little value in the diagnosis of renal diseases. Chandler *et al* (2007) documented ultrasonographic changes in 37 boxer dogs. Ultrasonographic findings included hyper-echoic renal cortices, loss of corticomedullary junction, dilated pelvis and irregularly shaped small kidneys. Tripathi and Mehta (2010) reported CKD in seven dogs out of 72 dogs. Out of which, four dogs showed loss of architecture, detail of renal parenchyma, indistinct contours of renal cortex, hyper-echoic periphery and small sized kidneys, lack of demarcation of corticomedullary junction and rest 3 dogs showed small sized kidneys, loss of architecture detail of renal parenchyma with defined irregular border. Kealy *et al* (2010) reported that small and irregular shaped kidneys in CKD can be better detected by survey radiographs. Girishkumar *et al* (2011) stated that ultrasonography was useful in evaluating the diffuse lesions of the parenchyma.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 28-34

Research Articles

Efficacy of anti-diarrhoeal herbal preparations in colibacillosis in goat kids

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Abstract

Evidence-based therapeutic trial revealed that the most effective regimens were Ciprofloxacin standard T_1, closely followed by combination herbals: dried methanolic extract of fruit rind of *Punica granatum* (Anar) + dried methanolic extract of bark powder of *Holarrhena antidysenterica* (Kutaj) T_4. Single ingredient herbal preparations: dried methanolic extract of KutajT_3, and dried methanolic extract of fruit rind of AnarT_2 were less effective.

Keywords: Colibacillosis, Goat kids, Therapy, Herbal preparations, Ciprofloxacin standard

Colibacillosis is one of the most important acute, infectious enzootic diseases of bacterial aetiology with high (15%-30%) mortality rate in the goat kids (Rajput *et al*., 2014). The itinerant gut bacterium *Escherichia coli* induces severe diarrhoea in the kids, especially during the first two weeks of life (Patel *et al*., 2017). Because of the resultant generalized tissue dehydration and severe metabolic acidosis, death ensues within 24-48 hr, if not treated in time. Colibacillosis is an opportunistic disease associated with degraded micro-environment, poor hygiene and sanitation and non-availability of adequate amounts of colostrums to neonates. The patho-biochemical alterations include deranged cell water-electrolytes homeostasis and acid-base balance leading to life-threatening situations (Singh *et al*., 2014).

The ethno-veterinary practices employed in the treatment of bacterial enteritis in goats involving the use of methanol extract of rind of *Punica granatum* (common name pomegranate, Hindi Anar) @ 200 to 400 mg/kg body weight (Akter *et al*., 2013), and in calves methanol extract of bark of *Holarrhena antidysenterica* (Hindi Kutaj) @ 10 mg/kg body weight bid PO for 3 to 5 days (Singh *et al*., 2016) are trend-setting. *P. granatum* revealed phytochemicals like alkaloids and polyphenols. The bark and rind of the fruit are used in the traditional Indian system of medicine, Ayurved in treating diarrhoea and dysentery in the humans. Methanolic extract of the seed and dried peels exhibits anti-diarrhoeal, anti-dysentery and antihelmintic activity. Extracts of rind of pomegranate exhibited antibacterial potency in *E. coli* infection (Khan and Hanee, 2011). Various parts of *H. antidysenterica* are stated to possess excellent antibacterial activity. Thus, the bark extracts were effective in the treatment of diarrhoea and dysentery, resulting from *E coli* infection (Sudhakar Rao, 2013). Chemical analysis has since established an assortment of the phyto-chemicals: flavonoids, alkaloids, tannins, phenolic compounds, resins, fatty acids and gum which promote cell functions (Kaundal and Sagar, 2016). This communication reports on the comparative therapeutic efficacy of the homemade herbal preparations: fruit rind extract of *P. granatum* and bark extract of *H. antidysenterica*, singly or in combination, *vs*. the reference antibiotic, Ciprofloxacin in clinical colibacillosis in goat kids.

Materials and Methods

Animals: Total 100 goat kids (up to 4 weeks post-partum), mainly from the Institute's Amanala Goat Farm and partly from the Teaching Veterinary Clinical Complex, and some small privately owned goat rearing units in and around Jabalpur, M.P. exhibiting typical signs of diarrhoea, poor body condition and dry muzzle were randomly selected for the present clinical trial of total six months duration (mid-July 2017 to mid-January 2018).

For the primary isolation of *E coli*, the faecal inoculum was enriched in MacConkey lactose broth and incubated (37°C, 24 hr).One loopful of the culture was then seeded uniformly on MacConkey agar plate and incubated (37°C, 24hr), according to Turkyilmaz *et al*. (2013). The characteristic lactose-fermenting pink coloured *E coli* colonies were inoculated into EMB agar plate and re-incubated The uniformly spread smears on glass slides from the colonies with typical greenish metallic sheen were stained with Grams stain. The

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isolates were identified as *E. coli* on IMVIC reaction: indole and methyl red test positive, and Voges-Proskauer test, and citrate utilization test negative to differentiate with certainty, the Gram-negative *Enterobacteriaceae*, *E. coli* from the *Klebsiella* group (Rajput *et al*., 2014).

Table 1: Characterization of *E. coli*

Parameters	Reaction
Grams staining	Gram –ve
Indole test	+
Methyl Red (MR) test	+
VogesProskauer test (VP) test	-
Citrate Utilization test	-

Home-made herbal medicaments

Punica granatum (pomegranate): The mass of peeled rind was washed, air dried and homogenized. Dried powder (10 g) was dissolved in 100 ml methanol in a 250 ml Erlenmeyer flask, plugged and gently agitated in a rotary shaker (24 hr). The material was filtered through wetted Whatman filter paper No. 1 cone, fitted inside a glass funnel, and centrifuged (5000 rpm, 10 min.). The solvent was slowly evaporated off till the final volume was reduced to ¼ of the original, and cold preserved (4°C) in airtight labeled glass bottles.

Therapeutic trial

Total 100 kids (up to 4 weeks age) from the Institute's Amanala Goat Farm, and Teaching Veterinary Clinical Complex (T.V.C.C.), and privately owned small goat rearing units in and around Jabalpur, M. P. were clinically screened for diarrhoea. Total 24 short-listed diarrhoeic kids of both sexes, irrespective of breed, were randomized into four equal treatment groups T_1-T_4, each comprising 6 animals. Six apparently normal kids were kept as the control group T_C.

Table I. Grouping of kids up to 4 weeks of age.

Group	No. of animals	Treatment schedule
T_c	6	Healthy control
T_1	6	Ciprofloxacin @ 5 mg/ kg body weight for 5 days o.d., PO
T_2	6	Methanolic extracts of fruit rind of *Punica granatum* (Pomgrenate, Anar) @ 400 mg/ kg body weight for 5 days o.d., PO
T_3	6	Methanolic extract of dry bark powder, *Holarrhena antidysentrica (*Kutaj) @ 400 mg/ kg body weight for 5 days o.d., PO
T_4	6	Methanolic extracts of fruit rind of *Punica granatum*, and the bark powder of *Holarrhena antidysenterica* (Kutaj), each @ 200 mg/ kg body weight concurrently for 5 days o.d., PO

*Holarrhena antidysentrica (*Kutaj): Dry bark powder was procured locally. Air dried powder (50 g) was transferred into 300 ml methanol in an Erlenmeyer flask, sealed with aluminum foil and allowed to stand for seven days to permit slow extraction of the medicinal phyto-chemicals in RT. The extract was filtered through wetted Whatman filter paper No. 1 cone fitted inside a glass funnel and evaporated off (40° C) in a rotary evaporator (Kaundal and Sagar, 2016). The extracts were pooled and stored in labeled glass bottles.

Haemato-biochemical profile

Five ml samples of blood were collected from the jugular vein of goat kids under the therapeutic trial on day 0 pre-treatment, and on days 3 and 6 post-treatment The haematological parameters included total erythrocyte count (TEC), haemoglobin (Hb) concentration, packed cell volume (PCV %), and total leukocyte count (TLC), estimated with. Medonic model M 32 Auto Analyzer. The concentrations (m Eq /L) of sodium (Na^+), potassium (K^+) and chloride (Cl^-) in serum were estimated with Cornley model ACCULYTE-3 Electrolytes Analyzer.

Serum bicarbonate (HCO_3^-) concentration was estimated with Semi-Biochemistry Analyzer Mini CHEM 100. Circulatory total protein and albumin concentrations (g/dL) were estimated with the standard diagnostic reagent kits on Blood Chemistry Auto Analyser Model Erba Mannheim CHEM-5 plus v2.

The experimental data were analyzed with the One way ANOVA, and the Mean values were compared with Duncan's Multiple Range Test (Snedecor and Cochran, 1984).

Table 1. Mean values of haemoglobin concentration (g/dl) in kids of different treatment groups at varying intervals.

Groups	Treatment intervals (Day)		
	Pre-treatment	Post-treatment	
	Day 0	Day 3	Day 6
T_c	$10.37^{aA} \pm 0.27$	$10.35^{aA} \pm 0.54$	$10.68^{aA} \pm 0.51$
T_1	$7.60^{bB} \pm 0.24$	$7.95^{abB} \pm 0.22$	$8.42^{aB} \pm 0.29$
T_2	$7.65^{B} \pm 0.19$	$7.75^{B} \pm 0.22$	$8.13^{B} \pm 0.16$
T_3	$7.58^{B} \pm 0.22$	$7.87^{B} \pm 0.20$	$8.18^{B} \pm 0.17$
T_4	$7.37^{bB} \pm 0.22$	$7.91^{abB} \pm 0.17$	$8.23^{aB} \pm 0.16$

The mean values between treatments (upper case) and between intervals (lower case) with different superscripts vary significantly ($P < 0.05$).

Table 2. Mean values of total erythrocyte count (10^{12}/l) in kids of different treatment groups at varying intervals.

Groups	Treatment intervals (Day)		
	Pre-treatment	Post-treatment	
	Day 0	Day 3	Day 6
T_c	$10.34^{aA} \pm 0.23$	$10.34^{aA} \pm 0.21$	$10.36^{aA} \pm 0.21$
T_1	$8.34^{bB} \pm 0.36$	$8.63^{bB} \pm 0.35$	$10.17^{aA} \pm 0.18$
T_2	$8.15^{B} \pm 0.30$	$8.19^{B} \pm 0.30$	$8.27^{B} \pm 0.30$
T_3	$8.11^{B} \pm 0.29$	$8.21^{B} \pm 0.29$	$8.29^{B} \pm 0.29$
T_4	$8.07^{B} \pm 0.30$	$8.18^{B} \pm 0.31$	$8.32^{B} \pm 0.30$

The mean values between treatments (upper case) and between intervals (lower case) with different superscripts vary significantly ($P < 0.05$).

Table 3. The mean value of total leukocyte count (10^9/l) in kids of different treatment groups at varying intervals.

Groups	Treatment intervals (Day)		
	Day 0	Day 3	Day 6
T_c	$6.19^{B} \pm 0.28$	$6.16^{B} \pm 0.31$	$6.26^{D} \pm 0.29$
T_1	$9.75^{aA} \pm 0.44$	$8.46^{bA} \pm 0.62$	$7.24^{bcCD} \pm 0.34$
T_2	$9.79^{A} \pm 0.52$	$9.20^{A} \pm 0.68$	$8.63^{A} \pm 0.42$
T_3	$9.86^{A} \pm 0.60$	$9.27^{A} \pm 0.53$	$8.37^{AB} \pm 0.40$
T_4	$10.17^{aA} \pm 0.63$	$8.73^{abA} \pm 0.44$	$7.55^{bBC} \pm 0.30$

The mean values between treatments (uppercase) and between intervals (lowercase) with different superscripts vary significantly ($P < 0.05$).

Table 4. Mean values of packed cell volume (l/l) of kids in different treatment groups at varying intervals.

Groups	Treatment intervals (Day)		
	Pre-treatment	Post-treatment	
	Day 0	Day 3	Day 6
T_c	$31.19^{aB} \pm 0.85$	$31.39^{aC} \pm 0.84$	$32.11^{aC} \pm 0.70$
T_1	$40.32^{aA} \pm 0.34$	$38.64^{bB} \pm 0.48$	$32.47^{cC} \pm 0.57$
T_2	$40.83^{A} \pm 0.56$	$40.56^{A} \pm 0.58$	$39.75^{A} \pm 0.54$
T_3	$40.50^{aA} \pm 0.33$	$39.89^{aAB} \pm 0.22$	$38.57^{bA} \pm 0.32$
T_4	$40.83^{aA} \pm 0.61$	$40.39^{aA} \pm 0.47$	$35.89^{bB} \pm 0.65$

The mean values between treatments (upper case) and between intervals (lower case) with different superscripts vary significantly ($P < 0.05$).

Table 5. Mean values of serum total protein concentration (g/dl) in kids of different treatment groups at varying intervals**.**

	Treatment intervals (Day)		
	Pre-treatment	Post-treatment	
	Day 0	Day 3	Day 6
T_c	6.81^{aA}±0.07	6.73^{aA}±0.059	6.79^{aAB}±0.10
T_1	5.57^{cB}±0.23	6.12^{bAB}±0.10	6.73^{aAB}±0.11
T_2	5.75^{B}±0.23	5.93^{B}±0.21	6.34^{B}±0.19
T_3	5.77^{bB}±0.18	6.14^{abB}±0.15	6.46^{aB}±0.18
T_4	5.63^{cB}±0.16	6.32^{bB}±0.13	6.87^{aA}±0.06

The mean values between treatments (upper case) and between intervals (lower case) with different superscripts vary significantly (P <0.05).

Table 6. The mean values of serum albumin concentration (g/dl) in kids of different treatment groups at varying intervals.

Groups	Treatment intervals (Day)		
	Pre-treatment	Post-treatment	
	Day 0	Day 3	Day 6
T_c	3.22^{aA}±0.08	3.21^{aA}±0.04	3.16^{aA}±0.07
T_1	2.12^{cB}±0.09	2.54^{bBC}±0.07	3.15^{aA}±0.05
T_2	2.18^{cB}±0.04	2.36^{bCD}±0.04	2.80^{aB}±0.07
T_3	1.82^{cC}±0.14	2.29^{bD}±0.10	2.79^{aB}±0.15
T_4	2.10^{cBC}±0.09	2.30^{bB}±0.08	3.30^{aA}±0.08

The mean values between treatments (upper case) and between intervals (lower case) with different superscripts vary significantly (P <0.05).

Table 7. Mean values of serum globulin concentration (g/dl) in kids of different treatment groups at varying intervals.

Groups	Treatment intervals (Day)		
	Pre-treatment	Post-treatment	
	Day 0	Day 3	Day 6
T_c	3.59^{AB} ± 0.02	3.42^{B} ± 0.05	3.36±0.06
T_1	3.36^{B} ± 0.17	3.57^{AB} ± 0.15	3.58±0.09
T_2	3.57^{AB} ± 0.25	3.54^{AB} ± 0.22	3.53±0.17
T_3	3.96^{A} ± 0.22	3.85^{A} ± 0.17	3.63±0.13
T_4	3.63^{AB} ± 0.15	3.62^{AB} ± 0.08	3.49±0.12

The mean values between treatments (upper case) and between intervals (lower case) with different superscripts vary significantly (P <0.05).

Table 8. Mean values of albumin: globulin ratio in kids of different treatment groups at varying intervals.

Groups	Treatment intervals (Day)		
	Pre-treatment	Post-treatment	
	Day 0	Day 3	Day 6
T_c	0.89^{aA}±0.02	0.93^{aA}± 0.02	0.94^{aA}±0.02
T_1	0.64^{bB}± 0.05	0.72^{bB}±0.05	0.88^{aA}±0.02
T_2	0.62^{bB}±0.05	0.67^{abBC}±0.04	0.79^{aB}±0.03
T_3	0.47^{bC}±0.06	0.60^{bC}±0.04	0.77^{aB}±0.04
T_4	0.56^{cBC}±0.04	0.75^{bB}±0.03	0.90^{aA}±0.02

The mean values between treatments (upper case) and between intervals (lower case) with different superscripts vary significantly (P <0.05).

Table 9. Mean values of serum sodium concentration (m Eq/l) in kids of different treatment groups at varying intervals**.**

Groups	Treatment intervals (Day)		
	Pre-treatment	Post-treatment	
	Day 0	Day 3	Day 6
T_c	$146.58^{aA}\pm1.35$	$146.71^{aA}\pm1.34$	$147.51^{aA}\pm1.4$
T_1	$131.76^{bB}\pm3.04$	$135.81^{abC}\pm2.54$	$140.86^{aB}\pm1.67$
T_2	$132.35^{B}\pm1.3$	$132.92^{C}\pm1.34$	$134.75^{C}\pm1.51$
T_3	$141.44^{A}\pm3.08$	$141.87^{AB}\pm3.11$	$143.29^{AB}\pm2.85$
T_4	$134.01^{bB}\pm1.05$	$136.34^{bBC}\pm1.04$	$141.87^{aB}\pm0.42$

The mean values between treatments (upper case) and between intervals (lower case) with different superscripts vary significantly (P <0.05).

Table 10. Mean values of serum potassium concentration (m Eq/l) in kids of different treatment groups at varying intervals.

Groups	Treatment intervals (Day)		
	Pre-treatment	Post-treatment	
	Day 0	Day 3	Day 6
T_c	$5.56^{aB}\pm0.17$	$5.59^{aB}\pm0.17$	$5.62^{aB}\pm0.17$
T_1	$6.95^{aA}\pm0.06$	$6.59^{bA}\pm0.06$	$5.48^{cB}\pm0.10$
T_2	$7.20^{A}\pm0.22$	$6.96^{A}\pm0.16$	$6.69^{A}\pm0.10$
T_3	$7.02^{A}\pm0.10$	$6.72^{A}\pm0.14$	$6.38^{A}\pm0.32$
T_4	$6.85^{aA}\pm0.03$	$6.64^{aA}\pm0.05$	$5.46^{bB}\pm0.12$

The mean values between treatments (upper case) and between intervals (lower case) with different superscripts vary significantly (P <0.05).

Table 11. Mean values of serum chloride concentration (m Eq/l) in kids of different treatment groups at varying intervals.

Groups	Treatment intervals (Day)		
	Pre-treatment	Post-treatment	
	Day 0	Day 3	Day 6
T_c	$104.04^{aA}\pm0.16$	$104.13^{aA}\pm0.18$	$104.71^{aA}\pm0.41$
T_1	$99.87^{cB}\pm0.14$	$101.44^{bB}\pm0.30$	$103.82^{aAB}\pm0.3$
T_2	$100.19^{B}\pm0.22$	$100.89^{B}\pm0.37$	$101.59^{B}\pm1.71$
T_3	$101.38^{B}\pm1.37$	$102.01^{B}\pm1.26$	$103.65^{AB}\pm1.02$
T_4	$100.17^{cB}\pm0.32$	$101.68^{bB}\pm0.35$	$102.86^{aAB}\pm0.21$

The mean values between treatments (upper case) and between intervals (lower case) with different superscripts vary significantly (P <0.05).

Table 12. Mean values of serum bicarbonate concentration (m Eq/l) in kids of different treatment groups at varying intervals.

	Pre-treatment	Post-treatment	
	Day 0	Day 3	Day 6
T_c	$29.00^{aA}\pm1.83$	$29.44^{aA}\pm1.94$	$30.15^{aA}\pm1.9$
T_1	$19.42^{bB}\pm1.22$	$22.02^{abB}\pm1.07$	$24.58^{aAB}\pm1.52$
T_2	$19.59^{B}\pm0.86$	$19.78^{B}\pm1.03$	$23.89^{B}\pm3.44$
T_3	$17.91^{bB}\pm0.62$	$19.15^{bB}\pm0.49$	$21.12^{aB}\pm0.44$
T_4	$17.3^{bB}\pm0.73$	$21.42^{aB}\pm1.67$	$22.83^{aB}\pm0.72$

The mean values between treatments (upper case) and between intervals (lower case) with different superscripts vary significantly (P <0.05).

Results and Discussion

Changes in the haemato-biochemical profile faithfully reflect the pathophysiological state of the sick animal, and also the quantum of the favourable response to different therapeutic regimens. In this study, the significantly ($P<0.05$) lower mean Hb concentration along with the reduced TEC in the *E. coli* infected kids of different treatment groups T_1-T_4, compared to the mean normal values, observed in the control group of normal kids, T_c (Tables 1, 2) signified the onset of anaemia. Zaki *et al*. (2010) also reported that the mean values of Hb concentration and TEC decreased significantly in the *E coli* infected kids. Following remedial therapy, the consistently increasing trend in Hb concentration in all the treatments groups, T_1-T_4 is noteworthy. On day 3 post-treatment, the increase was statistically significant ($P<0.05$) in the treatment groups T_1 (standard Ciprofloxacin group) and T_4 (combination of *H. antidysenterica* + *P. granatum*). The increasing trend in Hb concentration persisted on day 6 post-treatment. However, normalcy had not yet been restored. Similar observations were recorded by Meshram *et al*. (2009) in clinical bacterial enteritis in adult goats, treated with a new anti-diarrhoeal polyherbal preparation that included *H. antidysenterica* and *P. granatum*.

The TLC had increased significantly ($P<0.05$) in four treatment groups T_1-T_4, as compared to the normal control value in T_c, recorded on day 0 pre-treatment (Table 3). Leukocytosis was the consequence of enteric bacterial infection. Following treatment, in all treatment groups, abatement of leukocytosis was perceptible on day 3 and was statistically significant on day 6 post-treatment. The response was most pronounced in the treatment group T_1 (standard Ciprofloxacin group), followed by T_4, combination of *H. antidysenterica* + *P. granatum*, T_2, *P granatum* alone and T_3, *H. antidysenterica* alone. Comparable reports on the diarrhoeic buffalo calves are well-documented (Sharma, 2003; Fernandes *et al*., 2009; Kumar *et al*., 2010; Pandey, 2017).

Evaluation of the data on PCV% (Table 4) revealed significantly ($P<0.05$) increased values pre-treatment on day 0, followed by significant decline in all four treatment groups,T_1-T_4 towards partial restoration of normalcy on day 3 post-treatment. This declining trend persisted on day 6 post-treatment, and the value was at par with normal in all treatment groups, presumably because of restoration cell water homeostasis.

Serum total protein concentration (Table 5) showed significantly ($P< 0.05$) decreased values on day 0 pretreatment in T_1-T_4, *vs*. the normal value recorded in the control group, T_c. This observation is corroborated by the earlier reports (Meshram *et al*., 2009; Zaki *et al*., 2010). The values increased significantly on day 3 post-treatment, and on day 6, statistically significant ($P<0.05$) further increases were observed in T_1, T_3 and T_4. The favourable response to a polyherbal preparation including *H. antidysenterica* and *P. granatum* is on record (Meshram *et al*., 2009).

The pre-treatment serum albumin concentration in the treatment groups T_1-T_4 was significantly lower than the normal value, recorded in the control group, T_c. Following treatment, statistically significant ($P<0.5\%$) progressively increasing values were observed on day 3 and day 6, presumably because of accelerated biosynthesis in the hepatocytes. The significantly decreased circulatory albumin concentration in the pre-treated diarhoeic kids (present study) may be attributed to (i) reduced absorption of amino acids (ii) impaired synthesis in the liver cells, or both. Restoration of normal circulatory levels of albumin attests to the therapeutic efficacy of potent herbal preparations (present study), at par with the antibiotic regimens (Zaki *et al*., 2010).

Notably, in all pretreated diarrhoeic kids in T_1-T_4, the circulatory sodium (Na^+) titre (m Eq/L) had decreased significantly concurrent with significantly increased potassium (K^+) titre (m Eq/L), *vs*. the corresponding normal values, recorded in T_c. The increasing trends towards restoration of normalcy in the Na^+ titre and the reciprocal decline in the K^+ titre were significant on day 3 and day 6 post-treatment in T_1-T_4. No comparable published report on diarrhoeic goat kids is forthcoming. However, in the diarrhoeic calves the similar pattern of hyponatraemia with concurrent hyperkalaemia before treatment and restoration of normalcy in the cation (Na^+/ K^+) balance with herbal Kutaj therapy was reported by (Singh *et al*., 2016).

In the diarrhoeic kids of T_1-T_4, the pre-treatment serum chloride (Cl^-) and bicarbonate (HCO_3^-) concentrations were significantly lower than the corresponding normal values. However, the titres increased significantly post-treatment in all treatment groups on day 3. In diarrhoeic calves, hypochloraemia

before treatment and reversal following herbal treatment (Kutaj) was reported by Singh *et al*. (2016).

Conclusions

Restoration of homoeostasis in the goat kids with colibacillosis, corroborated with the haemato-biochemical parameters, revealed that the therapeutic efficacy of orally administered homemade combination herbal preparation: Kutaj bark+Pomgrenate fruit rind dried methanolic extracts in 1:1 ratio (w/w) T_4 was virtually at par with the reference standard antibiotic: Ciprofloxacin T_1. Single ingredient herbals: Kutaj bark dried methanolic extract T_2 and Pomgrenate fruit pulp dried methanolic extract T_3 were less effective. Further study in different geo-climatic regions with larger population size of diarrhoeic kids is recommended.

Acknowledgement

We sincerely thank Prof. (Dr.) I. C. Datta for valuable suggestions for improved presentation.

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Studies on biochemical alterations in hypothyroid dogs

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Abstract

Biochemical alterations in hypothyroid dogs was evaluated in the present study. Study population consisted 13 hypothyroid dogs referred to Bombay Veterinary College, Mumbai. Hypothyroid dogs were tentatively diagnosed on the basis of history and characteristic clinical signs (bilateral alopecia, lethargy, obesity and rat-tail) and were confirmed after estimation of thyroid hormone concentration (Total thyroxin, Total triiodothyronine and Free thyroxine) by radioimmunoassay. Serum biochemistry profile (Liver function, Kidney function, Cholesterol, Triglyceride and blood glucose) was performed in hypothyroid dogs and compared with clinically healthy dogs. Biochemical panel of dogs with hypothyroidism showed hypoglycemia, hypertriglyceremia, hypercholesteremia and increased alkaline phosphatase.

Keywords: Canine thyroid dysfunction, Hypothyroidism, Thyroxine, Triiodothyronine, and Free thyroxine

Thyroid hormones are iodine containing amino acids synthesized in thyroid gland that perform wide range of physiological effects. They are critically important for regulation of various metabolic processes. Calorigenic thyroid hormones are important in fetal life specifically for development of neural and skeletal system. Their deficiency or excess affects all systems in the body. Thyroid gland synthesizes and releases primarily three hormones viz. Total thyroxine (TT_4), Total tri-iodothyronine (TT_3) and free thyroxine (fT_4). Hypothyroidism is a common endocrinopathy in dogs. For diagnosis of hypothyroidism, estimation of TT_4, TT_3 and fT_4 levels in serum is essential and need to correlate with clinical symptoms and serum biochemical changes.

Part of M. V. Sc thesis submitted to Maharashtra Animal and Fishery Sciences University, Nagpur India for partial fulfillment of Master's Degree by first author.

Present research work was carried out with objective to study biochemical alterations in hypothyroid dogs. This establishment of association will help in diagnosis of suspected hypothyroid patients in veterinary clinical practice.

Material and Methods

Selection of Dogs

Study was approved from Institutional Ethics Committee for Veterinary clinical research (Project Approval No: IEC-VCR/2018/10 Dated:26.02.2018) and Institutional Bio-safety Committee (Project Approval No: IBSC, Resolution No: 2.2; Dated:15.12.2017) of Bombay Veterinary College, Mumbai, Maharashtra Animal and Fishery Sciences University, Nagpur. Maharashtra, India.

Dogs (n=59) were examined and considered healthy on the basis of history, clinical examination and biochemical parameters within reference range. Hypothyroid dogs (n=13) were selected on the basis of history, clinical signs (bilateral alopecia, lethargy, obesity and rat-tail) after due consent from owners. Study population was selected from cases referred to Bai Sakrabai Dinshaw petit hospital and Teaching Veterinary Clinical Complex Goregaon and Parel, Mumbai-12.

Biochemical Estimation

Five ml blood was collected from healthy dogs and hypothyroid dogs aseptically. Blood was transferred to plain tube for serum. Serum was collected by centrifugation at 5000rpm for 5 min within 1-2 hr of blood collection. Two aliquots of serum were prepared and one was used for biochemical analysis and other was stored at -20^{0}C for hormonal estimation. Serum biochemical parameters were analyzed using fully automated random-access chemistry analyzer (Falcon 260).

Hormone Estimation

Thyroid Hormone estimation was carried out at Radio Isotope Laboratory (Type II- Research purpose laboratory approved by Atomic Energy Regulatory Board, Govt. Of India) of Bombay Veterinary College. Hormone estimation (TT_4, TT_3 and fT_4) was carried out by using commercially available radioimmunoassay

kits. The method prescribed by manufacturer was followed for estimation of TT_4 and TT_3 whereas for estimation of fT_4 method was partially modified (One extra wash before addition of tracer).

Results and Discussion

Thyroid hormone concentrations of hypothyroid dogs were compared with clinically healthy dogs (Table 1). Results of hypothyroid dogs for total thyroxine, total triiodothyronine and free thyroxine were 18.46 ± 3.20 nmol/l, 0.56 ± 0.07 nmol/l and 5.68 ± 1.24 pmol/l respectively. Study recorded significant ($p<0.05$) lower concentrations of thyroid hormones (TT_4, TT_3 and fT_4) in hypothyroid dogs as compared to clinically healthy dogs.

The values of biochemical parameters in clinically healthy and hypothyroid dogs are presented in Table 1. The mean concentrations of total bilirubin, direct bilirubin and indirect bilirubin of healthy and hypothyroid dogs were 0.60 ± 0.03 mg/dl, 0.33 ± 0.02 mg/dl, 0.26 ± 0.03 mg/dl and 0.52±0.07 mg/dl, 0.25±0.04 mg/dl, 0.27±0.04 mg/dl respectively. The mean concentrations of BUN and creatinine of healthy and hypothyroid dogs were 23.52 ± 2.00 mg/dl, 0.84 ± 0.04 mg/dl and 15.04 ± 2.99 mg/dl, 1.00 ± 0.08 respectively. Mean concentrations of ALP, AST and ALT from healthy and hypothyroid dogs were 67.43 ± 18.23 IU/L, 52.94 ± 2.66 IU/L, 45.56 ± 5.05 IU/L and 349± 133 IU/L, 56.74± 5.44 IU/L, 49.38±7.69 IU/L respectively. The mean concentrations of total protein, albumin, globulin from healthy and hypothyroid dogs were 7.17 ± 0.15 gm/dl, 3.36 ± 0.46 gm/dl, 4.27 ± 0.15 gm/dl and 7.36±0.41 gm/dl, 2.43±0.10 gm/dl, 4.92±0.42 gm/dl respectively.

Mean concentrations of serum triglyceride and cholesterol in healthy and hypothyroid dogs were 67.80 ± 4.62 mg/dl, 193.58 ± 10.50 mg/dl and 82.58±9.68 mg/dl, 225.26 ± 18.72 mg/dl. Random blood glucose concentration in healthy and hypothyroid dogs was found to be 91.44 ± 1.88 mg/dl and 79.63 ± 6.70 mg/dl.

The biochemical profile values for clinically heathy dogs were within reference range as described by Brar *et al., 1999.* Study recorded nonsignificant ($p<0.05$) difference in values of all parameters except, ALP and Random blood glucose. Further, study also recorded considerable but nonsignificant ($p<0.05$) difference in concentrations of Triglyceride and Cholesterol.

Hypothyroidism is a result of inadequate circulating concentrations of the thyroid hormones. Adult-onset hypothyroidism is mostly because of thyroid gland malfunction resulting from either lymphocytic thyroiditis or idiopathic atrophy (Feldman and Nelson, 1996). Common signs of hypothyroidism in dogs include dermatological abnormalities.

Thyroid hormones play an important role in maintenance of dermal health (Panciera, 1990, Feldman and Nelson 1996). Aberrations in thyroid hormone results in changes in dermal health maintenance and results in dermatological changes. Hyperkeratosis causes scaling of skin and due to persistence of telogen growth phase alopecia develops (Panciera, 1990).

The application of present research findings is to establish suspicion of hypothyroidism on the basis of biochemical alterations. Present study reported hypercholestremia, hypertriglyceremia in hypothyroid dogs, which is in support with previously published work by other authors (Kaelin *et al.,* 1986, Panciera, 1990, Feldman and Nelson, 1996 and Dixon, 1999). Thyroid hormone plays important roles in lipid metabolism pertaining to production, mobilization and degradation which is affected in hyperthyroidism hence causes lipid accumulation. (Feldman and Nelson, 1996).

In comparison with standard laboratory reference ranges common alteration was increased concentration of triglyceride and cholesterol. Typical hypothyroidism has large increase in cholesterol concentration as compared to nonthyroidal illness (Barrie *et al.,* 1993). Increased level of liver enzymes and alkaline phosphatase observed in present study are in accordance with findings reported by Yousif *et al.* (2012) and Suraniti *et al* (2008)

Therefore, in veterinary clinical practice diagnosis of canine hypothyroidism can be made on the basis of clinical examination with classical dermatological signs and altered biochemical profile.

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Table 1 showing thyroid hormone and biochemical alterations in hypothyroid (n=13) and healthy (n=59) dogs

Sr. No	Parameter	Group	Concentration (Mean ± SE)	T (cal)
1	Total Thyroxine (nmol/l)	Healthy	29.67± 1.43[a]	3.2
		Hypothyroidism	18.46 ± 3.20[b]	
2	Total Triiodothyronine (nmol/l)	Healthy	1.03 ± 0.02a	6.7
		Hypothyroidism	0.56 ± 0.07[c]	
3	Free thyroxine (pmol/l)	Healthy	9.07 ± 0.52[a]	2.68
		Hypothyroidism	5.68 ± 1.24[b]	
4	Total bilirubin (mg/dl)	Healthy	0.60 ± 0.03	0.84
		Hypothyroidism	0.52 ± 0.07	
5	Direct bilirubin (mg/dl)	Healthy	0.33 ± 0.02	1.41
		Hypothyroidism	0.25 ± 0.04	
6	Indirect bilirubin (mg/dl)	Healthy	0.26 ± 0.03	0.15
		Hypothyroidism	0.27± 0.04	
7	BUN (mg/dl)	Healthy	23.52 ± 2.00	1.88
		Hypothyroidism	15.04 ± 2.99	
8	Creatinine (mg/dl)	Healthy	0.84 ± 0.04	1.36
		Hypothyroidism	1.00 ± 0.08	
9	ALP (IU/L)	Healthy	67.43 ± 18.23	**3.90**
		Hypothyroidism	349 ± 133	
10	AST (IU/L)	Healthy	52.94 ± 2.66	0.60
		Hypothyroidism	56.74 ± 5.44	
11	ALT (IU/L)	Healthy	45.56 ± 5.05	0.33
		Hypothyroidism	49.38 ± 7.69	
12	Total Protein (gm/dl)	Healthy	7.17 ± 0.15	0.49
		Hypothyroidism	7.36 ± 0.41	
13	Albumin (gm/dl)	Healthy	3.36 ± 0.46	0.93
		Hypothyroidism	2.43 ± 0.10	
14	Globulin (gm/dl)	Healthy	4.27 ± 0.15	1.73
		Hypothyroidism	4.92 ± 0.42	
15	Triglyceride (mg/dl)	Healthy	67.80 ± 4.62	1.36
		Hypothyroidism	82.58 ± 9.68	
16	Cholesterol (mg/dl)	Healthy	193.58 ± 10.50	1.31
		Hypothyroidism	225.26 ± 18.72	
17	Radom Blood Glucose (mg/dl)	Healthy	91.44 ± 1.88	**2.32**
		Hypothyroidism	79.63 ± 6.70	

Means showing dissimilar superscripts differ significantly ($p<0.05$).
t (0.05)=1.99,

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Prevalence and bacterial etiology of subclinical mastitis in Surti Buffaloes

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Abstract

In present investigation, total 115 quarter milk samples were collected from 29 apparently healthy Surti buffaloes in Udaipur district in southern part of Rajasthan. The prevalence of subclinical mastitis in Surti buffaloes was 23.48 per cent (27/115) and; 26.09 per cent (30/115) on quarter basis by culture examination and; CMT and SCC (both), respectively. The prevalence on animal basis was 37.93 per cent (11/29) and; 41.38 per cent (12/29) by culture examination and; CMT and SCC (both), respectively. The results showed highest prevalence of subclinical mastitis in Surti buffaloes in III lactation (35.71 per cent) on quarter basis. On animal basis, the lactation number wise prevalence was highest in V and VIII lactation (50 per cent each). Among the different isolates staphylococci was most prevalent organism accounting for 42.42 per cent (14/33) followed by streptococci as 24.24 per cent (8/33).

Keywords: Prevalence, Bacterial Etiology, Subclinical Mastitis, Surti Buffaloes

India has 299.9 million bovine population, out of which 92.5 million are female buffaloes. Rajasthan state has 12.97 million buffaloes and ranks second in the country (Livestock Census, 2012). In India, approximately 58 percent of total milk is produced by buffaloes. Surti is one of the important breeds of buffalo and is mostly distributed in Gujarat and southern part of Rajasthan. Surti buffaloes are medium sized buffaloes and known for high milk fat percentage and preparation of good quality milk products.

Mastitis is one of the most important diseases of buffaloes. Subclinical mastitis is more prevalent and causes huge economic losses due to loss of milk production and adverse effects on the quality of milk products. It is more hazardous because of lack of perceptible symptoms of inflammation and no observable changes in the secreted milk (Sharma *et al.,* 2009). Due to hidden nature and the huge impact of subclinical mastitis on health and production, it is imperative to know the prevalence and etiology of subclinical mastitis in different herds and locations.

There was no literature found on prevalence and bacterial etiology of subclinical mastitis in surti buffaloes in the region.

Materials and Methods

In present investigation, total 115 quarter milk samples were collected from 29 apparently healthy Surti buffaloes in Udaipur district in southern part of Rajasthan for the detection of subclinical mastitis. One buffalo had only three functional teats. Each apparently normal milk sample was screened for the presence of bacteria by cultivation, isolation and identification using standard procedures as per Cowan and Steel (1975). The total somatic cell count (TSCC) of milk samples was carried out as described by Prescott and Breed (1910) and california mastitis test (CMT) as per Schalm and Noorlander (1957). The statistical analysis of the data was done using statistical method described by Snedecor and Cochran (1994).

Results and Discussion

The prevalence of subclinical mastitis in Surti buffaloes was 23.48 per cent (27/115) and; 26.09 per cent (30/115) on quarter basis by bacteriological culture examination and; modified CMT and SCC (each), respectively. The prevalence on animal basis was 37.93 per cent (11/29) and; 41.38 per cent (12/29) by bacteriological culture examination and; modified CMT and SCC (each), respectively. Lower prevalence of subclinical mastitis in Surti buffaloes than other breeds may be because of flat udder and short teats. Three samples, which were positive with modified CMT and SCC, did not yield growth of bacteria. It might be due to presence of anaerobes, mycoplasma or fungi which were not attempted to culture.

Almost similar results have also been reported by Chander and Baxi (1975); Chavan *et al*. (2007); and Ramprabhu and Rajeshwar (2007).

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Table 1: Lactation number-wise prevalence of subclinical mastitis in Surti buffaloes on quarter basis

Lactation number	Total number of quarters examined	Number of quarters positive for SCM	Percentage
I	20	5	25
II	32	8	25
III	28	10	35.71
IV	20	5	25
V	7	1	14.29
VI	-	-	-
VII	-	-	-
VIII	8	1	12.5

Lactation number-wise prevalence of subclinical mastitis in Surti buffaloes is depicted in Table 1 and Table 2. Highest prevalence of subclinical mastitis in Surti buffaloes was observed in III lactation (35.71 per cent) followed by I, II and IV lactation (25 per cent in each), V lactation (14.29 per cent) and VIII lactation (12.5 per cent) on quarter basis. On animal basis, the lactation number wise prevalence was highest in V and VIII lactation (50 per cent in each) followed by III lactation (42.86 per cent), I and IV lactation (40 per cent in each) and II lactation (37.5 percent). There was no any buffalo in VI and VII lactation in the present study.

The prevalence of subclinical mastitis in Surti buffaloes decreased after III lactation on quarter basis. The high prevalence of subclinical mastitis in III lactation in present investigation might be because of higher milk yield as compared to other lactation and decreased immunity in the buffaloes.

Similar findings have also been reported by Sharma *et al*. (2007). The prevalence of subclinical mastitis varies from farm to farm, depending upon the management system and hygienic and sanitary measures (Radostitis, *et al.* 2007).

Out of 115 milk samples 27 were found positive for pathogenic bacteria on cultural examination. Out of these 27 bacteriological positive samples, 22.22 per cent (6/27) had mixed infection. In mixed infections combination of two types of bacteria were found. The bacteria identified in single infection included staphylococci, streptococci, *E. coli*, bacilli and *Corynebacterium* spp. whereas bacteria present in mixed infection were staphylococci in combination with streptococci, staphylococci in combination with *E. coli,* staphylococci in combination of bacilli, staphylococci in combination of *Corynebacterium* spp. and streptococci in combination of *E. coli.*

The sample wise occurrence of bacterial isolates in subclinical masitic milk sample is given in Table 3. Staphylococci alone was observed in 33.33 per cent samples, streptococci alone was found in 18.51 percent samples, *E. coli* alone was observed in 11.11 per cent samples and bacilli and *Corynebacterium* spp. alone was observed in 7.41 per cent samples each.

Table 2: Lactation number-wise prevalence of subclinical mastitis in Surti buffaloes on animal basis

Lactation number	Total number of animals examined	Number of animals positive for SCM	Percentage
I	5	2	40
II	8	3	37.5
III	7	3	42.86
IV	5	2	40.00
V	2	1	50.00
VI	-	-	-
VII	-	-	-
VIII	2	1	50.00

Table 3: Sample wise occurrence of bacterial isolates in subclinical mastitic milk in Surti buffaloes

S.N.	Bacterial isolates	Total number of sample (N=27)	Percentage
1.	Staphylococci	9	33.33
2.	Streptococci	5	18.51
3.	*E. coli*	3	11.11
4.	Bacilli	2	07.41
5.	*Corynebacterium* spp.	2	07.41
6.	Staphylococci + streptococci	2	07.41
7.	Staphylococci + *E. coli*	1	03.70
8.	*E. coli* + streptococci	1	03.70
9.	Bacilli + staphylococci	1	03.70
10.	Staphylococci + *Corynebacterium* spp.	1	03.70

Table 4: Total bacterial isolates in subclinical mastitic milk in Surti buffaloes

S.N.	Bacterial isolates	Total number of Samples (n=33)	Percentage
1.	Staphylococci	14	42.42
2.	Streptococci	8	24.24
3.	E. coli	5	15.15
4.	Bacilli	3	9.09
5.	*Corynebacterium* spp.	3	9.09

In the present investigation, among the different isolates staphylococci was most prevalent organism accounting for 42.42 per cent (14/33) followed by streptococci as 24.24 per cent (8/33), *E. coli* as 15.15 per cent (5/33) and bacilli and *Corynebacterium* spp. as 9.09 per cent (3/33) each (Table 4).

Almost similar findings were reported by Saini *et al.* (1994), Jha *et al*. (1994) and Sharma and Sindhu (2016).

Conclusions

The prevalence of subclinical mastitis in Surti buffaloes was 23.48 per cent and; 26.09 per cent on quarter basis by culture examination and; CMT and SCC (each), respectively. The prevalence on animal basis was 37.93 per cent and; 41.38 per cent by culture examination and; CMT and SCC (each), respectively. Lower prevalence of subclinical mastitis in Surti buffaloes than other breeds may be because of flat udder and short teats. Highest prevalence of subclinical mastitis in Surti buffaloes was in III lactation (35.71 per cent) on quarter basis. The high prevalence of subclinical mastitis in III lactation might be because of higher milk yield as compared to other lactations. On animal basis, the lactation number wise prevalence was highest in V and VIII lactation (50 per cent each). Among the different isolates staphylococci was most prevalent organism accounting for 42.42 per cent (14/33) followed by streptococci as 24.24 per cent (8/33).

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Comparative diagnostic efficacy of Ab-ELISA and traditional techniques in the detection of *Trypanosoma evansi* infection in naturally infected equines

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Abstract

The present study was conducted to compare the diagnostic efficacy of the routine parasitological examination techniques used for the detection of *T. evansi* in naturally infected Equines (Giemsa stained thin blood smear, Buffy coat technique (BCT) and Ab-ELISA. On examination of 515 equines suspected for trypanosomiasis (Surra), giemsa stained thin blood smear technique, revealed the presence of *T. evansi* in 36 (6.99%) while buffy coat examination revealed, the presence of *T. evansi* in 51 of suspected equines, showing an efficacy of 9.90%. Ab-ELISA examination of 515 number of suspected equines serum revealed the presence of *T. evansi* parasite antibodies in 115 equines, showing an efficacy of 22.33%. Therefore, in present study the order of relative diagnostic efficacy of various diagnostic tests applied, Ab-ELISA was found to be of maximum followed by buffy coat examination and giemsa stained thin blood smear examination.

Keywords: Ab-ELISA, Diagnostic efficacy, Equine, *T. evansi*,

Trypanosomiasis commonly known as surra caused by *Trypanosoma evansi* is most widely distributed in Asia, Africa and central and South America affecting domesticated livestock (Konnai *et al*., 2009). The typical signs are intermittent fever, progressive anaemia, weight loss, oedema of dependent parts, conjunctivitis, marked depression, abortion, petechial haemorrhages, neurological abnormalities and sudden death (Saleh *et al.,* 2009). T. *evansi* is usually detected routinely by the microscopical examination of infected blood (wet blood film, stained blood smears and buffy coat examination), mouse inoculation and immunological methods. However microscopic observation requires skilled technicians and has poor sensitivity and lower diagnostic efficacy. Mouse inoculation is impractical for routine clinical diagnosis and large-scale epidemiological study. Attention has recently been focused on the development of more sensitive and specific serological and DNA based tests, for that a number of tests have been described for the diagnosis of surra in domestic animals including ELISA (Desquesnes *et al*, 2009). The present study was conducted to compare the diagnostic efficacy of the routine parasitological examination techniques used for the detection of *T. evansi* in naturally infected Equines (Giemsa stained thin blood smear, Buffy coat technique (BCT) and Ab-ELISA).

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Materials and Methods

The study was performed on equines of Mathura and its adjoining districts from April, 2014 to March, 2015. The samples were collected individually from the field based on calls received from the owners explaining various clinical signs/illness of their animals in collaboration with Brooke hospital for animals. The animals exhibiting clinical signs suggestive of surra such as fever, anorexia, loss of body condition, neurological signs and lack of performance were selected for study. Blood samples were drawn from animal by usual technique of collection from ear tip and Jugular vein aseptically with a sterilized disposable syringe and needle. The sample from jugular vein was collected in two clean, dry blood collection vials one containing EDTA as anticoagulant and another without any anticoagulant. Diagnosis on the basis of parasitological examinations in the suspected cases was done by blood smear examination and Buffy coat examination technique.

A drop of blood was placed 20 mm from one end of a clean microscopic slide and a thin film was drawn. The film was air-dried briefly, fixed in absolute methanol for 2 minutes and allowed to dry. The smears were then stained by Giemsa (one drop Giemsa + 1 ml PBS, pH 7.2) for 25 minutes. The slide was washed in tap water and dried. Slides were visualized under microscope at 100x using immersion oil.

Blood was collected into micro-hematocrit centrifuge tubes containing anticoagulant and sealed with clay and centrifuged in micro-haematocrit centrifuge at 12,000 rpm for 5 minutes. A smear was prepared by scratching and breaking the capillary tube 1mm below the surface of the buffy-coat and one drop of the buffy coat was expelled onto microscope slide, smeared and covered with a cover slip and examined under microscope at 40x (Fig: 2).

The serum samples were tested by antibody ELISA using whole cell lysate (WCL) antigen as per method of Yadav *et al*., 2013. As per test protocol ELISA plates (Nunc) were coated with 50 µl of 1.0 µg/ml of antigen in 0.1 M carbonate/bicarbonate buffer (pH 9.6) per well and incubated overnight at 4 ºC. Plates were washed three times with phosphate buffered saline with tween 20 (PBST). Blocking was done with 100 µl of 5 % skim milk in PBST (SM-PBST) for 1 h at 37 ºC. Plates were washed three times with PBST. Subsequently, 50 µl of test serum (1:100 diluted in 5 % SM-PBST) were added to each well and incubated for 1 h at 37 ºC. Plates were washed five times with PBST. Thereafter, 50 µl of 1:10,000 diluted IgG-peroxidase conjugate (Sigma) was added to each well and the plates were incubated for 1 h at 37 ºC. Plates were washed five times with PBST. Finally, 50 µl per well of freshly prepared substrate tetramethyl benzidine (TMB) was added. Allow the color to develop and the reaction was stopped by adding 50 µl of 1M H_2SO_4 to each well. The absorbance was read at 450 nm on ELISA reader (Bio Tek, USA) and results were expressed as mean OD of duplicate samples. The cut off values were determined using mean OD ± 3SD of uninfected serum samples.

Diagnostic efficacies of Giemsa stained thin blood smear; Buffy coat method, and Ab-ELISA were evaluated on the basis of % positivity shown by individual diagnostic test.

$$\% \text{ Positivity} = \frac{\text{No of positive cases given by a particular diagnostic test (n)}}{\text{total no suspected cases (N)}} \times 100$$

Results and Discussion

In the present study a total of 515 equines suspected to be affected from trypanosomiasis (Surra) during the period of April 2014 to March 2015 were examined by giemsa stained thin blood smear, revealed the presence of *T. evansi* in 36 equines, showing an efficacy of 6.99% (Table-I). Buffy coat examination revealed, the presence of *T. evansi* in 51 of suspected equines, showing an efficacy of 9.90% (Table-I). Ab-ELISA examination of 515 number of suspected equines serum revealed the presence of *T. evansi* parasite antibody in 115 equines, showing an efficacy of 22.33% (Table-I). Serum samples of all the blood smear, buffy coat positive cases were also found positive with Ab-ELISA. Therefore, in present study the order of relative diagnostic efficacy of various diagnostic tests applied, Ab-ELISA was found to be of maximum efficacy followed by buffy coat examination and giemsa stained thin blood smear examination.

Table-I: Diagnostic efficacy (Percent positivity) of various tests in the detection of *T. evansi* infection in equines

Diagnostic tests	Suspected Equines (Total= 515)	
	No. of positive	Efficacy (% positive)
Giemsa stained blood smear	36	6.99
Buffy coat technique	51	9.90
Ab-ELISA	115	22.33

In present study, giemsa stained thin blood smear examinations in suspected cases of trypanosomiasis in equines revealed the presence of *T. evansi* with an efficacy of 6.99%. Mathura and its surrounding districts are more prone for exposure to biting flies (Tabanid flies); because of the fact that in this area there is high vector density due to its agro climatic condition, Ponds and Yamuna belt, therefore, the animals might be getting acute infection during grazing time. The present findings are similar with the findings of Agarwal *et al*. (2003). These findings of present investigation does not corroborates with the findings of Laha *et al*. (2009) and Shahzad *et al.* (2010).

Buffy coat examination in suspected cases of trypanosomiasis in equines revealed the presence of *T. evansi* with efficacy of 9.90%. Our findings were not in the agreement with the findings earlier reported by Hollanda *et al*. (2001). The buffy coat technique detected more number of cases of *T. evansi* infection compared with giemsa stained blood smears examination. It could be attributed to the reason that in most of the hosts, *T. evansi* can induce mild clinical infections with low parasitaemia and in such condition, concentrations methods like buffy coat technique become necessary. The application of parasite concentration methods like buffy coat techniques is recommended to diagnose the

T. evansi infection as an alternative method (OIE, 2012).

In present study Ab-ELISA examination revealed the presence of circulating *T. evansi* antibody in 115 cases showing a seroprevelence of 22.33% indicating endemicity of surra in equines in Mathura and its adjoining areas. All the blood smear, buffy coat positive cases were also found positive with Ab-ELISA however, healthy controls showed negative reaction. The findings of present investigations are similar with the findings of Kumar *et al.* (2013).

The order of decreasing diagnostic efficacy during present study was as follows: Ab-ELISA > Buffy Coat method > Giemsa stained thin blood smear. It may be due to the fact that microscopic detection of parasites in the blood are not always effective since trypanosomes are frequently absent from peripheral blood. Similar observations were made by various workers regarding diagnostic efficacy of routine parasitological examination techniques (Carlos *et al*., 1990; Paris *et al.,* 1982) Therefore it can be concluded from the present investigation that serological test like Ab-ELISA is of higher value in the detection of *T. evansi* infections than the routine parasitological examination methods hence, it can be used for seroprevalence studies of surra.

Acknowledgement

Authors are thankful to the Dean, COVSc &AH, Dean Post graduate studies, DUVASU, Mathura, Brooke hospital for animals and Director, NRC on Equine, Hisar, for providing necessary facilities and support during the investigation.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 46-48

Antibiogram against bacterial pathogens associated with subclinical mastitis in Surti Buffaloes

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Abstract

Present study was conducted to determine the antibiotic sensitivity pattern against bacterial isolates in subclinical mastitis in Surti buffaloes in Udaipur District of Rajasthan. Total 115 quarter milk samples of 29 apparently healthy Surti buffaloes of different parity and lactation status were collected. Out of which 27 samples were found positive for bacterial isolates on cultural examination viz. staphylococci, streptococci. *E. coli,* bacilli and *Corynebacterium* spp. and were subjected to antibiotic sensitivity test. All bacterial isolates were 100 percent sensitive to cefoperazone followed by amoxycillin-sulbactam, cefuroxime and ciprofloxacin. Overall highest resistant was observed against tetracycline (54.54 percent) followed by gentamicin, ceftriaxone and ciprofloxacin.

Keywords: Surti buffaloes, Subclinical mastitis, Bacterial isolates, Antibiogram.

Subclinical mastitis is a major problem affecting dairy animals throughout the world. It causes enormous losses for breeders and consequently influences the national income of the country (Ramachandrainh *et al*., 1990). Buffalo milk is considered as an important source of market milk as it meets certain specific food requirements of human population (Maniruzzaman*et al.,* 2010). The bacterial contamination of the milk renders it unfit for human consumption (Sharif *etal.*, 2009).

Antibiotic therapy is an important tool in the scheme of treatment and control of mastitis. The misuse or intensive use of antibiotics can lead to the development of resistance among different bacterial strains. Appropriate antibiotic selection can be enhanced using an antimicrobial susceptibility test. Therefore, regular studies on bacteriological culture examination and antibiotic sensitivity of bacterial isolates are mandatory for effective and economical treatment of the disease (Sanchez *et al*., 1988). In view of the above facts, present study was conducted to find out antibiotic sensitivity pattern against bacteria isolated from subclinical mastitis in Surti buffaloes.

Materials and Methods

Milk samples were screened for presence of bacterial isolates using standard procedure as per Cowan and Steel (1975) and antibiotic sensitivity test of the bacterial isolates was conducted as per Bauer *et al.* (1966) disc method. The result was recorded as sensitive, intermediate and resistant.

Result and Discussion

Results of *in vitro* antibiotic sensitivity test to different bacterial isolates from subclinical mastitic milk samples have been presented in Table-1.

On culture examination, 27 samples were found positive for various bacterial isolates viz. staphylococci, streptococci. *E. coli,* bacilli and *Corynebacterium* spp. as single or mixed infection. The antibiotic sensitivity pattern revealed that amongst 14 isolates of staphylococci, all (100 percent) were found sensitive to cefuroxime, chloramphenicol and cefoperazone, followed by 92.85, 85.71, 71.42, 64.28 and 42.85 percent sensitivity to amoxycillin-sulbactam, ciprofloxacin, ceftriaxone, gentamicin and tetracycline, respectively. Chahar *et al.* (2002) reported that various strains of *Staphylococcus* spp. were found 100% sensitive to gentamicin. All the streptococci isolates (100 percent) were found sensitive to amoxycillin-sulbactam, cefuroxime and cefoperazone followed by chloramphenicol and ciprofloxacin (87.5 percent each), gentamicin and ceftriaxone (75 percent each) and tetracycline (37.5 percent). All the isolates (total 5) of *E. coli* (100 percent) were found sensitive to Amoxycillin-sulbactam, cefuroxime, chloramphenicol, ciprofloxacin, gentamicin and cefoperazone followed

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Table 1: Antibiotic sensitivity of microorganisms isolated from subclinical mastitis samples.

Sr. No.	Antibiotic	Response of antibiotic	Staphylococci (14)	Streptococci (8)	*E. coli* (5)	Bacillus (3)	Corynebacterium (3)
1.	Amoxycillin-sulbactam	Sensitive	13 (92.85)	8 (100)	5 (100)	3 (100)	3 (100)
		Intermediate	1 (7.14)	-	-	-	-
		Resistant	-	-	-	-	-
2.	Cefuroxime	Sensitive	14 (100)	8 (100)	5 (100)	3 (100)	2 (66.66)
		Intermediate	-	-	-	-	1 (33.33)
		Resistant	-	-	-	-	-
3.	Chloramphenicol	Sensitive	14 (100)	7 (87.5)	5 (100)	2 (66.66)	3 (100)
		Intermediate	-	1 (12.5)	-	1 (33.33)	-
		Resistant	-	-	-	-	-
4.	Cefoperazone	Sensitive	14 (100)	8 (100)	5 (100)	3 (100)	3 (100)
		Intermediate	-	-	-	-	-
		Resistant	-	-	-	-	-
5.	ciprofloxacin	Sensitive	12 (85.71)	7 (87.5)	5 (100)	2 (66.66)	3 (100)
		Intermediate	1 (7.14)	-	-	1 (33.33)	-
		Resistant	1 (7.14)	1 (12.5)	-	-	-
6.	Gentamicin	Sensitive	9 (64.28)	6 (75)	5 (100)	1 (33.33)	2 (66.66)
		Intermediate	-	1 (12.5)	-	1 (33.33)	-
		Resistant	5 (35.71)	1 (12.5)	-	1 (33.33)	1 (33.33)
7.	Ceftriaxone	Sensitive	10 (71.42)	6 (75)	4 (80)	2 (66.66)	2 (66.66)
		Intermediate	2 (14.28)	2 (25)	-	1 (33.33)	1 (33.33)
		Resistant	2 (14.28)	-	1 (20)	-	-
8.	Tetracycline	Sensitive	6 (42.85)	3 (37.5)	3 (60)	1 (33.33)	1 (33.33)
		Intermediate	-	1 (12.5)	-	-	-
		Resistant	8 (57.14)	4 (50)	2 (40)	2 (66.66)	2 (66.66)

by ceftriaxone (80 percent) and tetracycline (60 percent). Bacilli isolates (total 3) were found 100 percent sensitive to amoxycillin-sulbactam, cefuroxime and cefoperazone followed by chloramphenicol, ciprofloxacin and ceftriaxone (66.66 percent each); and gentamicin and tetracycline (33.33 percent each). Isolates of *Corynebacterium* spp. (total 3) were found 100 percent sensitive to amoxycillin-sulbactam, chloramphenicol, cefoperazone, and ciprofloxacin followed by cefuroxime, gentamicin and ceftriaxone (66.66 percent each) and tetracycline (33.33 percent).

In the present study, overall highest bacterial sensitivity was found to cefoperazone (100 percent) followed by amoxycillin-sulbactam and cefuroxime (96.96 percent each), ciprofloxacin (87.87 percent), ceftriaxone (72.72 percent), gentamicin (69.69 percent) and tetracycline (42.42 percent). Overall highest resistant was observed to tetracycline (54.54 percent) followed by gentamicin (24.24 percent), ceftriaxone (9.09 percent) and ciprofloxacin (6.06 percent). There was no bacterial resistant against amoxycillin-sulbactam, cefurixime, chloramphenicol and cefuroxime. These antibiotics were found either sensitive or intermediate sensitive against different bacteria isolated from subclinical mastitic milk. The refractiveness of certain bacterial isolates to a particular antibiotic may be due to indiscriminate use of antibiotic therapy and involvement of large number of pathogenic bacteria.

Conclusions

It was concluded that overall highest bacterial

sensitivity was found to cefoperazone, amoxycillin-sulbactam and cefuroxime whereas highest resistant was observed against tetracycline.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 49-54

Reference values of electrocardiogram in healthy German Shepherd dogs

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Abstract

Breed-wise variation in the standard electrocardiographic values in dogs have been reported because of variation in body size and chest conformation leading to restrain of usage of values from one breed for data interpretation for another breed. The purpose of present study was to establish a comprehensive set of reference electrocardiographic values in healthy German shepherd dogs and to check effect of body weight, age and gender on various ECG parameters. Electrocardiogram was recorded in 30 clinically healthy German shepherd dogs using six channel ECG machine in right lateral recumbency, restrained manually without any chemical.Amplitude of P, Q, R, S and T waves were measured along with PR interval, duration of QRS and ST segment and heart rate. Non-significant effect of body weight and gender was observed on allthe ECG parameters. A positive correlation between heart rate & age and a negative correlation between PR interval & age was observed. It was concluded that age has a significant effect on various ECG parameters and should be considered while interpreting the data. This data can be used as baseline for the interpretation of theECG of German shepherd dogs.

Keywords: Dogs, Electrocardiography, German shepherd, Reference values

Heart is an electrically charged organ present in the thoracic cavity. Information related to heart rate, cardiac rhythm and conduction can be assessed through an electrocardiogram (Gugjoo *et al.*, 2014). An electrocardiogram is the recording of electric potentials generated by the cardiac impulse by placing electrodes on the skin on opposite sides of the heart (Guyton and Hall, 2006). However, in both veterinary and human medicine, ECG is also used to evaluate the presence of cardiac enlargement (Tsao*et al.,* 2008). Electrocardiography (ECG) is a widely available and cost effective diagnostic tool. The most robust application for electrocardiography is the evaluation of arrhythmias or conduction abnormalities. For ECG interpretation in healthy subjects, studying Lead II in the standard bipolar limb lead system has been widely used in the veterinary practice (HseihandHsu, 2012). Significant differences in various ECG parameters have been reported that may possibly occur due to variation in the chest size, thoracic conformation or due to genetic differences (Avizeh *et al.,* 2010).Analysis of the electrocardiograms in different dog breeds manifest that there can be differences betweenbreeds (Rezakhani*et al.,*1990).Reference ECG values have so far been established for different breeds of dogs like Labradors (Gugjoo*et al.,* 2014), Beagles (Hanton and Rabemampianina, 2006),Whippets (Bavegems*et al.,*2009) and Mongrels (Avizeh *et al.,*2010). Although data concerning values of electrocardiographic parameters (Rezakhani *et al.,* 1990)and effect of age on these parameters (Kosic *et al.,*2017) in healthy German shepherd dog were published, these studies were not drafted to analyze dependence of the ECG parameters on other factors like body weight and gender. The study presented here was performed to establish a comprehensive set of reference electrocardiographic values in healthy German shepherd dogsalong with assessment of effect of body weight, age and gender on various electrocardiographic parameters, which could be inscribed with the conventional diagnostic procedures extensively used in canine cardiology.

Material and Methods

The study was carried out on thirty clinically healthy German shepherd dogs of both the sexes with bodyweightranging from24-45kg,presentedatTeaching Veterinary Hospital of Guru AngadDev Veterinary and Animal Sciences University, Ludhiana, Indiafor routine health check-up. Before electrocardiographic recording, routine physical examination, cardiovascular examination, echocardiography was done and their health conditions were carefully validated and then all the dogs were further subjected to electrocardiography (ECG). None of the dogs had a clinical history of cardiac problem as described by the owner of the

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pet. Electrocardiogram was recorded in all dogs as described by Tilley*et al*.(1992)using BPL cardiart 8108 six channel ECG machine in right lateral recumbency and limbs held perpendicular to the body and parallel to the corresponding limb. The dogs were manually restrained without any anaesthetic or tranquilizer. Limb electrodes were placed either distal or proximal to the elbow (caudal surface) in the fore limbs and over the stifle in the hind limbs. ECG recording was taken at paper speed of 50 mm /sec and calibration of 10 mm equal to 1 mV.One small box on horizontal axis was taken equal to 0.02 sec and equal to 0.1 mV on vertical axis. The Bailey's hexaxial limb lead system involving lead I, II, III, aVR, aVL and aVF leads were used. The lead II rhythm strip was used for measurement of all the ECG parameters. The wave width was noted in seconds while the amplitude was noted in millivolts. Amplitude of P, Q, R, S and T waves were measured along with PR interval, duration of QRS and ST segment. Heart rate in beats per minute (bpm) was calculated as described by Tilley*et al*. (1992).The grouping of the animals was done on the basis of body weight, age and gender wherein Group I and Group II was categorised between 20-40 kg and 40-60 kg body weight respectively, Group III as 1-5yrs and Group IV as more than 5 years, Group V was designated for males and Group VI for females.

The data was analysed for statistical significance using SPSS software. Independent t test was used to compare the means among different groups based on body weight and gender. The differences were considered significant at a value of $p < 0.05$.

Results and Discussion

Mean±standard error (SEM) of different ECG parameters with range of extreme values (Lead II) in healthy German shepherd dogs (n=30) has been presented in Table-I.The ECG of Lead-II has also been depicted in Figure: I.Statistical analysis for comparison of sex, body weight and age in German shepherd dogs showed a number of significant differences, but most of the differences were of low magnitude and within the range of reading precision for the ECG parameters. Therefore, they do not indicate a real physiological difference.

Table I: Effect of body weight, age and gender on electrocardiographic parameters in healthy German shepherd dogs (n=30)

	Heart rate (bpm)	P wave amplitude (mV)	P wave duration (sec)	PR interval (sec)	R wave amplitude (mV)	QRS duration (sec)	Q wave (mV)
Body weight (kg)							
Group I (n=22)	120.9±3.41[1]A[2] (100-160)	0.18±.002A (0.1-0.4)	0.04±.008A (0.2-0.02)	0.11±0.003A (0.2-0.02)	1.5±0.09A (1.0-2.6)	0.07±0.02A (0.02-0.06)	0.29±0.19A (0.05-0.7)
Group II (n=8)	118.8±7.20A (90-140)	0.16±0.02A (0.1-0.3)	0.03±0.008A (0.01-0.06)	0.11±0.008A (0.08-0.14)	1.58±0.18A (0.9-2.7)	0.04±0.008A (0.02-0.08)	0.33±0.03A (0-0.5)
Age (years)							
Group III (n=18)	116.7±4.04A[3] (100-160)	0.17±0.01A (0.2-0.02)	0.04±0.01A (0.2-0.02)	0.12±0.004A (0.1-0.14)	1.7±0.10A (0.9-2.7)	0.07±0.03A (0.02-0.6)	0.35±0.04A (0.1-0.7)
Group IV (n=12)	131.7±4.05B (110-160)	0.17±0.02A (0.1-0.4)	0.03±0.005A (0.01-0.06)	0.10±0.006B (0.08-0.14)	1.30±0.07B (1.0-1.8)	0.04±0.004A (0.02-0.06)	0.23±0.04B (0.05-0.5)
Gender							
Group V (n=24)	122.9±3.69A[4] (100-160)	0.19±0.01A (0.1-0.4)	0.04±0.007A (0.02-0.2)	0.11±0.004A (0.08-0.14)	1.5±0.09A (0.9-2.7)	0.04±0.003A (0.02-0.08)	0.28±0.03A (0.05-0.7)
Group VI (n=6)	121.7±6.56A (100-140)	0.11±0.01A (0.1-0.2)	0.02±0.005A (0.01-0.04)	0.11±0.01A (0.08-0.14)	1.5±0.19A (1.0-2.4)	0.14±0.09A (0.02-0.6)	0.42±0.07A (0.1-0.6)
Over all							
(n=30)	122.66±3.18 (100-160)	0.17±0.01 (0.1-0.4)	0.03±0.006 (0.01-0.2)	0.11±0.003 (0.08-0.14)	1.5±0.08 (0.9-2.7)	0.06±0.018 (0.02-0.6)	0.30±0.03 (0.05-0.7)

[1]Values are mean±SEM

[2]Values with different upper case alphabets in same column differ significantly ($P \leq 0.05$) between different body weight groups.

[3]Values with different upper case alphabets in same column differ significantly ($P \leq 0.05$) between different age groups.

[4]Values with different upper case alphabets in same column differ significantly ($P \leq 0.05$) between different gender groups.

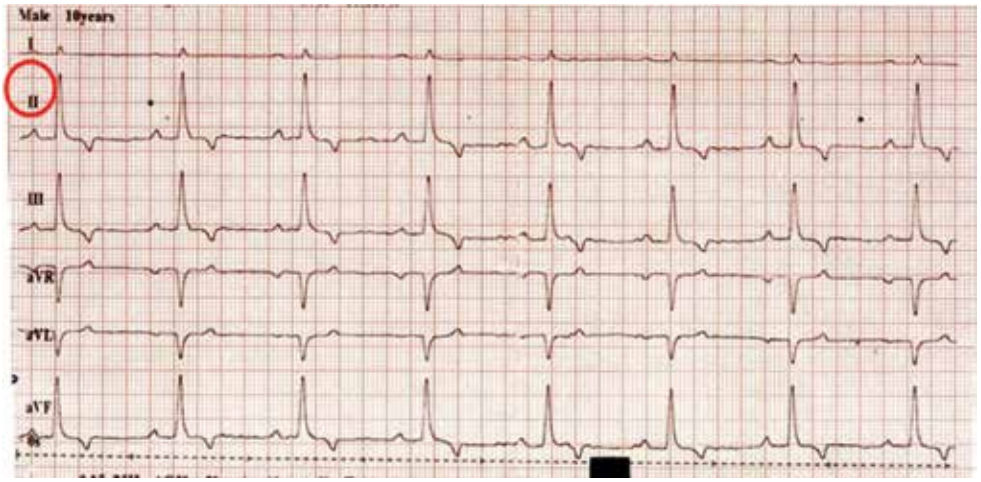

Fig I: Electrocardiogram in a healthy German shepherd dog depicting normal P, QRS complex and T wave

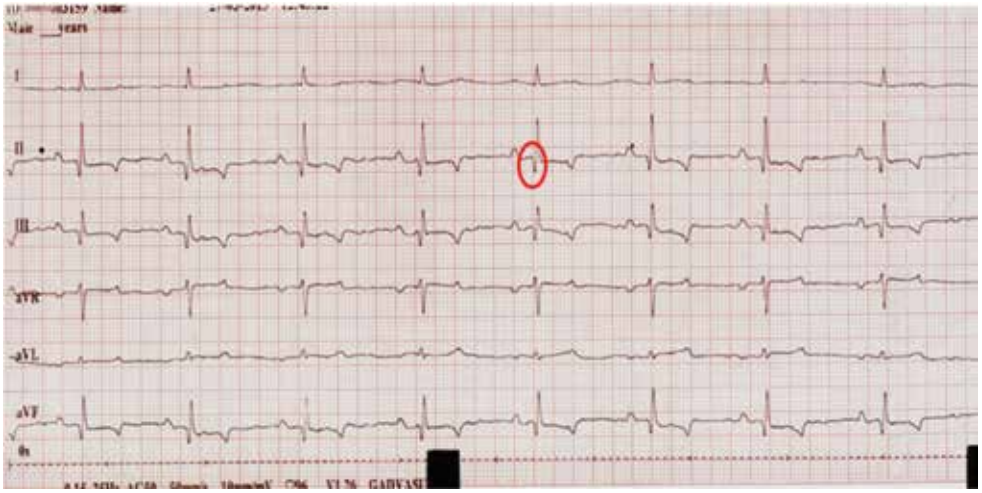

Fig II: Electrocardiogram in a healthy German shepherd dog showing inverted Q wave

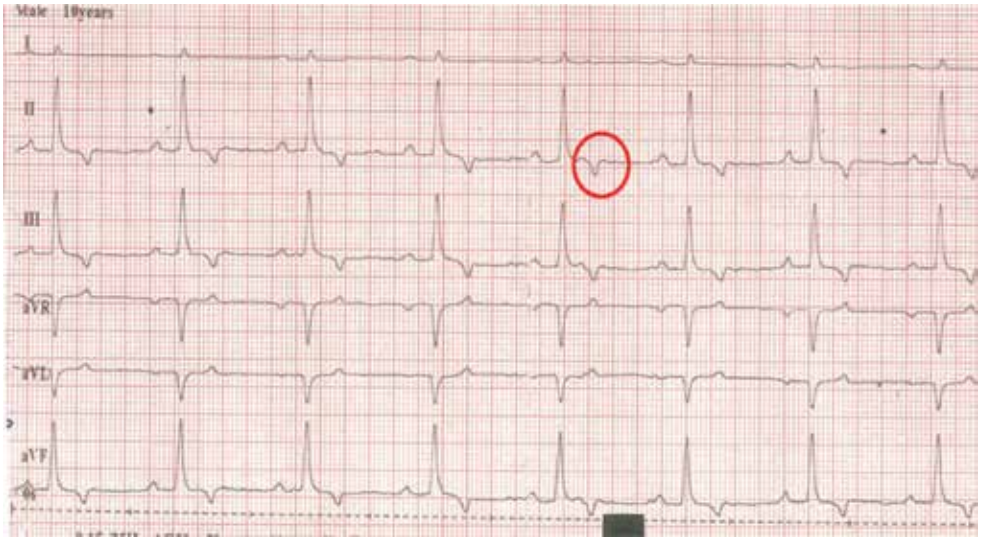

Fig III: Electrocardiogram in a healthy German shepherd dog showing negative T wave

Table I: continued

	ST segment (sec)	T wave Amplitude (mV)	T wave Duration (sec)	QT interval (sec)
Body weight (Kg)				
Group I (n=22)	0.01±0.08[1]A[2] (0.08-0.16)	0.31±0.02A (0.1-0.5)	0.05±0.004A (0.01-0.08)	0.15±0.006A (0.1-0.2)
Group II (n=8)	0.11±0.014A (0.04-0.18)	0.33±0.04A (0.2-0.5)	0.43±0.007A (0.02-0.08)	0.16±0.013A (0.12-0.22)
Age (years)				
Group III (n=18)	0.11±0.007A[3] (0.08-0.18)	0.35±0.02A (0.2-0.5)	0.05±0.004A (0.02-0.08)	0.16±0.006A (0.12-0.22)
Group IV (n=12)	0.09±.0.007A (0.04-0.12)	0.27±0.03A (0.1-0.5)	0.04±0.007A (0.01-0.08)	0.14±0.009B (0.1-0.18)
Gender				
Group V (n=24)	0.10±0.006A[4] (0.04-0.18)	0.3±0.02A (0.1-0.5)	0.04±0.004A (0.01-0.08)	0.15±0.006A (0.1-0.22)
Group VI (n=6)	0.11±0.01A (0.08-0.14)	0.4±0.05A (0.2-0.5)	0.06±0.005A (0.04-0.08)	0.16±0.014A (0.1-0.2)
Over all				
(n=30)	0.10±0.005 (0.04-0.18)	0.32±0.02 (0.1-0.5)	0.04±0.003 (0.01-0.08)	0.15±0.005 (0.1-0.22)

[1]Values are mean±SEM
[2]Values with different upper case alphabets in same column differ significantly (P ≤ 0.05) between different body weight groups.
[3]Values with different upper case alphabets in same column differ significantly (P ≤ 0.05) between different age groups.
[4]Values with different upper case alphabets in same column differ significantly (P ≤ 0.05) between different gender groups.

In the present study of ECG parameters in healthy German shepherd dogs, mean±SEM of heart rate was 122.66±3.18bpm, which ranged from 100-120 bpm (Table 1). However, Mukherjee *et al*. (2015) reported lower heart rate (104±5.6, 88-125 bpm) in trained German shepherd dogs used for security purposes. The reason of difference could be the dogs used in the present study were not trained for restrain and it is important to consider that heart rate is highly variable in dogs and may be altered due to stress and excitation during recording (HantonandRabemampianina, 2006).

P wave is the first positive deflection on an electrocardiogram representing magnitude of atrial depolarization which spreads from sinoatrial (SA) node to the atrioventricular (AV) node. The mean±SEM of P wave amplitudewas 0.17±0.01 (0.1-0.4 mV), within the normal range and in accordance with previous workers (Avizeh*et al.*, 2010). The P-wave amplitude is also dependent on the heart rate and breed of the animal (Gugjoo*et al.*, 2014). In the present study Q-wave of QRS complex, produced due to ventricular depolarization and transmission of electrical signal through interventricular septum after P-wave, was negatively directed in 29 dogs (absent in one dog) in Lead II(Fig: II) as supported by Gugjoo*et al.* (2014). Amplitude of R-wave is most commonly used to evaluate the left ventricular function and considered a good indicator for ventricular contractibility. R wave amplitude was found to be well within the range(0.9-2.7 mV). Similarly Mukherjee *et al.*(2015) reported range of R wave amplitude between 1.0-2.0 mV in trained German shepherd dogs.

QT interval is a dynamic physiological variable which can be affected by ventricular conduction and repolarisation. In the present investigation mean±SEM of QT interval was 0.15±0.005 sec, with a range of0.1-0.22 sec which is in accordance with the earlier work reported by Mukherjee *et al.*(2015) that gave the mean±SEM and range as 0.18±0.008 sec and 0.17-0.21sec, respectively.

T-wave amplitude and duration is directly related to the repolarization of the ventricular myocardial cells. In the present study, these were found to be within normal range as reported by other researchers (Su *et al.*, 2001). Inverted T-wave was observed in 73% (n=22) dogs (Fig:III), which is slightly

different from results given by (Mukherjee *et al.* (2015), wherein 66% of German shepherds had inverted T-waves. The determinants of T-wave polarity are yet to be understood andaltered polarity of T wave may be caused due to elevation of diaphragm during respiration (Tilley*et al.*, 1992).

Effect of body weight on various electrocardiographic parameters

In the present study, none of the electrocardiographic parameters showed any significant difference between Groups I and II (Table I). However the values of all the parameters were within the range specified for healthy German shepherd dogs. So it is evident from the present study that change in body weight has no influence on electrocardiographic parameters. Similarly, Gugjoo*et al.*(2014) reported no significant effect of body weight on any of the ECG parameters in healthy Labrador dogs.

Effect of age on various electrocardiographic parameters

The mean heart rate was significantly (P<0.05) lower in group III as compared to group IV. Mean PR interval was significantly (P<0.05) lower in group IV dogs as compared to group III (Table I). Similar to our findings, EckenfelsandTrieb (1979) reported negative correlation between heart rate and age and positive correlation between PR interval and age. However, Avizeh*et al.*(2010) reported a highly positive correlation between PR interval and age and negative correlation betweenheart rate and age. Mohapatra*et al*. (2013) reported increase in heart rate of healthy dogs with increasing age. The Q wave amplitude in group III was significantly (P<0.05) higher as compared to group IV dogs. Amplitude of R wave was significantly (P<0.05) higher in group III in comparison to group IV in consistency to the reported values of R-wave amplitudein German shepherd breed by Kosic*et al*. (2017). The Q-wave amplitude was found to increase with the advancement of age. Bernal *et al.*(1995) observed an increase in the amplitude of Q wave until 45 days of age, followed by a decline until the age of three months.In the present study, amplitude of Q wave was as high as 0.7mV in clinically healthy German shepherd dogs. Similarly, Bernal *et al.*(1995) also reported maximum amplitude of Q waves reaching 0.8 mV in healthy dogs. QT interval was significantly (P<0.05) higher in group III as compared to group IV. However, Kosic*et al.* (2017)reported no significant difference between QT interval of young dogs as compared to older dogs, although values of the present study were well within the range as described by Gugjoo*et al*. (2014).

Effect of gender on various electrocardiographic parameters

Mean ECG values of various parameters did not show any significant difference between groups V and VI (Table 1). (HantonandRabemampianina, 2006; Gugjoo*et al.,* 2014) also reported no significant difference in any of the ECG parameters between males and females in Labrador and in Beagle dogs respectively. The absence of significant differences in ECG parameters between sexes is consistent with the previous literature and thus allows that combination values from males and females can be used while interpreting the ECG parameters.

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Immunotherapeutic potential of *Tinospora cordifolia* stem powder in bovine subclinical mastitis: A clinical assessment

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Abstract

Present study was envisaged to evaluate the immunotherapeutic potential of an herb, *Tinospora cordifolia*, in mastitis in dairy cows. Twenty-Four HF × Sahiwal crossbred cows kept at an organized dairy farm, found positive in at least one quarter for specific mastitis as per International Dairy Federation criteria. The cows were divided into two equal groups: a control group and a group administered with *T. cordifolia* stem powder at 500mg/kg body weight daily divided into two doses orally for 7 days. The treatment could eliminate 65% and 75% of intramammary infections significantly at d15 and d30 post-treatment, respectively as compared to the control group on corresponding days. The treatment also resulted in a significant decline in somatic cell count and NAGase enzyme activity thus subsiding udder inflammation and improving milk quality. The udder immunity was also improved, as evidenced by the significant increase in phagocytic activity of milk neutrophils. The enhanced activity of milk neutrophils was observed for upto 90 days. Thus, the findings indicated the immunotherapeutic potential of *T. cordifolia* stem powder in treating bovine-specific subclinical mastitis.

Keywords: Immunotherapeutic potential, *Tinospora cordifolia* stem, Subclinical mastitis, Cow

Antibiotics in a modern therapeutic system have been enormously used in controlling the infectious diseases (Alekshun, 2005). Due to the extensive use of antibiotics, there has been an emergence of multidrug-resistant strains of many pathogens that are posing serious challenges to the clinicians (Waclaw, 2016). There is a dire need to find suitable replacements for some of the currently used antibiotics (Zaki and Karande, 2011). Currently antibiotics are being used extensively for the treatment and control of subclinical mastitis. But this approach is not sustainable due toresistance in the pathogens to such antibiotics andthe persistence of antibiotic residues in the milk. Therefore, a preventive approach at appropriate time is more suitable than control by treatment. One possible approach to control mastitis involves manipulation of host defense mechanism. Hence, recent strategies aimed at improving the immune cells of the diseased udder during immunosuppressive stages would greatly impact the ability of the animal to resist pathogenic infection. One such approach is based on enhancement of the animal's natural defense mechanism by use of some non-specific immuno-modulator such as herbs which, in turn minimizes the use of antibiotics. Even World Health Organization (WHO) has emphasized on the use of medicinal plants, as they are safer and effective than the synthetic drugs.

Giloy (*Tinospora cordifolia*), also known as *guduchi,* occupies the top spot in "*Ayurvedic Materia Medica*" and it has been designated as "*Rasayana*" (Bhattacharyya and Bhattacharyya, 2013). This plant finds mention in ancient Sanskrit literature like *Charak Samhita* and *Sushruta Samhita,* as a potential healer of many diseases. Mantena *et al.* (2006) found that one of the most important constituent present in stem of *T. cordifolia* is berberin which shows various pharmacological actions which enhances the therapeutic efficacy of this plant. (Singh, 2000) studied few herbal medicines e.g. oil extract of *Ocimum sanctum* with *Azardichta indica* and aqueous stem extract of *T. cordifolia* as intra-mammary infusion in SCM and observed immunopotentiation activity represented by enhancement of milk PMN cell phagocytosis. Though some information is available on the beneficial activities of *T. cordifolia* in human and animal medicine, data regarding its use as an antibacterial and immunomodulatory in bovine mastitis are scanty. Therefore, the present study was planned to evaluate the *in vivo* effectiveness of *T.cordifolia* stem powder in immunomodulation of the udder and therapy of mastitis in dairy cows.

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Materials and Methods

Design and animals allocated

Twenty-Four HF × Sahiwal crossbred cows kept at an organizeddairy farm, found positive in at least one quarter for specific mastitis (California Mastitis Test (CMT) score of ≥1 representing >200 × 10^3somatic cells/ml and positive for culture as per International Dairy Federation criteria), in early to middle lactation with average weight of over 400 kg and daily milk yield of above 15–20 kg, were included in the trial. The selected animals were divided randomly into two groups: a control group (n = 12) and a treatment group (n = 12) were treated with *T. cordifolia* crude stem powder i.e. 200g total dose P.O., divided in two parts morning and evening × 7 days.The dose of herb was calculated on the following observations: 1) when administered as a crude powder, the herb's dose was taken as four times the dose of herbal pure extract(Wynn and Fougere, 2007); 2) for safety reasons, the maximum dose of a chemical should not be more than 1/10 of the 50% lethal dose (LD50) dose of that particular chemical; 3) The 50% lethal dose (LD50) of *T. cordifolia* was found to be 1.6g/kg b. wt in rats by Rao *et al*.(2005). So, be on So, be on safer side, its dose was taken as 1/13th of LD50 i.e.~150 mg/ kg b wt. Since, the herb was fed as crude powder; it was administered at 4 times dose i.e. @~500 mg/kg body weight × 400 kg b. wt (~200 g total dose). The calculated dose was given separately from the animals' daily diet and the treatment did not show any adverse effects on the health of the cows.

Sampling and parameters studied

Sampling was done pre treatment (day 0) and at days 7, 15, 30, 60 and 90 post initiation of treatment during the routine morning milking hours to assess the quarter health status, milk quality, and immune status of the udder. Two types of milk samples, quarter foremilk and cow composite milk, were collected. During collection of milk samples, proper cleanliness and dryness of the udder were ensured. Quarter foremilk samples (about 10 ml) were collected in sterilized test tubes, analyzed for milk culture and California mastitis test (CMT). Cow composite samples (about80 ml) were collected in clean disposable plastic vials following cow milking which were analyzed for Somatic cell count (SCC), CMT, EC, pH, NAGase enzyme activity and phagocytic activity of milk PMN cells upto 90 days post initiation of treatment. The milk samples were packed in an ice box, transferred immediately to the laboratory, and analyzed for various parameters.

Isolation and identification of bacteria and CMT, pH, EC, SCC analyses

Isolation and identification of bacteria was performed as per the standard microbial procedures of the National Mastitis Council (1987). The CMT was conducted and interpreted as per the method described by (Pandit and Mehta, 1969). The results were read as negative (-), trace, one plus (+), two plus(++), and three plus (+++) depending upon the degree of gel formation. The pH of milk was recorded with the help of a digital pH meter (Mettler Toledo, Five Easy Plus). The electrical conductivity was recorded using Digital Conductivity Meter (CON 700, Eutech instruments). The results were expressed in milli Siemens per cm (mS/cm). The analysis of milk samples for SCC was done using milk somatic cell counter from DELTA Instrument, BV Kelvinlaan 3, 9207 JB Drachtenand results were expressed in ×10^3cells/ml. The NAGase

Table 1 : Elimination of intramammary infections with herbal therapy

Organism	Intramammary infections (IMI) Vs. TreatmentGroup					
	Control (G1)			*T. cordifolia*(G2)		
	0 d	15 d	30 d	0 d	15 d	30 d
Coagulase-positive staphylococci	30	8	8	32	19	23
Coagulase-negative staphylococci	7	3	3	5	2	4
Corynebacteria	6	1	2	1	1	1
Bacilli	_	_	_	2	2	2
Overall	43	12 (27.90)	13 (30.23)	40	24 (60)*	26 (65)#

Figures in parentheses indicate percentage
Significant differences existed in elimination of IMI between treatment and control group
*($\chi 2 = 8.69$; 01df; $p<0.05$), # ($\chi 2 = 16.63$; 01df; $p<0.05$)

enzyme activity in milk was analyzed by fluorometric microplate reader (Fluoroscan Ascent FL, Thermo scientific make) and results were expressed in nMoles/ml/min. Phagocytic activity of milk neutrophils was carried out using the same method as described elsewhere (Shafi *et al.*, 2016).

All numerical data were processed via SPSS 20.0. Analysis of parametric data was conducted byusing ANOVA, and when the main effect was significantthen Duncan's multiple range test was performed. The effectof treatment on elimination of intramammary infections was analyzed using the chi-square test. Significance levelwas set at $P \leq 0.05$.

Results and Discussion

The therapy with *T. cordifolia* could eliminate, in overall, 26/40 (65%) of intramammary infections at d15 post-treatment.as compared to 12/43 (27.90 %) in the control group whereas the therapy with *T. cordifolia* could eliminate, in overall, 30/40 (75%) of intramammary infections at d30 post-treatment. The differences in the elimination of intramammary infections in treatment vs. control were observed to be statistically significant ($p < 0.5$) at 15d and 30d respectively. The present findings are in agreement with (Ranjan ,2007) who evaluated the effects of intramammary use of antibiotic along with *T. cordifolia* extract (200mg/quarter) intramammary per day for 3 consecutive days in bovine clinical mastitis resulted higher recovery rate was noted in cows supplemented with extract of *T. cordifolia* (80.95%) than those treated with antibiotic alone (71.42%). Therapy with *T.cordifolia* showed a significant decline in CMT score from d15 (0.58±0.15, P<0.05) to d 90 (0.25±0.25) of treatment in comparison to the CMT score on d 0.The SCC of milk in treatment group showed significant (p<0.05) decline on day 7 (445.33±65.49 x 10^3 cells/ml) in comparison to the SCC on day 0 (677.42±101.03 × 10^3 cells/ml) as presented in Table 2. Similarly, the pH and EC values also decreased significantly (p<0.05) on day 15. The significant decline in CMT, SCC, pH, and EC in the present study is in agreement with(Mallick and Prakash, 2011) reported a significant reduction in milk somatic cell count post treatment in subclinical mastitis-affected animals treated with oral administration of *T. cordifolia.* However, (Mukherjee and Ram, 2010) found that Intramammary infusion of a hydro-methanolic extract of *T. cordifolia* treatment initially enhanced the SCC;

Table 2. Effect of therapy on inflammatory reaction of udder.

Parameter	Group	Days after initiation of treatment					
		0 d	7 d	15 d	30 d	60 d	90 d
CMT score	C	2.25 ± 0.13^{a}	1.83 ± 0.17^{b}	1.83 ± 0.21^{c}	1.58 ± 0.31^{d}	1.58 ± 0.31^{e}	1.75 ± 0.35^{f}
	T	1.92 ± 0.26^{a}	$1.25\pm0.18^{b,c}$	$0.58\pm0.15^{a,c,d}$	$0.08\pm0.08^{a,c,d,e}$	$0.25\pm0.25^{a,c,d,e,f}$	$0.25\pm0.25^{a,c,d,e,f}$
SCC ($\times10^3$/ml)	C	794.33 ± 60.35^{a} (503-1075)	712.92 ± 64.41^{b} (390-940)	663.92 ± 76.9^{e} (247-1125)	624.58 ± 96.6^{d} (200-1167)	604.17 ± 83.16^{e} (141-981)	624.08 ± 84.9^{f} (161-1028)
	T	677.42 ± 101.03^{a} (301-1387)	$445.33\pm65.49^{b,c}$ (140-898)	$305.17\pm56.48^{a,c,d}$ (67-611)	$175.42\pm40.13^{a,c,d,e}$ (34-423)	$189.17\pm69.45^{a,c,d,e,f}$ (36-899)	$172\pm69.38^{a,c,d,e,f}$ (35-904)
EC (mS/cm)	C	6.25 ± 0.26^{a}	6.34 ± 0.21^{b}	6.31 ± 0.24^{c}	5.97 ± 0.33^{d}	5.83 ± 0.31^{e}	5.74 ± 0.30^{f}
	T	6.64 ± 0.13^{a}	$5.88\pm0.18^{b,c}$	$5.38\pm0.13^{a,c,d}$	$4.94\pm0.07^{a,c,d,e}$	$4.62\pm0.08^{a,c,d,e,f}$	$4.69\pm0.05^{a,c,d,e,f}$
pH	C	6.99 ± 0.08^{a}	6.95 ± 0.15^{b}	6.94 ± 0.09^{c}	6.91 ± 0.09^{d}	6.88 ± 0.10^{e}	6.86 ± 0.07^{f}
	T	7.07 ± 0.06^{a}	$6.72\pm0.03^{b,c}$	$6.58\pm0.03^{a,c,d}$	$6.47\pm0.05^{a,c,d,e}$	$6.37\pm0.07^{a,c,d,e,f}$	$6.33\pm0.14^{a,c,d,e,f}$
NAGase (nMoles/ml/min)	C	298.89 ± 48.07^{a}	218.72 ± 35.35^{a}	159.94 ± 20.16^{a}	174.94 ± 21.9^{a}	156.59 ± 17.54^{a}	185.5 ± 23.65^{a}
	T	184.14 ± 30.04^{a}	$130.65\pm20.85^{a,b}$	$76.42\pm0.53^{a,b,c}$	$73.93\pm0.56^{a,b,c,d}$	$73.03\pm0.39^{a,b,c,d,e}$	$73.2\pm0.28^{a,b,c,d,e}$

The values having at least one same superscript (alphabets within row) differ significantly (p>0.05)

Table 3. Phagocytic activity and phagocytic index in control (C) and treatment groups (T).

Parameters	Group	Days after initiation of treatment					
		0 d	7 d	15 d	30 d	60 d	90 d
Phagocytic activity (%)	C	8.5±0.86^{a}	10.5±0.96^{a}	10.83±1.11^{a}	10±0.9^{a}	9.75±0.92^{a}	10.5±1.49^{a}
	T	12.33±1.67^{a}	29.33±1.83^{a,b}	31.83±1.6^{a,b,c}	26.08±2.93^{a,b,c,d}	25.33±2.02^{a,b,c,d,e}	23.75±1.92^{a,b,c,d,e}
Phagocytic index	C	1.07±0.03^{a}	1.03±0.01^{a}	1.07±0.02^{a}	1.08±0.02^{a}	1.06±0.03^{a}	1.08±0.04^{a}
	T	1.06±0.03^{a}	1.31±0.04^{a,b}	1.38±0.07^{a,b,c}	1.51±0.06^{a,b,c,d}	1.39±0.03^{a,b,c,d,e}	1.25±0.04^{a,b,c,d,e}

The values having at least one same superscript (alphabets within row) differ significantly (p>0.05)

thereafter, a significant reduction in cell count (P < .05) was observed on day 15 of the treatment period. Mir *et al.* (2015) who evaluated effect of supplementation of *Tinospora cordifolia* on lactation parameters in early lactating murrah buffaloes and found a significant increase (P<0.05) in milk yield of treatment group. He also observed a significant (p < 0.05) reduction in somatic cell count during the experimental period. Sharma *et al.* (2015) who treated animals infected with SCM with 250 mg of polysaccharide fraction of *T cordifolia* via intramammary route twice daily for five consecutive days, and found a significant reduction in SCC. Therapy with *T.cordifolia* showed a significant decline in NAGase from d 7 (82.21±7.16, P<0.05) to d 90 (74.91±0.76) of treatment in comparison to NAGase on d 0. No literature was found in support of our study but Pyorala *et al*. (2011) reported that NAGase activity was very low in the milk of healthy quarters, increased in subclinical mastitis and was highest in quarters with clinical mastitis.

The mean phagocytic activity and phagocytic index (PI) in control did not differ significantly throughout the course of study. In *T.cordifolia* treated cows, a significant increase (P<0.05) was observed in phagocytic activity and phagocytic index at d 7 of treatment, and it remained significantly elevated throughout the course of study as compared to d 0 (Table 3). Neutrophils are part of the innate defense cell mechanism in mammals which use phagocytosis and intracellular oxygen microbicide action (Bochsler and Slauson, 2002). During the early infection by *S. aureus* in milking cows, a considerable increase in somatic cell counts is produced, with a high proportion of PMN Peeler *et al.* (2003). The severity of the infection produced by *S. aureus* as well as the clinical evolution of mastitis considerably affect PMN functional activity in the mammary gland Tomita *et al.*(2000). Any imbalance in PMN effective functioning can result in the multiplication of pathogens in the udder, resulting in mastitis. This imbalance can be keptin check by enhancing phagocytic potential and migrationof PMNs from the circulation to an inflamed udder thathelps in removal of infective agents from the udder. Inthe present study, the significant increases in phagocyticactivity/phagocytic index and subsequent eliminationof intramammary infection suggest that the herb hasimmunomodulatory action, a finding in agreement with Sharma *et al.* (2015) who treated animals infected with subclinical mastitis with sterile 250 mg of a polysaccharide fraction of *T. cordifolia* via intramammary route twice daily for five consecutive days and enhanced phagocytic activity of milk polymorphonuclear cells (PMNs) posttreatment. Gupta *et al.*(2016) found that oral feeding of *T. cordifolia* stem significantly increase mean phagocytic index.

In conclusion, the data obtained from the presentstudy indicate beneficial effects of herbal therapy against subclinical mastitis in lactating dairy cows.The positive effects of these remedies may be due to their anti-bacterial, anti-inflammatory and immunomodulation potential as substantiated by elimination of intramammary infections, decrease of milk SCC and enhanced phagocytosis of milk leukocytes.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 60-63

Assessment of non-corpuscular markers of protein oxidation, lipid peroxidation and antioxidant status of calves with natural tropical theileriosis

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Abstract

Eight crossbred calves naturally infected with *Theileria annulata* and eight clinically healthy crossbred calves (healthy controls) to assess the non-corpuscular markers of protein oxidation, lipid peroxidation and antioxidant status, serum contents of protein carbonyls, malondialdehyde (MDA) and total antioxidant capacity (TAC) were included in the present study. Non-corpuscular TAC content of calves with theileriosis was significantly lower ($P \leq 0.0001$) in comparison with healthy calves. Contrarily, remarkably higher levels of MDA ($P \leq 0.0012$) and protein carbonyls ($P \leq 0.0001$) were recorded in serum samples of diseased calves when compared with healthy controls. The findings of the present study revealed that containment of systemic antioxidant defense and remarkable oxidative injuries might be associated with the patho-biology and progression of tropical theileriosis in newborn calves.

Keywords: Calf theileriosis, Malondialdehyde, Oxidative stress, Protein carbonyls, Total antioxidant capacity

Tropical theileriosis, a lymphoproliferative disease of cattle, is caused by an apicomplexan parasite *Theileria annulata*. The parasite acts as a serious constraint to the cattle production in endemic areas, causing lethal infections in exotic cattle and considerable mortality in indigenous and crossbred stock (Nazifi *et al*., 2009; Branco *et al*., 2010; Hassanpour *et al*., 2013; Woods *et al*., 2013). Calf mortality owing to theileriosis is one of the major impediments to the livestock upgrading programmes in the Indian subcontinent (Godara *et al*., 2009). Clinical manifestations and the fates of theileriosis mainly depend upon the damaging effects of the pathogen on lymphoid tissues and susceptibility of the host. The parasite infects bovine macrophages and transforms them into aggressively invasive tumours by complete hijacking over the regulation of infected leukocytes and inhibiting apoptosis of infected cells, conferring its over proliferation and clonal expansion (Woods *et al*., 2013; Metheni *et al*., 2014). Over-proliferating leukocytes produce soaring reactive oxygen species (ROS), escorting knock down of the antioxidant defense of diseased animal (Nazifi *et al*., 2009; Saleh *et al*., 2012). It is well known that ROS are produced by several pathological conditions and cause cellular damages such as lipid peroxidation and protein oxidation (Sordillo and Aitken, 2009). The biological oxidative effects of free radicals on lipids and proteins are controlled by a spectrum of antioxidants (Halliwell, 2006). The antioxidant status can be described by the analysis of single components in the defense systems against ROS; by the determination of total antioxidant capacity (TAC). The detection of oxidative stress/ antioxidant status parameters are important biomarkers for the host-parasite interactions. Although there are several biochemical studies on theileriosis in cattle and buffaloes (Ali and Radwan, 2011; Razavi *et al*., 2012; Turunc and Askar 2012; Fartashvand *et al*., 2013), few reports are available regarding assessment of non-corpuscular markers of protein oxidation, lipid peroxidation and antioxidative status of newborn calves during natural clinical infections of *T. annulata*. Considering these facts, the present study aimed to determine serum protein carbonyls, malondialdehyde (MDA) and TAC contents of newborn calves with natural tropical theileriosis.

Materials and Methods

Animal selection and sample collection

Eight naturally *Theileria annulata* infected crossbred calves of either sex, aged between 15 to 60 days, presented for clinical examination at Teaching Veterinary Clinical Complex of the University, were included in the present study. All calves were reported to suffering from the disease since 07-15 days before examination and were not treated with any of the anti-hemoprotozoal drugs. Clinical examination of diseased

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calves was performed ardently. Blood smear and lymph nodes aspirates smear examination were conducted by the routine methods. Lymph nodes aspirate smear examination revealed presence of schizonts in mononuclear cells of all calves, while blood smear examinations revealed presence of piroplasms only in six calves. Another eight clinically healthy crossbred calves (healthy control) free from any hemoprotozoa and gastrointestinal parasites on blood smear and fecal sample examinations were also included.

Blood samples were obtained from all calves by the usual technique of collection from jugular vein aseptically with a sterilized disposable syringe and needle. Approximately 5 ml of blood was transferred into vials containing clot activator and serum was harvested. Immediately after harvest, serum samples were transferred into cryovials and kept at -20 °C until the use.

Estimations of oxidative stress markers

The status of oxidative stress; total antioxidant capacity, lipid peroxides and protein carbonyl contents of the diseased and healthy calves, were estimated in serum samples by using specific calorimetric kits (Sigma-Aldrich).

Total Antioxidant Capacity (TAC) assay

Total Antioxidant Capacity (TAC) was determined by using antioxidant assay kit (Sigma-Aldrich) as per the kit protocols suggested by the manufacturer. The total antioxidant assay was based on the principle of formation of a ferryl myoglobin radical from metmyoglobin and hydrogen peroxide, which oxidizes the ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) to produce a radical cation, $ABTS^{\cdot+}$, a soluble chromogen that is green in color and can be determined spectrophotometrically at 405 nm. Antioxidants suppress the production of the radical cation in a concentration dependent manner and the color intensity decreases proportionally. Trolox™ water-soluble vitamin E analog, served as a standard or control antioxidant. The results are expressed as mmol/L of Trolox equvalent.

Lipid Peroxidation (MDA) Assay

Lipid peroxidation was determined using lipid peroxidation (MDA) assay kit (Sigma-Aldrich) by the reaction of MDA with thiobarbituric acid (TBA) to form a colorimetric (532 nm) product, proportional to the MDA present. Serum MDA content was determined as per the kit protocols suggested by the manufacturer.

Protein Carbonyl Contents Assay

Carbonyls contents was determined using the protein carbonyl content assay kit (Sigma-Aldrich) by the derivatization of protein carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH) leading to the formation of stable dinitrophenyl (DNP) hydrazone adducts, which was detected spectrophotometrically at 375 nm, proportional to the carbonyls present. Serum protein carbonyls contents were determined as per the kit protocols suggested by the manufacturer. The results are expressed as nmol of carbonyl/mg of protein.

Statistical differences between the two groups were evaluated by using unpaired *t* test on GraphPad Prism 8. Data are presented as the mean ± standard error (± SE) and P values less than 0.05 were considered significant.

Results and Discussion

Clinical examination revealed various clinical manifestations including generalized lymph nodes enlargement, pallor mucous membrane, reduced appetite, pyrexia, pica, coughing and respiratory distress, lacrimation, exophthalmia, recumbency and sub-mandibular edema. The values of TAC, protein carbonyls, and MDA contents of calves with tropical theileriosis and healthy control are presented in Table 1. Total antioxidant capacity of calves with theileriosis was significantly ($P \leq 0.0001$) lower in comparison with healthy calves. Contrarily, serum MDA contents of diseased calves were remarkably higher ($P \leq 0.0012$) in comparison with healthy calves. Additionally, protein carbonyls content of diseased calves was also significantly higher ($P \leq 0.0001$) when compared with healthy calves.

Clinical manifestations recorded in the present study clearly signify that lymph nodes enlargement, pallor mucous membrane, reduced appetite, respiratory distress, marked rise in the body temperature and exophthalmia are among the common clinical manifestations revealed by naturally *T. annulata* infected calves (Branco *et al*., 2010; Fartashvand *et al*., 2013; Singh *et al*., 2014). In the present study, significantly lowered TAC levels were detected in

Table 1. Total antioxidant capacity (TAC), malondialdehyde (MDA) and protein carbonyls (PC) contents in serum of calves with tropical theileriosis and healthy control (mean ± SE).

Oxidative Stress Markers	Calves with Theileriosis	Healthy Calves
Total Antioxidant Capacity (mmol/L)	0.122 ± 0.002[A]	0.217 ± 0.004
Protein Carbonyls (nM carbonyl/mg protein)	14.9 ± 2.07[B]	9.77 ± 1.21
Malondialdehyde (nmol/µL)	7.67 ± 2.71[C]	3.72 ± 0.57

[A]-Values were significantly lower ($P \leq 0.0001$), when compared with healthy calves.
[B]-Values were significantly higher ($P \leq 0.0001$), when compared with healthy calves.
[C]-Values were significantly higher ($P \leq 0.0012$), when compared with healthy calves.

calves with theileriosis as compared to healthy calves. In adult cattle and buffaloes infected with *T. annulata,* the antioxidant levels of RBC decrease during the progression of anaemia (Nazifi *et al*., 2009; Saleh *et al*., 2012). Containments of host antioxidants defense system for instance compromised corpuscular antioxidant enzymes have been demonstrated by previous scientific report in *T. annulata* infection (El-Deeb and Iacob, 2012; Razavi *et al*., 2012; Turunç and Aşkar, 2012). In agreement to the findings of the current study, remarkably decreased level of TAC in *T. annulata* infected cattle and buffaloes has been reported by various workers (Guzel *et al*., 2008; Ali and Radwan, 2011; El-Deeb and Iacob, 2012). Therefore, the findings of the present study evidently suggest a possible containment of antioxidant/oxidant balance of newborn calves owing to the oxidative stress and ROS generation in the course of Tropical Theileriosis. This reduction in TAC might be attributed to reduction in the levels of enzymatic and non-enzymatic antioxidants, which are the component of antioxidant-defense systems, as they are consumed by excessive free radicals generated in diseased calves.

Furthermore, results of the present study showed significant negative correlations between TAC and MDA levels in calves with theileriosis. Reactive oxygen species (ROS) lead to both lipid and protein oxidation and liberates MDA and protein carbonyls respectively. In tropical theileriosis; high fever, inflammation, oxidative stress and cellular injury occur which lead to formation of compound structured aldehydes such as MDA (Turunç and Aşkar, 2012). Razavi *et al*. (2012) also reported increased level of MDA in cattle with theileriosis. In the present study, high MDA levels in calves with theileriosis could be mainly result of lipid peroxidation and oxidative damage of erythrocytes due to parasitaemia (Saleh *et al*., 2012). Additionally, over proliferation and clonal expansion of schizont hijacked macrophages (Woods *et al*., 2013; Metheni *et al*., 2014) might be auxiliary to soaring ROS, escorting elevated MDA production and knock down of the antioxidant defense of diseased calves (Nazifi *et al*., 2009; Saleh *et al*., 2012).

The results of the current study have also revealed remarkable elevation in serum protein carbonyls content of calves with tropical theileriosis which strongly suggests the involvement of overproduced ROS in patho-biology and progression of tropical theileriosis in calves. Till date, there was no scientific report on estimating non-corpuscular protein carbonyls contents and demonstrating oxidative damage marker of protein during the course of tropical theileriosis in calves. The results of the present study demonstrated an oxidative damage of protein in calves with tropical theileriosis for the first time.

In conclusion, containment of systemic antioxidant defense and remarkable oxidative injuries might be associated with the patho-biology and progression of tropical theileriosis in calves. Veterinary clinicians may consider the compromised antioxidant defense of calves and can recommend adjunct antioxidants with anti-theilerial medicaments for therapeutic management of tropical theileriosis.

Acknowledgement

The authors are highly thankful to the Vice Chancellor, DUVASU, for the facilities and financial assistance provided.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 64-66

Mineral and haemato-biochemical status in buffaloes manifesting leucoderma in the fluoride endemic South-West Punjab, India

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Abstract

The present study was aimed to evaluate essential mineral and haemato-biochemical status of the buffaloes manifesting leucoderma (n=7) in comparison to the healthy control (n=99) in the fluoride endemic South-West Punjab, India. Blood samples of the buffaloes were analysed for plasma fluoride, calcium, phosphorus, magnesium, copper, molybdenum, zinc, iron, manganese, arsenic, ceruloplasmin and alkaline phosphatase activity, urea nitrogen, creatinine, glucose, cholesterol, triglycerides, serum total proteins and albumin, and Hb, PCV, TEC. The Cu status of leucodermic buffaloes was poor as compared to the healthy control as reflected by non-significantly lower plasma Cu and significantly ($p<0.05$) lower plasma ceruloplasmin levels in leucodermic buffaloes. Plasma Mn levels were significantly ($p<0.05$) lower, while the plasma As levels were significantly ($p<0.05$) higher in the leucodermic buffaloes. The Hb, PCV and TEC values did not vary between normal and leucodermic buffaloes. Leucodermic buffaloes had significantly ($p<0.05$) lower plasma glucose, and higher plasma triglycerides levels as compared to apparently healthy buffaloes. It can be inferred from the present results that leucoderma in buffaloes probably was due to Cu deficiency in these animals.

Keywords: Buffaloes, Leucoderma, Fluoride, Minerals, Haemato-biochemical

Depigmentation of coat colour in ruminants is indicative of underlying nutritional deficiency particularly that of trace minerals viz. copper (Cu) and zinc (Zn) (Suttle, 2010) as these minerals play crucial part in melanin synthesis. In fluoride endemic areas, ruminants may suffer from Cu and Zn deficiency due to antagonistic effects of F on Cu and Zn metabolism. The present work was aimed to study essential minerals and haemato-biochemical status in buffaloes manifesting leucoderma in the fluoride endemic South-West Punjab, India.

Materials and Methods

The South-west region of Punjab is characterised by high environmental temperature, low rain fall, shorter fodder growing period and poor quality of groundwater having high fluoride (F) contents. A random survey was conducted in few villages (n=24) of the fluoride endemic South-West Punjab, India. The adult buffaloes manifesting leucoderma (n=7) were selected and for comparative analysis healthy buffaloes (n=99) were taken as control. Blood samples of the buffaloes were collected from jugular vein in heparin for plasma separation, without anticoagulant for serum harvesting and in EDTA for haemogram analysis. Fecal samples were collected and examined for parasitic ova/cyst by standard methods. Concentrations of plasma copper (Cu), molybdenum (Mo), zinc (Zn), iron (Fe) and manganese (Mn) were analysed by atomic absorption spectrophotometer (AAS, PerkinElmer A Analyst 700, USA). For this, the plasma samples were wet digested as per Kolmer *et al.* (1951). For digestion, 3 ml of plasma and equal volume of concentrated nitric acid (HNO_3 98% GR, Merck Specialties Pvt. Ltd., Mumbai) were mixed in the digestion flask, kept overnight at room temperature followed by digestion on low heat (70-80°C) using digestion bench until volume of the mixture reduced to 1 ml. To this, 3 ml of double acid mixture of concentrated HNO_3 and perchloric acid ($HClO_4$ 70% GR, Merck Specialties Pvt. Ltd., Mumbai) in 3:1 ratio was added and low heat digestion continued until the digested sample became watery clear and emitted white fumes. Final volume of the digestate was made up to 10 ml with double distilled water. Concentrations of arsenic (As) in the digested plasma were estimated on graphite furnace AAS using matrix modifiers pladinum (1%, Merck KGA, Germany) and magnesium nitrate (1%, Sigma Aldrich, USA) to control chemical interferences. Concentrations of calcium (Ca) and magnesium (Mg) in plasma were estimated on AAS without digestion of the samples as described by PerkinElmer (2000). For

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this, the plasma samples were diluted to 1:100 with 0.1 per cent (w/v) lanthanum chloride to control strong phosphate interference. Plasma fluoride was analysed by digital ion analyser (Orion 4 star bench top pH ISE meter, Thermo Scientific, Singapore) and fluoride electrodes (Thermo Scientific, USA). For the fluoride estimation, the plasma samples were diluted 1:1 with total ionic strength adjustment buffer II (TISAB II). Plasma inorganic phosphorous (Pi) was measured by method of Taussky and Shorr, (1953).

Activity of ceruloplasmin (Cp) in plasma was analysed by the method of Houchin, (1958). Total proteins and albumin in serum and plasma urea nitrogen, creatinine, glucose, cholesterol and triglycerides were analysed on Microlab 300 (Merck, Netherlands) using diagnostic kits (Merck Specialties Pvt. Ltd., Goa). Plasma alkaline phosphatase activity (ALP) was estimated on System Vitros DT6011 Chemistry (Ortho-Clinical diagnostics, Johnson and Johnson, USA). Red cell parameters (Hb, PCV, TEC) were estimated by Advia 2120 Haematology System (Siemens Medical Solutions Diagnostics, USA). Mean values for different parameters were calculated and compared between the leucoderma and control group by student's t-test using Statistical Package for Social Sciences (SPSS) for Window version 11.0.1© SPSS Inc. USA computer software program.

Results and Discussion

The Cu status of leucodermic buffaloes was poor as compared to the normal buffaloes as reflected by non-significantly lower plasma Cu and significantly ($p<0.05$) lower plasma Cp levels in the earlier group. In the leucodermic buffaloes, the plasma Cp levels varied from 2.20 to 7.00 $mgdl^{-1}$, which were considerably lower than the lower critical limit of 10.00 $mgdl^{-1}$ suggested by Gay *et al* (1988). Apart from poor Cu status, plasma Mn levels were significantly ($p<0.05$) lower, while the plasma As levels were significantly ($p<0.05$) higher in the leucodermic buffaloes as compared to the normal buffaloes. Similar to present findings, Singh *et al* (2003) and Randhawa *et al* (2006) had observed leucoderma in hypocupraemic dairy animals from Punjab. Reduced amino oxidase activity in Cu deficient animals, resulting in failure of conversion of tyrosine to melanin is the probable metabolic disturbance that leads to occurrence of leucoderma in animals (Underwood and Suttle 1999). However, Randhawa *et al* (2009) found no variation in plasma Cu, but he found significantly higher plasma Mo levels in the leucodermic buffaloes as compared to the control buffaloes. The significance of marginally elevated As levels in blood on ruminant health is not known, however, As had been associated with reducing hepatic and plasma Cu, and increasing Cu deficiency in rats (Uthus 2001).

The Hb, PCV and TEC values did not vary between normal and leucodermic buffaloes, which was in agreement with Randhawa *et al* (2009). The protein profile of leucodermic buffaloes as suggested by serum total proteins, albumin, globulin, and plasma urea nitrogen and creatinine levels was comparable to that of apparently healthy buffaloes, suggesting no role of

Table 1. Plasma mineral concentrations and ceruloplasmin activity of buffaloes manifesting leucoderma (Mean±SE).

Minerals	Apparently healthy (n = 99)	Leucoderma (n = 7)
Ca ($mgdl^{-1}$)	10.46 ± 0.22	9.64 ± 0.75
Pi ($mgdl^{-1}$)	5.56 ± 0.12	5.00 ± 0.59
Mg ($mgdl^{-1}$)	2.95 ± 0.09	2.67 ± 0.31
Cu (μgml^{-1})	0.66 ± 0.03	0.50 ± 0.08
Mo ($ngml^{-1}$)	114.95 ± 15.36	30.31 ± 6.20
Zn (μgml^{-1})	1.18 ± 0.07	1.06 ± 0.08
Fe (μgml^{-1})	3.74 ± 0.26	4.15 ± 1.47
Mn ($ngml^{-1}$)	66.42 ± 4.96	9.33 ± 9.17*
I (μgml^{-1})	1.38 ± 0.04	1.44 ± 0.17
F (μgml^{-1})	0.29 ± 0.02	0.27 ± 0.05
As ($ngml^{-1}$)	11.95 ± 2.07	42.87 ± 22.00*
Ceruloplasmin ($mgdl^{-1}$)	9.94 ± 0.60	5.16 ± 0.62*

*Significant at ($p<0.05$).

Table 2. Haemato-biochemical profile of buffaloes manifesting leucoderma (Mean±SE).

Parameter	Apparently healthy (n = 99)	Leucoderma (n = 7)
Hb (gdl^{-1})	10.08 ± 0.15	9.78 ± 0.73
PCV (%)	30.72 ± 0.49	32.10 ± 1.66
TEC ($x10^6 \mu l^{-1}$)	6.78 ± 0.13	6.33 ± 0.36
Total proteins (gdl^{-1})	7.48 ± 0.11	7.74 ± 0.48
Albumin (gdl^{-1})	3.33 ± 0.07	3.73 ± 0.31
Globulin (gdl^{-1})	4.15 ± 0.10	4.01 ± 0.34
A:G ratio	0.86 ± 0.03	0.98 ± 0.15
Urea nitrogen ($mgdl^{-1}$)	11.81 ± 0.47	10.91 ± 1.98
Creatinine ($mgdl^{-1}$)	1.32 ± 0.04	1.52 ± 0.09
Creatine kinase (uL^{-1})	181.32 ± 11.28	235.50 ± 66.50
Glucose ($mgdl^{-1}$)	70.98 ± 1.49	48.86 ± 7.45*
Cholesterol ($mgdl^{-1}$)	136.53 ± 3.72	145.00 ± 15.96
Triglycerides ($mgdl^{-1}$)	13.36 ± 1.12	23.60 ± 8.20*
ALP (uL^{-1})	130.26 ± 9.93	171.14 ± 41.27
AST (uL^{-1})	60.13 ± 2.31	64.40 ± 9.26
GGT (uL^{-1})	7.62 ± 0.37	8.40 ± 1.44
Total bilirubin ($mgdl^{-1}$)	0.47 ± 0.04	0.30 ± 0.06

*Significant at ($p<0.05$).

proteins in development of leucoderma in buffaloes. These results were in agreement with those reported by Randhawa *et al* (2009). Leucodermic buffaloes had significantly ($p<0.05$) lower plasma glucose, and higher plasma triglycerides levels as compared to apparently healthy buffaloes. Lower plasma glucose levels in leucodermic buffaloes could be due to reduced intakes of dietary carbohydrates that subsequently lead to release of stored triglycerides from the liver resulting in elevation of triglycerides in plasma. Plasma creatine kinase, ALP, AST and GGT levels did not vary between leucodermic and apparently healthy buffaloes, suggesting absence of internal tissue damage in the leucodermic buffaloes. However, Randhawa *et al* (2009) had observed significantly higher creatine kinase, ALP and AST values in the leucodermic buffaloes as compared to the healthy controls. It can be inferred from the present results that leucoderma in buffaloes probably was due to Cu deficiency in these animals.

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Assessment of cytokine profile in the peripheral blood mononuclear cells of dogs with generalized demodicosis

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Abstract

The objective of this study was the assessment of the cytokine profile in the dogs suffering from chronic generalized demodicosis.The mechanism of cytokines secretion from T-Lymphocytes plays an important role in the immune response of dogs suffering with parasitic skin infestations. Assessment of cytokine profile of*Demodex canis* infested dogs could augment understanding of pathology of canine demodectic mange. Twelve dogs (six diseased treatment group and six untreated control group) naturally infested with *Demodex canis*were included in the study. Another six clinically healthy dogs were kept as controls. Peripheral blood mononuclear cells were isolated from heparinized blood samples and used for extraction of m-RNA. Further,cDNA was synthesized from mRNA by reverse transcription. Relative levels of cytokine expression were compared with normalized glyceraldehyde-3- phosphate dehydrogenase (GAPDH) transcripts. The levels of transforming growth factor-beta (TGF-β) mRNA expression in dogs with demodectic mange was significantly higherwhereas, the expression of interleukin-2 and interleukin-5 also showed up-regulation than control (healthy) dogs but it was statistically non-significant. After treatment of demodectic dogs, the expression of IL-2 and IL-5 indicating progress of clinical signs. However, TGF-β was not found appropriate for monitoring the progress of the condition.

Keywords*:* Canine generalized demodicosis,Cytokine, Dogs, IL-2, IL-5, TGF-β.

Cutaneous ectoparasitosis is one of the important skin manifestations of dogs.Mange is a very common ectoparasitic infestation. It has two types viz. sarcorptic mange or canine scabies caused by *Sarcoptes scabei var. canis* and demodectic mange or canine demodicosis caused by *Demodex canis.* Canine demodicosis is a common, non-contagious, inflammatory parasitic dermatosis characterized by excessive proliferation of the commensal mite *Demodex canis*within the hair follicles and sebaceous glands (Shipstone, 2000). Demodicosis is a complex disease whose exact pathogenesis remains unclear. Immunosuppression is directly associated with the development of the disease (Gortel, 2006). The mechanism of cytokine secretion from T lymphocytes plays an important role in the immune response of the dog against parasitic skin infestations (Tani *et al.,* 2002; Yarim *et al.,* 2003; Singh and Dimri, 2014). Interleukin-2 (IL-2)plays a critical role in the regulating both cellular and humoral chronic inflammatory responses (Herrod, 1889). IL-2 mediates its effects by binding to the IL-2 receptor on T lymphocytes leads to cell proliferation, increased lymphokine secretion (lymphotoxin, IL-4, IL-3, IL-5 and GM-CSF) and enhanced expression of class II MHC molecules. It has a well-documented role in induction of pruritus (Fallahzadeh*et al.,* 2011). IL-5 and TGF-β are involved in the humoral inflammatory response. IL-5 is produced by T helper cells and mast cells. Its functions are to stimulate B cell growth and increase immunoglobulin secretion (Milburn *et al.,* 1993). Transforming growth factor β (TGF-β) is a protein that controls proliferation, cellular differentiation, and other function in most cells (Khalil, 1999).

Material and methods

Animal selection criteria and design of the study

The dogs enrolled in the study were recruited among the patients presented for clinical and dermatological examination. Twelve dogs naturally infested with *D. canis*were included in the study. Demodicosis in dogs was diagnosed by deep skin scraping examination (DSS), trichography/ hair plucking (HP) microscopy and exudates (E) microscopy. None of the dog was treated with ectoparasiticides or steroidal anti-inflammatory drugs in the last 30 days before obtaining blood samples. The diseased dogs were also free from mites. Another six dogs clinically healthy and free of mites on microscopic examination were kept as healthy controls. Approximately 5ml

blood sample was obtained from each dog in heparin and used for isolation of peripheral blood mononuclear cells (PBMC).

Extraction of mRNA from PBMC and cDNA synthesis

PBMC isolated from pooled heparinized blood collected from dogs was layered on Histopaque (Sigma, USA) and centrifuged for 15 minutes at 1500 × g. The mononuclear cell fraction was washed twice in PBS at 1000 × g. These cells were adjusted to 1×10^6 cells and were pelleted (4000×g for 10 minutes). Total mRNA was extracted from PBMC as per the manufacturer's recommendation using TRI Reagent®. The samples were treated with DNase toremove possible DNA contamination. Synthesis of cDNA was performed by using 3 µg of mRNA as per the manufacturer's recommendation (Verso™ Reverse Transcriptase Kit).

Reverse Transcription Cycling Program

	Temp.	Time	Number of cycles
cDNA Synthesis	42°C	30 min	1 cycle
Inactivation	95°C	2 min	1 cycle

Oligonucleotide primers

The following primers of IL-2, IL-5, TGF-β and housekeeping protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene were used as reported by Tani *et al.* (2002):

Sr. No.	Genes	Primer Sequence (5'-3')	Amplicon size (bp)
1	GAPDH	AACATCATCCCTGCTTCCAC TGCCTGCTTCACTACCTTCTT	183
2	IL-2	GTGCGCCTATTACTTCAAGCTC GCTGTCTCGTTCATCATATTC	344
3	IL-5	TTGCTAGCTCTTGGGGCTGCCT TCCCCGTGGGCAGTTTGGTT	221
4	TGF-β	TTCCTGCTCCTCATGGCCAC GCAGGAGCGCACGATCATGT	393

Real Time Quantitative Polymerase Chain Reaction

Expression of cytokines mRNA was quantified by Real Time PCR and analysed using Applied Biosystems 7500 SDS software. All PCR reactions were performed in optical 96 well plates. The amplification was carried out in a final reaction volume of 25µl containing 1X Maxima SYBR Green PCR master mix, 10 pmol of each gene specific primer and 3µl of cDNA template. The PCR protocol design for 40 cycles for cytokine expression in dog pre and post treatment. For each gene of interest, negative and positive controls were used. Untreated demodectic dog PBMCs reverse transcribed RNA was used as positive control. Negative control were the samples in which cDNA was not added. To confirm the targeted PCR amplification, initially 5 µl of PCR product or each amplified target tube was mixed with 1 µl of 6X gel loading buffer and electrophoresed along with 50 bp DNA molecular weight marker (GeneRuler, MBI Fermentas) on 2.0 % agarose gel containing ethidium bromide (at the rate of 0.5 µg/ml) at constant 80 V for 30 minutes in 0.5X TBE buffer. The amplified product was visualized as asingle compact band of expected size under UV light and documented by gel documentation system (SynGene, Gene Genius Bio Imaging System, UK). Cytokine quantification was achieved using the Ct (Cycle threshold) comparative method and was expressed as "n-fold up regulation of cytokine transcription" in relation to calibrator which is represented by the smallest signal detectable for that specific cytokine. The expression of each gene was analyzed using the relative quantification method described by Pfaffl (2001).

Statistical analysis

Statistical analysis was performed using SPSS software to determine the Pearson's correlation analysis of cytokine expression before and after treatment in demodectic dogs. The results were presented as mean ± standard error (S.E.).

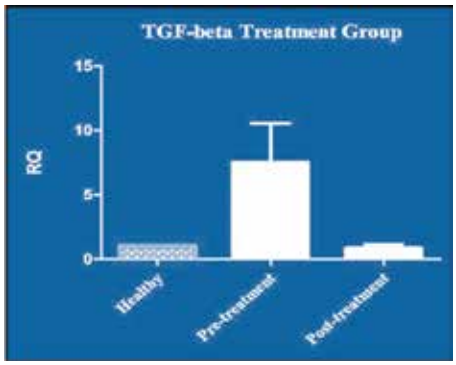
Results and Discussion

In the present study, TGF-β mRNA levels were found to be statistically up regulated (9.33 ± 3.80 folds, $p< 0.05$) in demodectic dogs as compared to healthy dogs. Post- treatment, the expression of the gene was found to be significantly lower (0.76± 0.33 folds, $p<0.05$) as compared to pre-treatment diseased dogs. The expression of TGF- β mRNA in the dogs was found to be significantly higher (12.04 ± 5.38 folds, $p<0.05$) on day 21 than on day 0 in untreated control group. Comparative evaluation of the expression of TGF- β gene showed a significant down regulation in the demodectic dogs post-treatment, whereas there was a significant up regulation in the untreated control group dogs at day 21 of sample collection. IL-5 mRNA expressions were found up-regulated (2.09 ± 0.85 folds) in dogs with demodicosis in treatment group as compared to healthy dogs. Post- treatment, gene

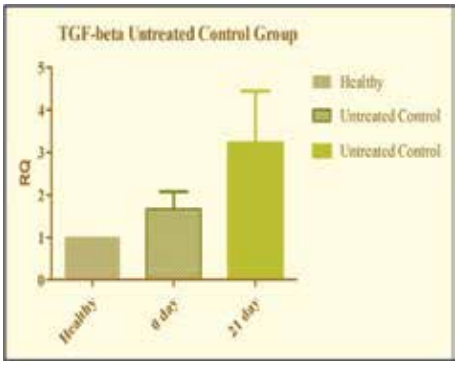
expression was found to be down regulated (0.71±0.31 folds) in treatment group as compared to pre-treatment diseased dogs. The IL-5 mRNA expression showed no significant difference between the two groups. The gene expression of untreated control group was significantly up-regulated (3.28 ± 1.68 folds, $p<0.05$) on day 21 than on day 0. The expression of IL-2 mRNA levels was found to be up-regulated (2.82 ± 1.26 folds) in dogs with demodicosis as compared to healthy dogs. In post-treatment group the gene expression of the IL-2 mRNA levels was found to be lower (0.61 ± 0.27 folds) as compared to pre-treatment. The IL-2 mRNA expression showed no significant difference between the above two groups.

The present study demonstrates mRNA expression of cytokine in PBMC of dogs directly *ex vivo*without *in vitro* stimulation. The mechanism of cytokine secretion from T lymphocytes plays important role in immune response of the host against the mange mites (Walton, 2010; Singh and Dimri, 2014). T helper 1 (Th1) and T helper 2 (Th2) cells seem to moderate virtually all the known patterns of the immune response. Th1 cells are hypothesized to lead the attack against intracellular pathogens such as viruses, raise the classic delayed –type hypersensitivity (DTH) skin response to viral and bacterial antigens and fight cancer cells.Th1 cells produce IL-2 and interferon gamma (Cher and Mosmann, 1987), whereas Th2 cells produce IL-4, IL-5 and IL-10 (Cherwinski *et al.,* 1990). Th2 cells are believed to provide protection against extracellular pathogens such as multicellular parasites (Kidd, 2003).Transforming growth factor (TGF)-β is an anti-inflammatory cytokine designated as Th3, which suppresses the growth of Th1 and Th2 and also B cells (Tani *et al.,* 2002).

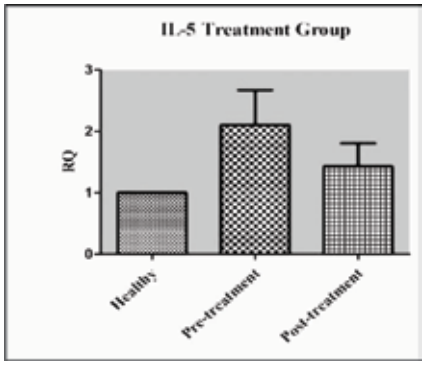
The results of the present study demonstrated regulated expression of TGF-β in dogs with demodectic mange. TGF-β acts as a potent immunosuppressor byregulating the proliferation and survival of many cells ofthe immune system.The TGF-β familyis part of a superfamily of proteins known as the transforming growth factor β superfamily. Increased TGF-β mRNA



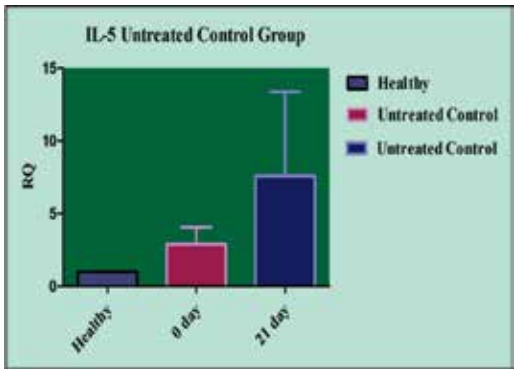
Expression of TGF-β mRNA in Demodectic Dogs (treatment group) and Healthy Dogs



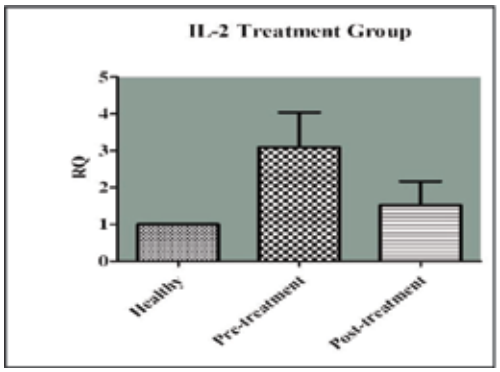
Expression of TGF-β mRNA in Demodectic Dogs (untreated control group) and Healthy Dogs



Expression of IL-5 mRNA in Demodectic Dogs (treatment group) and Healthy Dogs



Expression of IL-5 mRNA in Demodectic Dogs (untreated control group) and Healthy Dogs



Expression of IL-2 mRNA in Demodectic Dogs (treatment group) and Healthy Dogs

may be associated with the immunosuppression seen in generalized demodicosis (Barriga*et al.,* 1992). It is also possible that TGF-β might be secreted to protect cytotoxic immunity, such as CD8+ cells. TGF-β inhibits T cell and NK cell proliferation and activation and may play an important role in inflammation. Sporn*et al.* (1986) and Tani*et al.* (2002) demonstrated that TGF-β showed regular variation in clinical signs and suggested that the response does not always reflect the clinical signs. Nuttall *et al.* (2002) reported that the TGF-β mRNA expression was lower in diseased dogs as compared to healthy ones. Therefore, the role of TGF-β needs to be further investigated.

Interleukin -2 (IL-2) is an interleukin, a type of cytokine signaling molecule in the immune system. Tani *et al.* (2002) reportedlower expression of IL-2 mRNA expression compared to healthy dogs. Lemarie and Horohov (1996) suggested that the Th1 response is decreased in the dogs with generalized demodicosis due to decreased IL-2 and IL-2 receptor expression, even at an early stage of the disease when the Th1 response might have decreased. The Th1 response may decrease in localized demodicosis dogs and possibly in those with generalized demodicosis, due to deceased production ofIFN-γrather than the IL-2 *in vivo* status (Tani *et al.,* 2002).

IL-5 is an interleukin produced by T helper -2 cells and mast cells. It is also a key mediator in eosinophil activation (Yokota *et al.*, 1988). The results indicated that the expression of IL-5 gene is induced by *Demodex* mites. It stimulates and activates eosinophils. Eosinophilia is a common feature in demodicosis of canines (Ballari*et al.,* 2009; Singh *et al.,* 2011). The findings are in agreement with those earlier reported by Tani *et al.* (2002) who reported that the expression of IL-5 mRNA was up-regulated in diseased group as compared to healthy and post-treatment dogs.

Conclusions

The expression of IL-2, IL-5 and TGF-β revealed that though IL-2 and IL-5 are used for the expression of the gene and progress of the clinical signs, TGF-β is not suitable for monitoring the progress of the canine demodicosis. Thus, IL-2 and IL-5 gene expression are most suitable for monitoring canine demodicosis.

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Prevalence and risk factors associated with seropositivity to bluetongue in small ruminants of Punjab

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Abstract

The present study was conducted to ascertain the seroprevalence of bluetongue in sheep and goat and identify risk factors associated with seropositivity. A total of 368 samples from sheep and goat were collected from 37 flocks belonging to 3 agro-climatic zones of Punjab and subjected to commercial cELISA kit (VMRD) for detection of anti-bluetongue antibodies. The epidemiological history of animals was collected and association of various risk factors such as species, sex, feeding pattern, stage of lactation, age and geographical location with seropositivity was determined. The overall apparent prevalence of bluetongue disease was found to be 52.99%. Species wise, seroprevalence was found to be higher in sheep (58.56%) as compared to goats (50.58%), though difference was not statistically significant. The prevalence of disease was found to vary significantly (γ^2= 5.465, $p<0.05$) with respect to sex of animals (male-39.34% vs. female-55.75%). No difference in prevalence rates of flocks was observed with respect to feeding pattern, age of animals or stage of lactation in female animals of both species. The seroprevalence of bluetongue in central plain region (64.39%) was significantly higher as compared to submountainous zone (51.85%) and southwestern region (45.05%). There has been absence of clinical reports on bluetongue in Punjab though very high seroprevalence has been observed. The possible reasons such as prevalent serotypes of BTV and *Culicoides* spp. of the region need to be identified so that appropriate control and preventive measures may be suggested.

Keywords: Bluetongue, ELISA, Prevalence, Risk factor, Small ruminants.

Small ruminants play an important role in enhancing income generation, capital storage, employment and improving household nutrition. These species are traditionally reared by small and marginal farmers and landless laborers under extensive range management with top feed supplementation during lean season. Infectious diseases pose a significant threat to rearing of small ruminants, besides bluetongue is reported to be a major disease particularly of sheep. It is an infectious, non-contagious, arthropod borne, viral disease caused by bluetongue virus (BTV), a type specific virus of the genus *Orbivirus* of family *Reoviridae*. Bluetongue typically occurs when susceptible animal species are introduced into areas with circulating virulent BTV strains, or when virulent BTV strains extend their range to previously unexposed populations of ruminants [1].

The worldwide economic losses due to bluetongue has been estimated as $3 billion annually due to death, abortions, weight loss, reduced milk yield, meat efficiency and export restrictions for live animals, their semen and some products such as fetal bovine serum [2]. Competitive enzyme linked immuno sorbent assay (c-ELISA) is prescribed test for International Trade in the OIE Manual of Standards for Diagnostic Test and Vaccines [3]. Therefore, in the present study, it was decided to employ cELISA to establish seroprevalence of bluetongue and understand associated risk factors.

Materials and Methods

Punjab is north-western state of India situated between the 29.30°N to 32.32°N latitude and 73.55°E to 76.50°E longitude with Pakistan on the western border. Approximately 70% of human population lives in villages and agriculture is their main occupation. The statistical calculation of samples size has been carried out using standard methods [4]. A computerized list of the villages of the state was used as sampling frame and 37 flocks from 31 villages were selected from the whole of the state using simple random sampling, *without replacement* and *without stratification* with 'Random Village' programme of Survey toolbox [5]. Approximately 10% of samples were collected from each flock. Blood samples (5ml) were collected from the jugular vein in serum separation vials; the sera were separated and stored at -20°C until they were screened for antibodies to bluetongue virus. The serum samples were analyzed with commercial cELISA kits

(Bluetongue virus antibody test kit, VMRD) with technique as described by manufacturer. Finally, optical density was measured at 630nm in iMark Microplate Reader (BIORAD) and test samples were considered positive or negative if optical density was less or more than 50% of the mean of the negative controls, respectively.

A standardized questionnaire for animals sampled at the farms was filled at the time of blood collection. The presence or absence of disease was statistically correlated using appropriate statistical tools, with various risk factors, such as species, age, sex, stage of lactation, history of mastitis, arthritis and conjunctivitis, geographical location and feeding pattern i.e. stall fed/ grazing. True prevalence was calculated at 95% confidence interval (CI) using the 'True prevalence' program of survey toolbox, in which sensitivity, specificity and sample size were taken into consideration. The data was analyzed using SPSS (Statistical Package for Social Sciences) for Window version 11.0.1©SPSS Inc. USA computer software program. The associations were evaluated between binary outcome variable and a variety of risk factors such as species, age, sex, and feeding patterns.

Results and Discussion

Out of 368 samples screened for antibodies to bluetongue virus using cELISA, overall apparent prevalence of disease was estimated to be 52.99%. With sensitivity and specificity of bluetongue virus antibody test cELISA kit reported as 100% and 99%, true prevalence was calculated to be 52.51%. Seroprevalence was found to be higher in sheep (58.56%) as compared to goats (50.58%), though difference was not statistically significant (Table 1). In present study, 39.3% of males were positive, while 55.75% in the females with risk of detection of antibodies found to be almost twice higher in females. There was no difference in prevalence between grazing (52.74%) and stall-fed (55.00%) animals (γ^2=0.073, p>0.05) and there was no significant different with respect to stage of lactation and age. The seroprevalence of bluetongue was found to be

Table 1: Risk factor analysis of serpositivity to bluetongue in Punjab

Risk factors		Positive	Total	Prevalence (%)	Statistical analysis	Odds ratio	Relative risk
Species	Goat	130	257	50.58%	1.979 (p>0.05)[a] 1.974 (p>0.05)[b] 1.672 (p>0.05)[c]	0.724 (95% CI 0.450-1.161)	0.864 (95% CI 0.710-1.075)
	Sheep	65	111	58.56%			
Sex	Female	158	307	51.46%	1.725 (p>0.05)[a] 1.721 (p>0.05)[b] 1.376 (p>0.05)[c]	0.688 (95% CI 0.378-1.247)	0.848 (95% CI 0.684-1.114)
	Male	37	61	60.65%			
Feeding pattern	Grazing	173	328	52.74%	0.073 (p>0.05)[a] 0.073 (p>0.05)[b] 0.010(P>0.05)[c]	0.913 (95% CI 0.449- 1.851)	0.959 (95% CI 0.730-1.386)
	Stall fed	22	40	55.00%			
Stage of lactation	Not lactating	46	89	51.68%	5.793 (p>0.05)[a]	-	-
	<1.5 month	42	66	63.64%			
	1.5-3 months	29	65	44.61%			
	≥3 months	41	87	47.13%			
Age	< 1 year	15	22	68.18%	6.95 (p>0.05)[a]	-	-
	1-3 years	61	102	59.80%			
	3-5 years	88	188	46.81%			
	≥5 years	31	56	55.36%			
Agroclimatic zones	Submountainous	28	54	51.85%	11.52 (p<0.01)	-	-
	Central plain	85	132	64.39%			
	South western	82	182	45.05%			

[a]Chi square uncorrected; [b]Chi square Mantel-Haenszel; [c]Chi square-Yates corrected

significantly (γ^2= 11.52, p<0.01) higher in central plain region as compared to other two zones of the region.

The overall apparent seroprevalence of bluetongue was 52.99% in the present study was found to be quite high compared to previous reports [6] as 6.64% in sheep were positive for BTV antibodies by the immunodiffusion test. The higher prevalence in present study might be due to application of more sensitive cELISA technique in the present study or the infection might have built up during these 35 years. However, another serological study in north southern and central regions of West Bengal reported prevalence in range from 40 to 80% [7].

In present study, 39.3% of males were found to be positive in comparison to 55.75% females quite higher than in a study [8] reporting an overall seroprevalence of 31.8% in females as compared to 26.4% in males. There was no significant difference in prevalence based on feeding pattern, stage of lactation and age, though the seroprevalence was significantly (γ^2= 11.52, p<0.01) higher in central plain regions as reported by Tigga *et al.* [9].

The distribution and intensity of infection in different regions as determined by the climate, geography and altitude might affect the occurrence and activity of the *Culicoides spp.* vectors [10]. The climate is a major risk factor as *Culicoides* require warmth and moisture for breeding and calm, warm humid weather for feeding. A cold winter or a dry summer can markedly reduce vector numbers and risk for the disease as temperature above a mean of 12.5ºC for the cooler months and temperatures in the range of 18 to 30ºC in the summer and autumn are optimum for activity of *Culicoides spp.*

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 75-77

Short Communications

Comparative efficacy of single and three days injection protocols of marbofloxacin in treatment of mastitis

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Abstract

Mastitis is a common problem of dairy cows and different treatment protocols have been tried. Sensitivity to commonly used antimicrobials has reduced with time. The present study was designed to evaluate the therapeutic efficacy of two different protocols of marbofloxacin use in mastitis i.e. single dose of injection marbofloxacin (Marbomet*) @ 8 mg /Kg body weight intra muscular and three successive injections of marbofloxacin (Marbomet) at a dose rate of 2 mg/kg body weight intra muscular. Highest recovery was recorded in cows given injections of marbofloxacin for 3 consecutive days i.e. group II (88.89 %) in comparison to 77.78% in group I animals who received single injection of marbofloxacin.

Keywords: Cows, Mastitis, Marbofloxacin

Mastitis, a disease complex, is a persistent enigmatic problem affecting the dairy herds. Early and specific use of antimicrobials significantly limits the severity of the disease and in many cases prevent the appearance of any visible signs of infection. The goals of therapy include the return of the cow to normal milk production and composition, prevention of mortality in per acute cases, elimination of infectious microorganisms and diminution of somatic cell counts and reduction of contamination of other cows. The present study was planned to study the therapeutic efficacy of marbofloxacin in management of mastitis. Marbofloxacin (Marbomet*)exhibits concentration-dependent bactericidal activity against gram-positive and gram-negative bacteria (Aliabadi and Lees, 2002). Marbofloxacin is used in the treatment of bacterial infections in animals (Kroemer *et al.*, 2012 and Lee *et al.*, 2011). It is recommended in two different dosage forms i.e. single injection or 3 consecutive injections. The present study was designed to study the comparative efficacy of different dosages protocols of marbofloxacin in treatment of mastitis.

Present study was conducted on 18 cows showing acute mastitis immediately after calving. Udder, individual teats and quarters were examined immediately after milking for presence of any swelling, hardness and pain. Severely inflamed, reddish, hot to touch and painful udder were considered acute. Milk from affected quarters was discolored, watery in consistency, with or without flakes or curdled. Milk was collected aseptically from all quarters for physical examination as well as for battery of tests namely California mastitis test and Somatic cell count using standard protocols. The animals were subdivided in two treatment groups of 9 animals each. Group I animals were administered single dose of injection Marbofloxacin @ 8 mg /Kg body weight intra muscular and animals of group II were given three successive injections of Marbofloxacin at a dose rate of 2 mg/kg body weight intra muscular

Supportive treatment included Inj. Meloxicam (Melonex*) @ 10 ml IM and Inj. Chlorpheniramine maleate (Anistamin*) @ 10 ml intramuscularly for 3 days. The owner was also advised to perform completed frequent milking at every 8 hours. The recovery was assessed based on changes in milk and udder and screening tests - California mastitis test (CMT) and somatic cell count (SCC). Observations were recorded on day 3, day 5 and day 7.

Highest recovery was recorded in cows given injections of marbofloxacin for 3 consecutive days i.e. group II (88.89 %) in comparison to 77.78% in group I animals who received single injection of marbofloxacin. In group II maximum recovery was recorded on day 3 (77.78 %) followed by11.11% on day 5. One cow (11.11%) remained affected although improvement was noticed. In group I maximum recovery was also reported on day 3 (55.56 %) but the percent recovery was less than day 3 results of group I. Recovery percent on day 5 and 7 in group I was (11.11%) and (11.11%) respectively. Two animals (22.22 %) could not be recovered.

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Table 1: Screening test result pre and post therapy

Group	Scores	CMT score point		SCC(10^4 cells / ml)	
		Pre treatment	Post treatment	Pre treatment	Post treatment
I	+1	-	2	193.27 ± 15.70	7.95** ± 0.54
	+2	5	1		
	+3	3	-		
	+4	1	-		
II	+1	-	1	171.32 ± 13.35	4.31** ± 0.12
	+2	6	1		
	+3	2	-		
	+4	1	-		

**Highly Significant

The mean pre treatment milk SCC of cows under group I and II were 193.27 ± 15.70 x 10^4 / ml and 171.32 ± 13.35 x 10^4 / ml respectively. The post treatment values of SCC in group I and II reduced significantly to 7.95 ± 0.54 x 10^4 / ml and 4.31 ± 0.12 x 10^4 / ml when compared to the pre treatment values. The post treatment values of group II were more towards normal range. The CMT score pre and post treatment values in cows with mastitis are given in Table 1. The reduction in the scores was more pronounced in group II followed by group I cows following therapy. CMT scores in mastitis affected cows varied from +1 to +4 in various treatment groups of cows (Table 1). After treatment with antibiotic in most of the cases, milk was restored to normalcy in cows. However, 2 cows in group II and 3 cows in group I were still reacting to CMT with +1 or +2 score. These observations are indicative of slow return of biochemical changes in subclinical mastitis affected milk.

The results are in accordance with the study of Grandemange and Davot (2002) who concluded that 3-day marbofloxacin treatment at the dosage of 2 mg/kg/day (intramuscular injections) was shown to be statistically more efficient than a 3-day systemic administration of amoxicillin-clavulanic acid. The efficacy of daily dosage of 2 mg/ kg with Marbocyl® (marbofloxacin)10% for the treatment of acute clinical mastitis was already demonstrated in vitro (Schneide *et al., 2004*) and in vivo after experimental (Heurtin, 2004) or natural infection (Grandemange, 2002). Rajnikanth (2016) studied the effect of morbofloxacin in 18 Holstein Friesian cross bred cows, affected with clinical mastitis and observed clinical improvement in 16 animals within 48 hr and two animals within 72 hr. Normal milk production and complete recovery were observed in all the cases. Following the introduction of new concepts for the use of fluoroquinolones at higher doses for reduced treatment duration and reduction of the risk of resistance selection among pathogenic bacteria (Zhao and Drlica, 2008), Single Injection Short Acting Anti Biotic [SISAAB] protocols have been developed for the treatment of acute BRD and mastitis with marbofloxacin in cattle (Grandemange *et al.*, 2012a; Grandemange *et al.,* 2012b and Valle *et al.,* 2012). Grandemange (2017) observed 73.6% cure rates in animals administered single injection of marbofloxacin (10 mg/kg, intramuscular (IM).

Highest recovery was recorded in cows given injections of marbofloxacin for 3 consecutive in comparison group I animals who received single injection of marbofloxacin. Thus three day protocol can be used in field for better results.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 78-81

Fatal traumatic peritonitis in sheep: A report of three unusual cases

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Abstract

Peritonitis especially traumatic peritonitis is common in cattle and buffaloes but rare in sheep and goats. The present report describes a case series of unfamiliar traumatic peritonitis in sheep presented to Veterinary Clinical Services Complex, Faculty of Veterinary Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), over a period of two years. The sheep were presented individually but with a similar history of fighting with other sheep, hematuria or blood in faeces and fever followed by loss of defecation. Clinical and laboratory examinations were suggestive of toxemia. None of the animals responded to treatment. Necropsy revealed generalized adhesive peritonitis due to intestinal rupture. The most probable cause of intestinal rupture was trauma due to fighting. This case series widens the horizon of gastrointestinal disorders in sheep.

Keywords: Sheep, Peritonitis, Intestinal rupture, Trauma

Affections of the ruminant forestomach especially due to peritonitis are the subject of attention almost all over the world and of major economic importance due to severe loss of production. Peritonitis resulting from perforation of the reticular wall by a sharp foreign material causes a fatal digestive disease. It may result in local or diffuse peritonitis and foreign bodies may also penetrate into the thoracic cavity and the adjacent abdominal anatomic structures including the liver and spleen. Although peritonitis is reportedly common in cattle, its occurrence has rarely been documented in pigs, sheep and goats (Radostits *et al.,* 2007; Jones and Smith 2008). Perusal of available literature did not show any report on this condition in sheep from Kashmir. Although, many veterinarians assume that the occurrence of peritonitis is rare in small ruminants, we describe three unusual occurrences in sheep in the present report.

Within a two year period, three female sheep from different herds were referred to the Veterinary Clinical Services Complex, Faculty of Veterinary Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, for treatment. The history of the cases according to the owner declaration was as follows:

Case 1: A three-year-old, non pregnant ewe, weighing 27 kg, with a history of eight days of sudden anorexia, fever, reluctance to move and rise, hematuria, decreased fecal output with mild abdominal distention and then loss of defecation. One herd mate had died of hemonchosis three days back and owner suspected same for the current sheep. The animal had been treated for hemonchosis and cystitis, at the farm level. There was no response to the on farm treatment. On further inquiring, the owner revealed that the ewe showed first signs of illness a day after she had fight with a ram in the same herd.

Case 2: A four-year-old, non-pregnant ewe, weighing 33 kg, with a twenty-day history of anorexia, fever, hemorrhagic loose faeces, reluctance to move and mild abdominal distention followed by complete loss of defecation and recumbency. This sheep also had a history of fight with other sheep before the illness. The owner had treated the sheep for hemorrhagic enteritis without any favorable outcome.

C**ase 3**: A three-year-old ewe, weighing 25 kg, showing symptoms of anorexia, fever, bruxism and reluctance to move, hematuria and loss of defecation, during ten days. Like the first two cases, this ewe also had a history of fight with other sheep before the illness. The on farm treatment without any significant clinical improvement was for Babesiosis and hypophosphatemia. The sheep became recumbent and was referred to the University Referral Hospital.

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Clinical and Hematology Findings, and Treatment

The animals were depressed, dehydrated and recumbent (except case 1) with mild to moderate abdominal distension. The mucous membranes were pale (Figure 1), rumen was atonic and rectal temperature was >104°F. Heart rates were variable (86, 68 and 78 beats per minute, respectively). Simultaneous auscultation and percussion of ventral abdomen on right side revealed fluid filled intestines in case 1 and 3. Abdominocentesis revealed yellow colored turbid peritoneal fluid with fibrin clots, in case 3 (Figure 2). Hematological examination invariably revealed anemia, neutrophilia and left shift. Blood smear was negative for any hemoprotozoa.

Case 1 died before any treatment. On the basis of clinical evaluation, diagnosis could not be established in case 2 and was treated symptomatically with intravenous fluids, antibiotics and analgesic. This sheep died on next day without any clinical response to the treatment. Case 3 was treated for peritonitis (with antibiotics, hematinics and other supportive care) for 3 days without any clinical improvement.

On postmortem examination, the abdominal cavity contained yellowish turbid peritoneal fluid with fibrin clots, adhesive peritonitis and intestinal rupture in all three sheep. The margins of the ruptured intestinal area were necrotic (Figure 3). The reticulo-rumen was adhered ventrally or laterally but there was no evidence of traumatic reticuloperitonitis (Figure 4). The intestinal loops were adhered together or were encapsulated in fibrinous clots. Urinary bladder showed no apparent abnormality while kidney had a few ecchymosed hemorrhages. In all the three sheep, abomasums were free of haemonchus worms but had few Type 1a ulcers in case 2 and 3 (Figure 5). Thoracic findings were insignificant except froth in trachea and a few epicardial and/or endocardial hemorrhages.

These findings supported the diagnosis of unusual traumatic peritonitis, as no other cause of peritonitis (other than intestinal rupture) could be established even on necropsy. Accordingly, death most likely occurred due to chronic peritonitis in all the three ewes.

Peritonitis of specific cause is uncommon in sheep. An unusual case series of peritonitis in sheep is reported herein. To the authors best knowledge there is no report of peritonitis due to traumatic intestinal rupture in sheep. Cattle commonly ingest foreign objects because they do not discriminate against metal materials in feed and do not completely masticate feed before swallowing (Braun *et al.,* 2002). There are few case reports about traumatic reticuloperitonitis and its complications in sheep and goats (Akkoc 2007; Torki *et al.,* 2011). In this short communication, we determined peritonitis by necropsy in two sheep and by clinical and peritoneal fluid examinations in one sheep. Abdominocentesis was performed in case 3 only because at first instance we did not suspect peritonitis in first two cases. It was only after the experience from first two cases that we performed abdominocentesis in case 3 and were able to diagnose and establish peritonitis in this case. History/observations of the owners, clinical

Fig. 1: Pale conjunctiva and sunken eye balls in a sheep with fatal peritonitis

Fig. 2: Yellow colored turbid peritoneal fluid with fibrin clots, obtained by abdominocentesis from Case 3

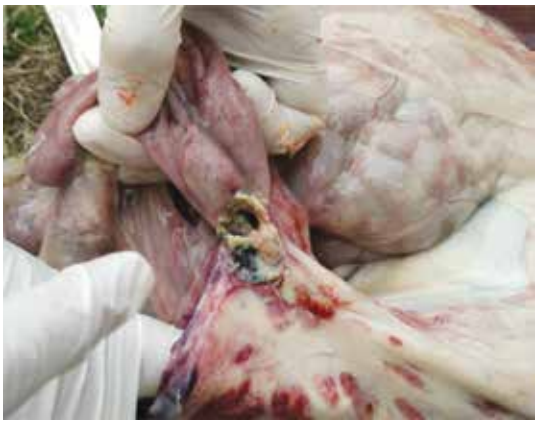

Fig. 3: Intestinal rupture with necrotic margins, on necropsy of a sheep with fatal peritonitis

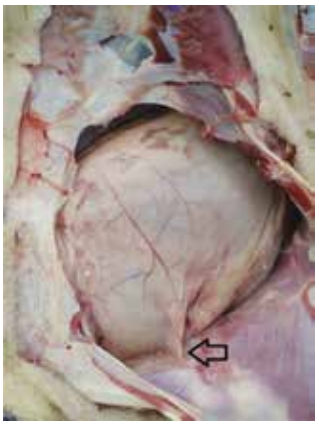

Fig. 4: Adhesions of reticulo-rumen with ventro-lateral abdominal wall (arrow), on necropsy of a sheep with fatal peritonitis

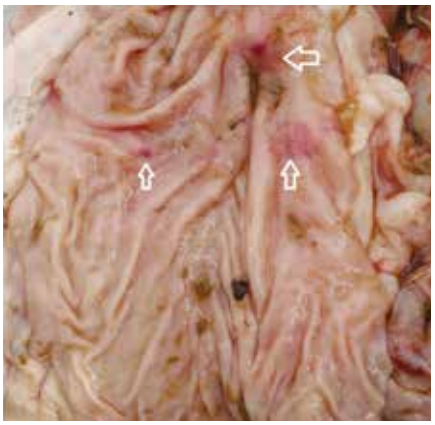

Fig. 5: Type 1ulcers (White arrows) in abomasum, on necropsy of a sheep with fatal peritonitis

signs and the postmortem observations were similar in all the three sheep. Diseases with similar signs such as ruminal atony, hematuria, fever and abdominal discomfort should be the important differential diseases.

Incidences of traumatic reticuloperitonitis are low in sheep because ingestion of sharp non-food items such as nails and needles is extremely rare (Radostits *et al.,* 2007; Jones and Smith 2008). From India, there seems to be a single report of traumatic reticuloperitonitis in sheep, due to wooden stick (Thilakan *et al.,* 2002). But the present cases were of unfamiliar traumatic origin. Sheep commonly graze on pasture in our area. Contaminated pasture with sharp materials and pica in the sheep seemed unlikely as no foreign body or lesion due to a foreign body could be observed in the abdominal cavity on necropsy. Foreign bodies commonly encountered in grazing sheep of this area are plastic objects (unpublished observation). So, we assume that the most probable cause for peritonitis in this case series was intestinal trauma as all the affected sheep had history of fighting with other animals, in the recent past. The trauma could have resulted in ischemic necrosis of intestinal wall followed by intestinal rupture or direct rupture. In ruminants, peritonitis is usually localized but in these sheep, it was generalized and hence peritonitis proved fatal.

The authors suggest that these cases are of special significance because the intestines are lined by shock proof peritoneum and peritonitis of this nature is extremely rare, even in cattle and buffaloes. Practitioners interested in small ruminants should be aware of this condition while investigating gastrointestinal problems.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 82-84

Arginine status in healthy dogs

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Abstract

A total 7 dogsof different age, breed and sex presented for a yearly vaccination at Teaching Veterinary Clinical Complex, Bombay Veterinary College, Parel were selected for the study on the basis of normal history and physical examination in combination with testing by CBC and serum biochemistry profile. Quantitative Plasma Amino acid Analysis was performed by High Performance Liquid Chromatography (HPLC) technique to estimate the levels of free amino acidarginine.

Keywords: canine, healthy dogs, plasma free arginine, HPLC

The amino acid Arginine was first isolated from lupin seedlings in 1886 (Schulze &Steiger, 1886). It was identified as an animal protein component in 1985(Hedin, 1895).The amino acid "Arginine" plays a variety of roles in many different cell types. In addition to serving as substrate for protein synthesis, Arginine is a precursor to Nitric Oxide (NO), Polyamines, Proline, Glutamate, Creatine, and Agmatine (Morris, 2007). Besides serving as a building block for tissue proteins, it plays a critical role in ammonia detoxification (Morris, 2002). This amino acid is an allosteric activator of N-acetylglutamate synthase, the enzyme catalyzing the synthesis of N acetylglutamate from glutamate and acetyl-CoA. N-acetylglutamate is an allosteric activator of carbamoylphosphate synthase–I (CPS-I), the first enzyme of the urea cycle that converts ammonia and bicarbonate into carbamoylphosphate (Meijer *et al.,* 1990).Arginine is assumed to occupy a position intermediate between the indispensable and dispensable amino acids in most mammals since it is required for optimum growth and nitrogen retention. The present study was undertaken to estimate the Arginine levels in healthy dogs.

Seven healthy dogs of various breeds, ages and sexes presented for a yearly vaccination at Teaching Veterinary Clinical Complex, Bombay Veterinary College, Parelwere selected for the study. Healthiness of the dogs were ascertained on the basis of normal history and physical examination in combination with testing by CBC and serum biochemistry profile. Quantitative plasma amino acid analysis was performed by High Performance Liquid Chromatography (HPLC) technique to estimate the levels of free amino acidArginine(Azuma *et al.,* 2016).Plasma samples were collected after an 8 hour fast to minimize dietary influences on circulating amino acids and was deproteinized with 400 µl methanol (plasma: methanol (v/v) = 1:4) in 1.5 ml eppendorf tubes. The mixture was vortex mixed for 3 min and kept overnight at -20^{o}C and centrifuged for 10 min at 15,000 rpm to remove the precipitated proteins. 200 µl of the supernatant was dried in a speed-vac. The dried residues were dissolved in 50 µl of 0.1N HCl and then 1 µl of the mixture supernatant was subjected to pre column derivatization method using an Agilent 1290 series UHPLC system with a binary pump delivery system coupled to a Agilent 1260 Infinity Diode Array Detector.

Plasma concentrations of arginine are reduced in every type of urea cycle disorder except in arginase deficiency leading to hyperammonemia (Machado and Silva, 2014).The hematological and biochemical values of the 7 dogs under study are presented in Table 1 and 2 respectively. The values werefound to be within reference range established by Brar*et al.*(1999). Table 3 presents the clinical data and plasma level of free amino acid arginine in all 7 dogs under study. Mean ± SE of argininewas 77.55 ± 1.82 nmol/ml.This findingwas in agreement with Chan *et al.* (2009) who reported the level of amino acid arginine in healthy dog to range from 64.8 to 165.9nmol/ml.

The sources of free arginine within the body are dietary protein, endogenous synthesis, and turnover of body proteins. About 40% of dietary arginine is catabolized by the intestine before it can enter the circulation. During the fasting state, ≈85 % of the arginine entering the circulation is derived from protein turnover, and the remainder originates from de novo synthesis (Wu and Morris, 1998). In adults, the

Table 1. presents the hematological parameters of 7 dogs under study

Sr. No	Parameter	Mean ± S.E	Reference Range (Brar et al,1999)
1	Hemoglobin (Hb, gm%)	13.58 ± 0.38	10-16
2	Packed Cell Volume (PCV, %)	39.68 ± 1.32	30-50
3	Total Erythrocyte Count (TEC, 10^6/cmm)	5.97 ± 0.25	5-8
4	Total Leucocyte Count (TLC, 10^3/cmm)	11.87 ± 0.99	6.0-16.0
5	Differential Leucocyte Count(%) Neutrophils Lymphocyte Monocyte Eosinophils	 67.40 ± 2.17 24.70 ± 1.20 3.14 ± 0.14 3.71 ± 1.90	 60-70 15-30 3-8 2-10
6	Mean Corpuscular Volume (MCV, fl)	66.79 ± 2	55-75
7	Mean Corpuscular Hemoglobin (MCH, pg)	22.88 ± 0.68	19-24
8	Mean Corpuscular Hemoglobin Concentration (MCHC, gm/dl)	34.28 ± 0.43	30-36
9	Platelets (PLT, lacks/cumm)	323714.3 ± 38284.47	200000-850000

majority of endogenous arginine synthesis involves an interorgan pathway (also known as the intestinal±renal axis), in which the small intestine releases citrulline into the blood circulation which is then extracted primarily by the kidney for conversion into arginine (Dhanakoti*et al.,* 1990).The magnitude of endogenous synthesis is sufficiently great that arginine is not an essential dietary amino acid for healthy adults. However, endogenous arginine synthesis cannot fully meet the needs of infants and growing children or of adults under catabolic stress or with dysfunction of the small intestine or kidney; thus, arginine is classified as a semiessential or conditionally essential amino acid (Flynn *et al.,* 2002).

There are a number of limitations to the study. Circulating plasma amino acid Arginine concentrations were evaluated and these concentrations do not necessarily reflect intracellular concentrations of amino acid Arginine.

In conclusion, arginine levels in dogs represents the efficiency of the urea cycle and ammonia detoxification. Further studies should aim at the standardization of arginine levels for the canines in India as it is one of the factor causing hyperammonemia.

Table 2. presents the biochemical parameters of 7 dogs under study

Sr. No	Parameter	Mean ± S.E	Reference Range (Brar *et al.*, 1999)
1	Blood Urea Nitrogen (BUN, mg/dl)	16.94 ± 0.88	8-25
2	Creatinine (mg/dl)	1.16 ± 0.11	0.5-1.6
3	Total Bilirubin (mg/dl)	0.4± 0.04	0-0.6
4	Direct Bilirubin (mg/dl)	0.18± 0.02	0-0.3
5	Indirect Bilirubin (mg/dl)	0.22± 0.03	0-0.3
6	Alkaline Phosphatase (ALP, U/L)	76.4± 15.64	10-94
7	Aspartate transaminase (AST, IU/L)	48.5± 5.03	10-62
8	Alanine transaminase (ALT, IU/L)	45.28 ± 2.81	25-92
9	Total Protein (gm/dl)	6.59± 0.24	5.0-7.0
10	Albumin (gm/dl)	3.31± 0.2	2.5-4.0
11	Globulin (gm/dl)	3.27± 0.19	2.3-4.5
12	A/G ratio	1.03± 0.09	0.8-2.0

Table 3 presents the clinical data and plasma level of free amino acid arginine

Dog no	Breed	Gender	Age (yrs)	Body weight (kg)	Arginine (nmol/ml)
1	Mongrel	Female	10	25	74.43
2	Labrador	Male	4	30	73.15
3	Labrador	Male	5	32	82.87
4	Labrador	Male	2	35	79.19
5	Mongrel	Female	7	28	80.71
6	Mongrel	Female	3	20	82.08
7	Labrador	Male	6	34	70.48

Acknowledgement

Authors are thankful to Indian Institute of Technology Bombay for providing technical support.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 85-87

Epidemiological studies in canine ascites

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Abstract

A total of 386 cases of dogs reported with the symptoms of gastroenteropathies registered at TVCSC between January 2010 to December 2010 were screened for ascites. 31 dogs were found to be affected with ascites of different organ origin indicating the overall incidence of ascites to be 8.03 per cent. Of these, 18 (58.06%) cases were found to be harbouring ascites associated with hepatic involvement. Highest number, 14 (45.16%) of cases were recorded in dogs aged between 5-10 yrs. Mongrels showed greater (58.06%) involvement. The distribution of clinical cases revealed 70.96% male and 29.04% in female dogs affected with ascites. Dogs fed on home-made diet were more affected.

Keywords: Ascites, Canine, Incidence, Hepatic, Cardiac, Renal, Hepatobiliary, Diet.

Ascites is described as accumulation of fluid within the peritoneal cavity. It is not a disease *per se* but represents clinical manifestation of multifactorial disease entity involving various organs of the body singly or in combination. Thus, cardiac, renal and hepatic disorders, separately or conjointly, play an important predisposing role in the etiology of ascites. Factors contributing to ascites include age, sex, continuous exposure to high levels of toxins, etc. (Muller et al., 2000; Aitken *et al.,* 2003). The present investigation was undertaken to study the epidemiology of ascites in dogs.

The research work was carried out in the Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, MPPCVV, Jabalpur, Madhya Pradesh. The research was conducted to study the incidence of ascites due to hepatic, renal and cardiac disorders separately or in conjunction, to study the correlation of ascites with different feeding habits of animals and to study the efficacy of different drug regimen in hepatic origin ascites. Details of each affected dog with regard to the breed, age, sex, course of illness, previous illness, if any, and its treatment, environmental hygiene, nutritional and vaccination status, were recorded in a drawn out proforma to work out the incidence.

Out of the total 386 cases of dogs (both male and female dogs), reported with the symptoms of gastroenteropathies registered at TVCSC between January 2010 to December 2010, thirty one dogs were found to be affected with ascites of different organ origin indicating the overall incidence of ascites to be 8.03%. Of these, 18 (58.06%) cases were found to be harbouring ascites associated with hepatic involvement.

Age wise incidence of ascites in the present study indicated a higher incidence in the age group between 5 -10 years (Table 1). Out of the total 31 dogs affected with ascites, 14 dogs were in the age between 5-10 yrs indicating an overall 45.16% followed by 8 in the age between 1-5 yrs indicating an overall 25.80%. 6 dogs (19.35%) were above 10 yrs, and three dogs (9.67%) below one year of age.

Table 1: Frequency distribution in dogs with ascites in different age groups

S. No.	Age group	Incidence (%)
1.	below 1 year	9.67
2.	1 year to 5 year	25.80
3.	5 -10 years	45.16
4.	10 years & above	19.35

The greater incidence of ascites encountered in higher age group in our study corroborates with the findings of Silva *et al.* (2007) who reported higher susceptibility of dogs aged 5 year and above. Nair *et al.* (1973), Baumwart *et al.* (2005) and Chohan *et al.* (2007) also reported ascites in middle age group (4-6.8/ years). Dogs above 10 years showed a lower incidence as few dogs survive at this age.

Breed wise incidence indicated higher incidence (58.06%) in Mongrels (non-descript dogs) followed by spitz (25.80%). Incidence was comparatively low in other breeds (Table 2). These findings are comparable to those of Ogbe *et al.* (2003) and Mohammad *et al.* (2003) who reported ascites to be more common among

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Table 2: Frequency distribution in dogs with ascites in different breeds and sex

Breed	Spitz	German Shepherd	Labrador	Non-descript	Total	Incidence (%)
Male	6	1	2	11	22	70.96
Female	2	-	-	7	9	29.04
Incidence (%)	25.80%	3.22%	6.45%	58.06%	31	100

local non-descript breeds of dog than exotic breeds.

The higher incidence in Mongrels may be attributable mainly to dominance of these animals in the area of experiment. It may also be partly related to increased exposure to scavenging and wandering habits of these dogs, with improper nutrition as well as irregular or no deworming and vaccination. The sex wise incidence of ascites revealed 22 in male dogs (70.96%) and 9 in female dogs (29.04%) The sex wise incidence of ascites is presented in Table 2. These observations fall in agreement with the findings of Vijayan *et al.* (2006) who reported higher incidence in male dogs (81.67%). The selective preferences of pet owners for male dogs may be a possible explanation for the greater susceptibility of male dogs as recorded in our study.

The present study revealed more dogs with only hepatic origin ascites (58.06%) than any other single affected organ or a combination of two or more organs (Table 3). The findings are in accordance with the findings of Sunil *et al.* (2003) who reported 17 (85%) of the 20 dogs with suspected liver origin ascites and 3 (15%) with suspected kidney origin ascites. Rajan *et al. (*1991), Hunt (1993) and Bhagat *et al.,* (2011) also reported much higher incidence of hepatic onset ascites as compared to cardiac or renal onset ascites.

The higher incidence of the hepatobiliary disease in dogs may be attributable to its multifactorial etiology. Incidence of hereditary portosystemic shunt are reported upto 1% (Strombeck 1991), while tumours and chronic active hepatitis account for over 60% of patients with hepatobiliary disease (Rothuizen and Meyer 2000). Other etiological agents include toxins, drug induced hepatotoxicity (Kristal *et al.,* 2004). Infectious agents like *Leptospira* spp., *Salmonella* spp., adenovirus I, canine herpes virus, *Toxoplasma* sp., *Ancyltostoma* sp., and *Baberia* sp. (Utulas *et al.,* 2003) also lead to gastrointestinal and hepatic afflictions.

Table 3: Frequency distribution in dogs with ascites vis-à-vis the organ(s) involved

S. No.	Origin	No. of cases	Incidence (%)
1.	Hepatic	18	58.06
2.	Cardiac	2	6.45
3.	Renal	3	9.69
4.	Hepatic + Renal	4	12.90
5.	Hepatic + Renal + Cardiac	4	12.90

The present study in dogs revealed close association of different feeding habits with ascites. The information collected indicated that dogs fed on home-made diet were more affected than other groups (Table 4).

Table 4: Frequency distribution in dogs with ascites vis-à-vis nature of diet

S. No	Nature of diet	No. of dogs affected	Incidence (%)
1.	Vegetarian/Home made diet (Milk, Chapati, Bread, Biscuit, Porridge & Pulses)	17	54.83
2.	Vegetarian (Eggs with normal home made feed)	5	16.12
3.	Non-vegetarian (Eggs, Chicken, Mutton & Soya with home made diet)	9	29.03

Similar findings of higher incidence of ascites in dogs fed with vegetarian diet were reported by Upadhyaya (2007). Greater incidence in vegetarian diet group (without eggs) may be attributed to lack of balanced diet with insufficient proteins and nutrients which leads to low osmotic pressure within the capillaries resulting in oozing out of fluid to extravascular space. Non-vegetarian diet contains sufficient amount of essential amino acids necessary for proper growth and functioning of tissues especially liver.

Acknowledgements

Authors are grateful to the Dean, College of Veterinary Sci. & A.H., Jabalpur, NDVSU, Jabalpur

for providing the necessary facilities to carry out the research work and the personals of TVCSC for their cooperation.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 88-89

Clinical Articles

Clinical management of hypothermia in infants of endangered Kashmir Gray Langur (*Semnopithecus ajax*)

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Abstract

Two infants of Kashmir gray langur found abandoned in the Dachigam National Park were brought to Wild Animal Health Care Centre. The animals were under shock and showing very less response to external stimuli. Clinical examination revealed hypothermia in both the infants (Body temperature 90^0F) with pale mucous membrane depressed respiration (25/min and 26/min) and bradycardia **(**27/min and 29 min**).** The cases were managed successfully with life saving drugs and fluid and electrolytes therapy.

Keywords: Kashmir gray langur, Hypothermia, Langur

Kashmir gray langur, fondly described as a 'handsome langur' inhabits the steep, rugged and unscrupulous mountains of western Himalayas (Sharma and Ahmad, 2017) is placed in the endangered category of the IUCN red list (IUCN, 2012). Hypothermia is a less common cause of sudden death in primates than hyperthermia, although it may contribute significantly to other types of illness. It is more likely to occur in abandoned infants (Coote, 2005). The present case record describes hypothermia in two abandoned Kashmir gray langur infants and their successful management.

Clinical Observations and Treatment

Two infants of Kashmir gray langur were found abandoned in the Dachigam National Park near erstwhile sheep breeding farm. They appeared sluggish and less active and weighed two kilograms each. Clinical examination revealed severe hypothermia in both the infant (Body temperature 90^0F) with pale mucous membrane, depressed respiration (25/min and 26/min) and bradycardia **(**27/min and 29 min**).** Apparently, animals were under shock and showing very less response to external stimuli. One infant had a fresh wound on the medial aspect of the thigh region, about 2 cm in length and had smooth, even, well-defined edges with gaping in the centre. Quick emergency medical treatment was started to save the life of animals.

Treatment was initiated with the injection of Dexamethasone (1mg/kg) 0.5 ml I/V into the femoral vein using scalp vein 22 G, to stabilise and speed up recovery from the shock (Magre 2009). Warm towels were wrapped around the infants (Fig. 1). Warm normal saline (50 ml) was administered intravenously to each infant to further encourage quick reversal from hypothermia. The body temperature was closely monitored till it reached to normal. The wound was irrigated with normal saline, dressed with betadine solution (5%) and subsequently sutured (simple interrupted pattern) using Vicryl 1#, under local infiltration anaesthesia using 2% Lignocaine Hydrochloride (Fig. 2). Both the infants were given Ceftriaxone @ 50mg/kg (0.2gm IM) (Magre 2009) and Polybion (0.25 ml IM). 10ml of 50% dextrose was given orally to each infant to overcome possible hypoglycaemia. After a few hours the infants became more active and stable, gentle body massage was given as physiotherapy to improve the body circulation. On the 2nd day, clinical examination revealed marked improvement in the condition of both the animals. The treatment was continued for three days. During the treatment, the animals were fed as per protocol of Central Zoo Authority of India (Fig. 3). On 3rd day all the vital parameters were within the normal range with the better and stable condition of both animals (Fig. 4). The wound was dry. It was dressed with antiseptics and herbal fly repellant (Topicure, Natural Remedies Private) before releasing the animals into wild.

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Fig. 1: Langurs wrapped in warm towels

Fig. 2: Antisepsis wound dressing

Fig. 3: Feeding of abandoned langurs

Fig. 4: Recovered infant langurs

Discussion

To the authors best knowledge this is the first report of clinical management of hypothermia in Kashmir gray langur infants. Kashmir gray langur like many other non-human primates is a gregarious animal. The gregarious behaviour and strong social integration make them more adapted to frigid winter (Mcfarland and Majolo, 2013). The possible explanation for this is that they have more excellent opportunities for huddling particularly at night making them less susceptible to heat loss and energy expenditure during winters (Satinoff, 2011). The abandoned Kashmir Langur infants were found to be separated from their social group in midwinter season which is harsh part of winter in the Himalayan region making them very much prone to severe hypothermia. Management and treatment for hypothermia, in this case, was initiated according to standard protocol which includes gradual rewarming by use of blankets, warm towels, recirculating electric blankets, warm water bottles, incubator type cages and warm intravenous fluids depending on local availability. Dexamethasone mitigates complications arising due to hypothermia. Entezarisl and Isazadehfar (2013) have also recommended the use of dexamethasone in hypothermic patients as it reduces the gradient between body core and skin. Antibiotics were prescribed as a prophylactic measure. 50% dextrose was given orally in drops to overcome possible hypoglycaemia. Reunion of abandoned infant langurs with their troop without any delay was carried out as suggested by Sugiyama (1966).

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 90-93

Concurrent occurrence of prostate enlargement with hyperlipidemia and hypercholesterolaemia in a dog: A case report

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Abstract

An eight years old male crossbred spitz dog was presented to RVP, IVRI with the history of anorexia, stiff gait and hematuria. Clinical examination revealed pale and moist conjunctival mucous membrane, rectal temperature of 102.5ºF, heart rate of 85 bpm and respiratory rate of 34/min. Abnormality in posture and gait was noticed. Pain evinced on abdominal palpation. Digital rectal palpation revealed swollen prostate gland. Ultrasonographic examination revealed distended urinary bladder, enlarged prostate gland with anechoic spot within the parenchyma. Large amount of fatty infiltration was also evident in liver by ultrascan examination. Serum biochemistry revealed increased concentration of cholesterol, lipids and triglycerides. The dog was treated with Tab. Clindamycin 600 mg, Tab. Prednisolone 20 mg, Tab L-Carnitine 300 mg, Tab. Atorvastatin 20 mg and Silymarin 5ml for 15 days. The dog recovered uneventfully after the course of treatment.

Keywords: Prostate gland, Clindamycin, Hyperlipidemia, Atorvastatin

Benign prostatic hyperplasia (BPH) is one of the most common reproductive disorders in dogs (Krawiec and Heflin, 1992). It is an age-related disorder in intact male dog that is associated with an increase in the prostatic size. More than 80% of the intact male dogs over 5 years of age exhibit BPH, and prostatic volume in affected dogs is 2 to 6.5 times greater than that of normal size (Paclikova *et al*., 2006). It is associated with the increasing concentrations of dihydrotestosterone hormone stimulates the growth and regulates the secretion of the prostatic epithelial cells.

Pathogenesis of the disease is related to the secretion of testosterone. It is required by the prostatic gland for development and functioning. The enzyme, 5-alpha-reductase, converts testosterone to dihydrotestosterone, a hormone which interacts with prostate receptors causing its growth. As a dog ages, the number of receptors as well as testosterone secretion increase. Furthermore, chronic inflammations also predispose dogs to BPH (Branam *et al*., 1984). The present case deals with BPH with hypercholesterolemia and hyperlipidemia.

Clinical History and Observations

An eight years old intact male crossbred spitz dog was presented to RVP, IVRI with the history of anorexia, difficulty in walking and hematuria (Fig.1). Clinical examination revealed pale and moist conjuctival mucous membrane, rectal temperature of 102.5ºF, heart rate of 85 bpm and respiratory rate of 34/min. Abnormality in posture and gait was noticed. Pain evinced on abdominal palpation. Digital rectal palpation revealed swollen prostate gland.

Serum biochemistry revealed increased concentration of cholesterol, lipids and triglycerides (Table.1) Ultrasonographic examination revealed distended urinary bladder, enlarged prostate gland with anechoic spot within the parenchyma (Fig.2). Normal architecture of kidneys and spleen was noticed. Large amount of fatty infiltration was evident in liver during ultrascan examination suggestive of hepatic lipidosis (Fig. 3).

Treatment and Discussion

The dog was treated with Tab. Clindamycin 600mg, Tab. Prednisolone 20mg(dose tapered a week later), Tab L-Carnitine 3000mg, Tab. Atorvastatin 20mg sid and Sy.Silymarin 5ml bid PO for 15 days. Significant differences were noticed in post therapy serum biochemistry. Marked reduction was noticed in the size of the prostate gland. It was advised to continue the same dose of Atorvastatin, L-carnitine and silymarin syrup for another 10 days. The dog recovered uneventfully after the course of treatment.

Prostate is the only accessory sex gland present in the dog. It produces secretions which forms seminal fluid which constitutes 90% of the entire ejaculate volume (Hewitt, 2001). Seminal fluid helps in the transportation of sperm and its constituent influences

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Table 1. Biochemical parameters of the dog before and after therapy.

Parameters	Before treatment	After treatment	Reference range
Serum triglycerides(mg/dl)	176	166	22-125
Total cholesterol(mg/dl)	334	232	135-278
HDL(mg/dl)	42	44	60-93
LDL(mg/dl)	256	154	36-197
VLDL(mg/dl)	35	33	17-21
S.G.P.T(IU/L)	94	58	10-109

sperm motility and metabolism (Arienti *et al*., 2004). Prostatic hyperplasia is an age related disorder which is mainly seen in intact dog and is associated with the increase in prostatic size **(**Holst & Holmroos *et al*., 2017). The etiology and patho-physiology of prostatic hyperplasia is not known well but the androgen dependent and dihydrotestosterone hormone play a major role (Andriole *et al*., 2004). The clinical signs include sanguineous urethral discharge, decreased fertility, constipation, dyschezia, lameness and systemic signs such as anorexia (Krawiec *et al*., 1992; Polisca *et al*., 2016). Diagnosing BPF is very challenging, it depends on the clinical signs, transrectal digital examination and ultrasonography. Ultrasonography is often the method of choice for determining prostatic size (Ruel *et al*., 1998). It has the additional advantage of showing the internal parenchyma, allowing visualization of e.g. mineralization and cystlike lesions, and evaluation of echogenicity and echotexture (Ruel *et al*., 1998; Moxon *et al*., 2015). Circulating biomarkers can also be used for examination of prostatic status. The biomarker like canine prostate specific esterase (CPSE) is the major secretory product of the canine prostate **(**Chapdelaine *et al*., 1984). Increased level of CPSE observed in dogs with BPH than normal dogs (Wolf *et al*., 2012).

It has been suggested that there is some relationship between the metabolic syndrome and BPH but the exact biological pathways are still unclear. Reports have been documented for the relationship between MetS and BPH (Zang *et al.,* 2014). The exact mechanisms for the relationship between BPH and MetS have not been clarified. But the majority of the studies suggest that inflammation is responsible for this relationship

Treatment for BPH includes the suppression/ prevention of androgen synthesis or action. Castration is the most effective treatment for removing the hormone influence on dogs with BPH. Surgical castration causes a 70% reduction in size after surgery. Although the prostate begins to shrink within 7–14 d after castration, complete involution may require 4 months (Kutzler *et al.,* 2005). Currently the most common medical treatment for BPH is finasteride. It is a synthetic steroid type-II 5a- reductase inhibitor that converts testosterone to dihydrotestosterone. Dihydrotestosterone is biologically active hormone that promotes prostatic hyperplasia in both humans and dogs. Finasteride decreases prostatic diameter, prostatic volume, and serum DHT (Smith, 2008). Reports on its successful use in dogs suffering from BPH by daily oral administration of 0.1–0.5 mg/kg BW for 16 weeks (Sirinarumitr *et al*., 2001) or 1 mg/dog for 3–21 weeks (Lange *et al*. 2001) are available.

Fig. 1. Male Spitz Dog

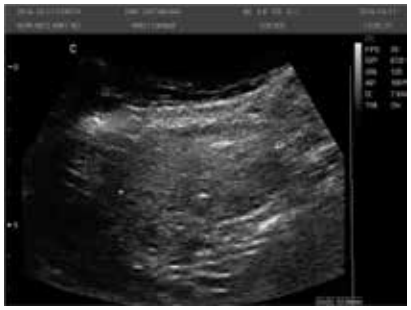

Fig. 2. Enlarged Prostate gland

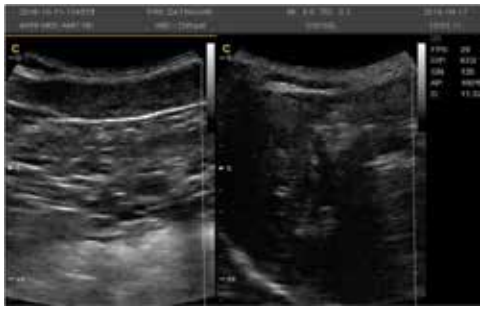

Fig. 3. Fatty infiltration in liver

Atorvastatin (AT), a highly substituted pyrrole, is an inhibitor of HMG-CoA reductase (HMGRI) that has decreased low-density lipoprotein (LDL) cholesterol in humans by 60% at doses of 80 mg/day (Walsh *et al*., 1996). Statins also inhibit the formation and reduce the levels of cholesterol in the prostate, which affects prostate cell growth and survival **(**Solomon and Freeman, 2008). Atorvastatin significantly reduces the prostate volume, improved LUTS, and slowed the clinical progression of BPH possibly by lowering cholesterol and anti-inflammatory factors (Lee *et al*., 2013).

Antibiotic for the prostate infection should be based on culture and sensitivity, as well as the pharmacokinetics of the antibiotic. The prostate is difficult for many antibiotics to penetrate, due to pH differences in the blood versus the prostatic fluid, lipid solubility, and protein binding characteristics of antibiotics (Kutzler *et al*., 2005). The pH of the prostatic fluid is typically <7.4 (lower than that of blood). This acidic environment makes it easier for drugs with a higher pH to enter the prostate. Antibiotics those are more basic than 7.4, e.g. erythromycin and trimethoprim, will cross the blood–prostate barrier easier than their acidic counterparts (Barsanti *et al*., 1986). However, the fluoroquinolones can also penetrate the prostate regardless of their pH, due to their zwitterions characteristics. Additionally, highly lipid soluble drugs, e.g. the fluoroquinolones, chloramphenicol, clindamycin and trimethoprim-sulfa, cross the prostatic acini easily, whereas poorly lipid soluble drugs cannot cross the prostatic acini (Memon *et al*., 2007).

Conclusions

A case of prostate enlargement with metabolic syndrome was diagnosed and treated successfully with specific and symptomatic treatment.

Acknowledgements

The authors are thankful to the Director, ICAR-IVRI for providing facilities for conducting this work. We also acknowledge the effort of Dr. A.C. Saxena, Sci, (SS) for ultrasonographic examination.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 94-95

The confirmed occurrence of classical swine fever in a Large White Yorkshire pig

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Abstract

Classical swine fever (CSF), a highly contagious and potentially fatal disease of pigs causing serious losses in the pig industry. A six month old female Large White Yorkshire was presented to the Teaching Veterinary Clinical Complex, GADVASU, Ludhiana with the complaint of anorexia, depression, epistaxis, erythema all over the body, puffiness of eyes, pyrexia, respiratory distress, and sudden death of animals in the herd. The serum sample was subjected to sandwich ELISA using IDEXX's enzyme immunoassay and the case was diagnosed as Classical Swine Fever.

Keywords: Classical swine fever, pig, sandwich ELISA

Classical swine fever (CSF) or hog cholera, is a highly contagious, potentially fatal disease of pigs and is classed as a List A disease by the Office International des Epizooties (Quinn, 2002). It causes serious losses in the pig industry because it is highly pathogenic and may cause widespread deaths. Pigs infected with highly virulent CSFV strains may shed high amounts of virus before showing clinical signs of the disease. Animals that survive an acute or subacute infection develop antibodies and will no longer spread the virus. Moderately virulent, less pathogenic strains may lead to chronic infection when pigs excrete the virus continuously or intermittently until death. Congenital infection may result in abortion, mummified fetuses, stillborn and/or weak piglets, or embryonic malformations. Thus the present case report stresses upon the rapid and precise detection of CSFV for disease containment.

Case History and Observations

A six month old female Large White Yorkshire weighing 94 kg was presented to the Teaching Veterinary Clinical Complex, GADVASU, Ludhiana with the complaint of anorexia, depression, epistaxis, erythema all over the body, puffiness of eyes, pyrexia, respiratory distress, and sudden death of animals in the herd. Other animals which died also showed similar signs. On clinical examination, conjunctival mucus membrane was found moist and congested. The rectal temperature was 104.2°F and heart rate was 71/min. Physical examination revealed hyperemia of ears, face (Fig 1), ventral abdomen and legs, subcutaneous haemorrhage all over the body and mild convulsions (Fig 2). On oral examination there were severe pinpoint hemorrhages and button ulcers.

Haematological examination showed significant leucopenia and anaemia. The haemoglobin, TLC, TEC, PCV and platelet were recorded as 6.9g%, 7260/cmm, 5.04×10^6/cmm, 26% and 485×10^3/µl, respectively.

The serum sample was subjected to sandwich ELISA and the sample was positive for Classical Swine Fever Virus specific antibodies using IDEXX's enzyme immunoassay. The assay is a blocking ELISA which utilizes microplates coated with CSFV antigen. Antibodies present in the sample blocks the binding of Horseradish Peroxidase conjugated CSFV specific monoclonal antibodies. The bound monoclonal antibodies are detected by a substrate reactive with Horseradish Peroxidase. The result is indicated by color development. The optical density is measured by a microplate reader at a single wavelength of 450 nm, or dual wavelengths of 450 nm and 650 nm. Color development is weak (positive result), when CSFV specific antibodies are present in the test sample (Fig 3). Color development is maximal (negative result) in the absence of specific antibodies. The blocking percentage of the sample is calculated from the optical density (OD450) obtained with the test sample and mean OD450 of the negative control (NC) with the formula: Blocking % = (Mean OD of NC – Sample OD)*100/ Mean OD of NC. In this study, mean OD of NC was 1.393 and the sample (Fig.3, well B3) was 0.144 and the blocking percentage (from the formula) was found to be 89.66% which clearly indicated the presence of CSF specific antibodies in the tested serum sample.

Fig. 1. Hyperemia of ears and face with puffiness of eyes and epistaxis

Fig. 2. Hyperemia of ventral abdomen and legs, subcutaneous haemorrhage all over the body

Fig. 3. Showing positive for CSFV specific Antibodies

Discussion

Classical swine fever may occur in peracute, acute, subacute, and chronic forms, with the acute form occurring most commonly (Radostits, 2003). In the acute form, high fever, depression, anorexia, and conjunctivitis appear 2 to 4 days post exposure, followed by vomiting, bacterial pneumonia, paresis, paralysis, tremor, and convulsions. The clinical findings in the present report are in corroboration with those of Chintu Ravishankar *et al.*, 2007 who described that hyperemia and purpura of the abdomen and ears may be observed in light-skinned pigs. Clinical signs of CSF can remain undetected, particularly during infections with CSFV strains of low virulence (Terpstra C., 1991). Moreover, gross lesions observed at necropsy are diverse and often not pathognomonic (Van Oirschot 1999, Carbrey *et al*, 1996, Moennig 1992). Thus a rapid and precise detection of CSFV is critical and necessary for disease control and prevention. The case presented in the clinics was the representation of the affected lot of pigs which was confirmed by sandwich ELISA and the owner of the pig was advised accordingly to control the disease on the farm. As such, the affected pigs must be slaughtered and the carcasses and bedding properly disposed of. This should be followed by thorough disinfection of the farm and control of pig movement. The source of infection must be identified and herds on farms near the infected premise should be tested serologically (OIE, 2006). The present case report concludes the necessity of the hygiene on the farm and timely vaccination of pigs to avert the fiscal losses incurred due to this highly pathogenic disease.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 96-99

Rare case of milk fever in a non-descript indigenous cow and its successful management

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Abstract

A nine-year-old non-descript indigenous cow presented in sternal recumbency was admitted to the clinics. History revealed parturition 3 days ago, milk yield of 4 L/day, with sudden onset of anorexia and sternal recumbency since last two hours. Clinical examination revealed sub-normal body temperature (99.6 ^{0}F), normal respiration (28/min) and tachycardia (64/min), ruminal atony and tympany, dullness, sluggish eye preservation reflex and dry muzzle. Hematology revealed normal blood cell count while serum biochemistry revealed lowered calcium (2.7 mg/dl) and normal inorganic phosphorus (5.6 mg/dl). Based on clinical signs and lowered levels of serum calcium, the case was diagnosed for milk fever and treated with 225 ml of calcium borogluconate (25%) slow intravenously. Immediately during administration of calcium borogluconate, the cow revealed clinical recovery in the form of increased alertness, restoration of eye preservation reflex, lifting of tail, belching and resumption of moisture on the muzzle. By the end of administration of calcium treatment cow immediately got up and urinated followed by resumption of normal feed intake and rumination. The next dose of 225 ml of calcium boroborogluconate was administered 12 hours later and case was discharged with advice for supplementation of mineral-mixture daily. In this way, a rare case of hypocalcemia (milk fever) in non-descript indigenous cow has been reported and successfully treated with calcium boroborogluconate.

Keywords: Calcium boroborogluconate, hypocalcemia, indigenous cow, milk fever, sternal recumbency

Calcium (Ca) is the most important macro-mineral in terms of relative requirement and the diversity of functions in animal body. Circulatory calcium deficit in the plasma pool with sudden excess loss of Ca in milk/colostrum of high yielder parturient cows is mainly attributed to milk fever (Radostitis *et al.,* 2007). The postpartum drop in blood Ca levels in cows reduces the frequency and amplitude of the ruminal contractions and hence reduces the feed intake which further aggravates hypocalcaemia in parturient cows (Daniel, 1983). Moreover, milk fever also may culminate in several complications and can act as gateway for various production and infectious diseases, thereby hampering the milk production of lactating cows. In recent past, the role of milk fever as a paramount predisposing factor for development of various diseases including abomasal displacement, ketosis, increased risk of mastitis, impairment of immune defense, retention of placenta, metritis ultimately reducing the milk production has been demonstrated (Kimura *et al.,* 2006; Goff, 2008; Radostitis *et al.,* 2007). Milk fever in dairy cows can be diagnosed based on history of recent calving, clinical signs like anorexia, apprehension, sub-normal body temperature, ruminal atony, the sternal recumbency with lateral kink of the neck and lateral recumbency in third stage (Radostitis *et al.,* 2007). The condition could be confirmed by estimation of serum calcium (Reinhardt *et al.,* 2011) or immediate response to treatment with calcium boroborogluconate (Radostitis *et al.,* 2007). Incidences of clinical hypocalcaemia (milk fever) in indigenous cows are scarcely available. The present case report elucidates a rare case of milk fever in non-descript indigenous cow and its successful therapeutic management.

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Case History and Observations

A nine-year-old non-descript indigenous cow weighing 308 kg presented in sternal recumbency since 2 hours was admitted to the clinics. An ardent clinical inspection revealed the history of parturition two days ago (fifth lactation) with milk yield of 4 L/day. Managemental history showed reliance on dry roughages as feed source since last two months without provision of greens and concentrates in diet. The cow revealed sudden onset of anorexia followed by sternal recumbency and lateral kink of the neck since last two hours (Fig. 1). Clinical examination revealed sub-normal rectal temperature (99.6 ^{0}F), normal respiratory

rate (28/ minute) and tachycardia (64 beats/ minute), ruminal atony and tympany. Other clinical signs observed were dullness, sluggish eye preservation reflex and dry muzzle. 5 ml of blood sample was collected into a vacutainer (EDTA) and used for hematology analysis. Moreover, to harvest serum for the estimation of calcium and inorganic phosphorus, 5 ml blood sample was also collected into a tube containing clot activator. Heamtologcial analysis was done on veterinary specific fully automated hematology cell counter (Model: Abacus Junior Vet. Diatron, GMBH, Austria) while biochemical analysis wad done on semi-automated biochemical analyzer (Chemistry Analyzer-CA 2005 B4B Diagnostic division, China, Model no. CA 2005) with kits of Agappe Diagnostics Ltd., Kerala, India.

Laboratory investigations showed normal complete blood count (Table 1) whereas biochemical analysis revealed remarkably decreased serum Ca (2.7 mg/dl) level and normal inorganic phosphorous (5. 6 mg/dl) concentration. The diagnosis of milk fever in the ailing cow was made on the basis of precise history of recent parturition, sole feeding of dry roughages for longer duration, clinical signs like sub-normal body temperature (99.6 ^{0}F), sternal recumbency with lateral kink of the neck, and decreased serum calcium levels (2.7 mg/dl).

Treatment and Discussion

The cow was immediately treated with 225 ml of 25% calcium borogluconate slow intravenously. During infusion of calcium borogluconate, cow exhibited typical clinical recovery response in the form of resumption of moisture onto the muzzle, belching, regain of muscles strength exhibited by tail movement and normal balancing the neck. Immediately after completion of calcium infusion, the cow was able to stand without any assistance and passed urine and dung. Ailing cow showed normal nervous demeanor of alertness and active behavior (Figs. 2, 3) and within half hour of treatment, the cow had resumed feed and water intake (Fig. 4).

Milk fever is an acute to per acute, afebrile paresis of adult dairy cows usually occurring within 48-72 hours of calving (Roche and Berry, 2006). Parturient paresis is an economically important disease of dairy cattle characterized by depression of levels of calcium in tissue fluids. Calcium level in tissue fluid is depleted due to excessive loss of calcium in colostrum beyond capacity of its intestinal absorption and rate of the bone resorption. The Ca absorption from intestine at the time of parturition and mobilization of Ca from skeletal storage may not be sufficiently rapid to maintain normal serum Ca. Around 5-20% of adult lactating dairy cows are unable to maintain normal plasma calcium (9.7-12.4 mg/dl) and succumb to milk fever, which requires treatment (Radostitis *et al.,* 2007).

Adult dairy cows in 5-10 year age group and in their 3rd to 7th parturition frequently suffer from milk fever (Radostits *et al.,* 2007). The findings of the present report are in agreement where 9-year cow in fifth parity suffered from milk fever. Typical clinical signs of milk fever like anorexia, apprehension, rumen atony, weakness, stage of sternal recumbency with lateral kink of the neck were also observed in the ailing cow. No significant variation in hematological parameters were observed compared to normal healthy cows, however biochemical analysis revealed marked decrease in serum calcium level and normal inorganic phosphorus level.

Standard line of treatment for milk fever is immediate intravenous administration of calcium preparations to correct blood calcium levels and to prevent the complications arising due to prolonged recumbency like downer cow syndrome. Most common preparations for treatment of milk fever includes calcium boroborogluconate along with phosphorus, magnesium and glucose to correct simultaneous deficiencies of other minerals and energy (Goff, 2008). Abdullah *et al.* (2014) reported stage 2 milk fever in 7-year-old Jersey cross cow parturited two months ago and successfully treated with 400 ml calcium boroborogluconate along

Table 1: Hematological parameters of non-descript indigenous cow ailing with milk fever.

Parameter	WBC	GRA	MON	LYM	RBC	Hb	PCV	PLT
Unit	(109/L)	(109/L)	(109/L)	(109/L)	(1012/L)	(gm/dl)	(%)	(109/L)
Case values	8.52	4.41	0.78	3.33	7.57	12.9	31.3	324
Reference range (Radostitis *et al*., 2007)	4-12	0.6-6.7	0-0.84	2.5-7.5	5-10	8-15	24-46	100-800

Fig. 1: Non-descript indigenous cow ailing from milk fever in sternal recumbency (second stage of milk fever).

Fig. 2: Recovered standing non-descript indigenous cow after treatment with calcium. borogluconate.

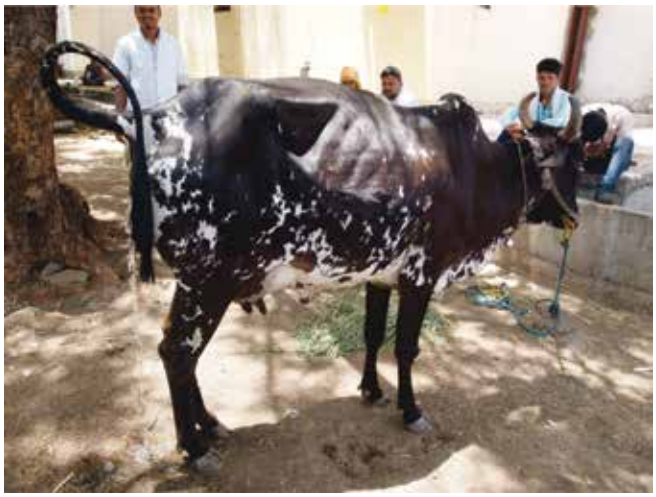

Fig. 3: Recovered non-descript indigenous cow passing urine and dung after completion of treatment with calcium borogluconate.

Fig. 4: Recovered indigenous cow with resumption of feed intake after completion of treatment with calcium borogluconate.

with supportive therapy. Occurrence of milk fever in exotic breeds and crossbreeds is common but literature on milk fever in indigenous cows are meager. To conclude a very rare case of milk fever in non-descript indigenous cow has been reported within 48 hours of parturition which might be due to failure of the indigenous cow in regaining the normocalcaemic state with a faster rate after parturition. Being economically important disease, it is essential to assess incidence of milk fever in indigenous cattle of Maharashtra state to recommend scientific feeding strategies for its prevention.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 100-101

Successful reversal of blindness in a dog associated with hepatic encephalopathy

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Abstract

A three year old pug dog was presented to the Small Animal Clinics, College of Veterinary Sciences and Animal Husbandry, GADVASU, Ludhiana, India with a history of ataxia, partial blindness and fits from last three days. On clinical examination dog was disoriented and dull. Complete blood count and estimation of biochemical parameters (SGPT, ALKP, total protein, albumin, BUN and creatinine) revealed elevated level of liver enzymes and neutrophilic leukocytosis. Ultrasonographic examination of abdomen revealed Hepatomegaly. On the basis of serum biochemistry and ultrasonographic examination dog was diagnosed to be suffering from 'Type A' hepatic encephalopathy (HE) i.e. associated with acute liver failure. The dog was treated with lactulose containing syrup, herbal syrup for controlling hepatic damage and dysfunction containing *silybum marianum* and acetyl cysteine alongwith metronidazole and ampicillin. Dog recovered from nervous signs after five days of treatment. Treatment for hepatic damage was continued as preventive measure.

Keywords: Dog, Hepatic encephalopathy, Lactulose

Liver dysfunction and porto-systemic bypasses result in accumulation of toxins and this systemic accumulation of toxins leads to altered neurotransmission in brain manifestation which is called as hepatic encephalopathy. Hepatic encephalopathy can be categorized into 3 types in type A which is associated with acute liver failure there will be sudden onset of signs and disease will be rapid in progression, Type B associated with portosystemic bypass without intrinsic liver disease in this type signs are episodic and disease progresses gradually and type C associated with severe hepatic parenchymal disease and portal hypertension (Ferenci *et al,* 2002). Depending on the nervous clinical signs disease is characterized into four stages. Stage 1 in which animal will show mild confusion, irritability and dull behaviour; Stage 2 in which head pressing, ataxia, partial blindness, disorientation and lethargies will be typical signs, in Stage 3 seizures, severe ptyalism, inactiveness and occasional aggression will be seen and in stage 4, which is terminal one, animal will be recumbent and in comatose condition and will eventually die. Presences of nervous signs in hepatic encephalopathy are attributed to elevated serum levels of ammonia. The pathogenesis of HE is incompletely understood although ammonia, manganese and inflammatory cytokines have all been implicated (Mas, 2006).

Case History and Observations

A 3 year old male Pug with acute nervous signs was presented to Small Animal Clinics, Guru Angad Dev University of Veterinary and Animal Sciences, Ludhiana with chief complaint of ataxia, partial bilateral blindness, tick infestation and fits. Complete blood count and estimation of biochemical parameters (SGPT, ALKP, total protein, albumin, BUN and creatinine) was done using serum sample. Blood was also examined for haemoprotozoa irrespective of normal rectal temperature. Eyes were examined for cause of blindness, if any Complete anamnesis of dog revealed presence of clinical signs from last three days. General and physical examination of dogs disclosed markedly dull behaviour, disorientation, rectal temperature within range and normal mucous membranes.

Haematological findings revealed haemoglobin 14.8 g percent, Total leukocyte count 21400 cu mm, Total erythrocytic count 6.53 X 10^6 cu mm and packed cell volume 44.3 percent, platelet count. On differential leukocytic count, neutrophils were found to be 92 percent and lymphocytes to be 8 percent. Haematological analysis report showed dog having neutrophilic leukocytosis with most neutrophils mature, mild dehydration and shift to left. Biochemical analysis report of serum depicted bilirubin 0.5 mg/dl, SGPT 124 U/L, ALKP 70 U/L , total protein 7.5 g/dl,

BUN 11mg/dl, creatinine 0.7 mg/dl and glucose 145 mg/dl. Elevated SGPT level indicated towards liver involvement. Clinical examination of eye revealed pigmentry keratitis with TLR of both eye intact.

Ultrasonographic examination of abdomen region was done which revealed hepatomegaly. On the basis of clinical signs, serum biochemistry and ultrasonography the case was diagnosed as 'Type A' hepatic encephalopathy i.e. associated with acute liver failure. The dog was supposed to be in transition phase from stage 2 to stage 3. Therapeutic diagnosis confirmed the case to be of hepatic encephalopathy.

Discussion

Treatment was initiated with lactulose containing syrup, herbal syrup containing *silybum marianum* and acetyl cysteine alongwith antibiotics to combat hepatic damage and reduce ammonia concentration leading to nervous signs. Lactulose, a non-absorbable disaccharide, is used to reduce ammonia production in gut. It causes acidification of lumen leading to reduced ammonia uptake, increased faecal nitrogen excretion by facilitation of incorporation of ammonia into bacteria and its cathartic effect as also reported by Butterworth (2002). Dietary protein was also restricted as HE is precipitated by agents producing more ammonia. Intravenous administration of DNS (5%), metronidazole @ 15 mg/kg body weight intravenously twice a day and ampicillin @ 20mg/kg body weight intramuscularly twice a day was administered for five days to counteract the dehydration and infection status of the dog. Regular resampling was done to ensure hepatic enzyme level which shows reduction in values of SGPT and clinically animal was also followed to recover from partial blindness. Animal recovered completely from nervous signs after five days of the treatment. Treatment for hepatic damage was continued as preventive measure. Therapeutic diagnosis as seen by response to the treatment confirms the presumptive diagnosis of case to be of hepatic encephalopathy.

Case was diagnosed to be of hepatic encephalopathy and spontaneous recovery was observed following lactulose therapy.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 102-103

Therapeutic management of juvenile idiopathic epilepsy in a foal

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Abstract

One week old foal was presented with history of multiple generalized seizures since birth. On the basis of history and clinico-pathological observations the case was diagnosed as juvenile idiopathic epilepsy. The foal was treated successfully with a combination of anti-epileptic drugs, phenobarbital sodium orally at loading dose @ 5 mg/kg followed by maintenance dose of 2.5 mg/kg bid and potassium bromide orally at loading dose @120 mg/kg followed by maintenance dose of 60 mg/kg.

Keywords: Foal, epilepsy, anti-epileptic drugs

Epilepsy refers not to a specific disease but to a heterogeneous group of chronic disorders characterized by a tendency to have recurrent seizures without precipitating factors (Sengoku, 2002). Extensive descriptions of epilepsy are available in the medical literature. However, in Veterinary medicine, information is almost exclusively limited to dogs and cats and relatively rare in horses as compared to other species (Berendt and Gram, 1998). Present case report deals with a rare occurrence of juvenile idiopathic epilepsy in a foal and its therapeutic management.

Clinical History and Observations

One week old foal was presented to teaching veterinary clinical complex (TVCC) with history of multiple generalized seizures since birth. Clinical signs noted at presentation included absence of menace response, and abnormal mentation (Fig.1), nystagmus, the seizures ranged from focal head twitches, progressing to generalized tonic seizures followed by clonic motor activity. The duration of the episodes lasts for <1 minute. Postictal signs were blindness followed by obtundation, lethargy, disorientation, hyperesthesia, ataxia, mydriasis and salivation. Routine hematology revealed normal hematological finding and biochemical examinations revealed slightly higher levels of serum creatinine and blood glucose (SC-580 U/L (normal range- 81–585 U/L) and blood glucose-140 mg/dl). No any cytological changes were observed in cerebrospinal fluid. Therefore on the basis of history of recurrent episodes of seizure without any underlying pathological abnormality the case was diagnosed as juvenile idiopathic epilepsy and treated accordingly.

Treatment was done with anti-epileptic drugs, Phenobarbital sodium was given orally @ 5 mg/kg body weight as loading dose followed by 2.5 mg/kg body weight every 12 hrs along with Potassium bromide (KBr) at loading dosage of 120 mg/kg body weight followed by a maintenance dosage of 60 mg/kg orally for seven days. Other treatments included supportive therapy with intravenous fluids (DNS and RL), vitamin E, antibiotics (Intacef tazo 1gm), and anti-inflammatory drugs for three days to reduce the secondary complications. After three days of the treatment and no episode of seizure was observed. So the owner was advised to maintain the oral doses of Phenobarbital sodium and potasium bromide as recommended for next seven days. After that owner again reported that there was no seizure and animal started behaving normally with normal physical activity and appetite. So the owner was advised to maintain the animal on half of the oral doses of Phenobarbital and potassium bromide for next one month and after that oral dosing was stopped. After next two months of follow up no further seizure was reported in the foal.

Discussion

Juvenile idiopathic epilepsy is clinically characterized by recurrent generalized seizures that are manageable with commonly used anti-epileptic drugs with good long-term prognosis and no apparent permanent sequel (Aleman *et al.,* 2006). The idiopathic epilepsies in humans have no specific cause and are thought to be associated with a genetic etiology, normal brain, early onset (childhood or adolescence), and good

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Fig. 1. A week old foal with signs of disorientation and lethargy

antiepileptic drug (AED) response with a favorable prognosis. In Veterinary medicine, classification of epilepsy has been attempted in dogs (Podell *et al.,* 1995). Although there are numerous case reports of known causes of seizures in horses, but there is limited information regarding idiopathic epilepsy (Sweeney and Hanson, 1987). The history of juvenile onset, clinical manifestations and unremarkable laboratory findings in the present case supported the diagnosis of juvenile idiopathic epilepsy as reported by Aleman *et al*. (2006). Clinically, the seizures episode in the foal was characterized by generalized tonic seizures followed by clonic seizures that lasted for <1 min followed by postictal signs, as reported earlier (Mittel, 1987). Phenobarbital has been the drug of choice to manage seizures in horses (Furr, 1996). Potassium bromide is used in present case for management of seizures as it is very safe and recommended therapy along with phenobarbiatal sodium in multiple and frequent seizures cases as antiepileptic medication (Mayhew, 1998). In the present case, prompt and accurate diagnosis and suitable line of treatment leads to successful recovery without any further complications.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 104-106

Therapeutic management of uroperitoneum in a Labrador dog

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Abstract

Uroperitoneum due to urinary bladder rupture with blunt trauma in male Labrador dog reported to TVCC with history of abdominal distention, vomiting and difficulty in walking, urine output was absent and animal started vomiting just after water intake. On thorough clinical examination, animal was found dull and dehydrated; there was moderate degree of abdominal distention with fluid thrill. The radiographic examination of abdomen was done on lateral recumbency reveals ground glass appearance of abdomen, on catheterization of urinary bladder only few drops of urine come out. On the basis history of anuria, abdominal distension, radiography and catherizattion findings the case was diagnosed as uroperitoneum due to rupture of urinary bladder with blunt trauma and successfully treated by medico-surgical approach.

Keywords: Dog, Uroperitoneum, Anuria

Urinary bladder rupture is the most common cause of uroperitoneum in cats, dogs, and humans (Rieser, 2005). As the bladder fills, it moves into the abdomen and makes it more vulnerable to be ruptured or injured (Bartges and Polzin, 2011). Currently, urinary bladder rupture is the most common traumatic urinary injury in dogs and cats, and mostly occurs in male dogs which often results in mortality (Selcer, 1982). Present case deals with urinary bladder rupture with blunt trauma in male Labrador dog with its successful therapeutic management.

Clinical History and Observations

Eleven month old male labrador dog from NTCD, Border Security Force, Tekanpur, was presented to TVCC with complain of abdominal distention, vomiting and difficulty in walking. On detail investigation of history it was reported that animal was fallen on the edge of drainage wall, after that animal felt slight pain and given analgesics by local veterinarian of the unit, but after 4 days the animal stopped taking food, urine output was absent and animal started vomiting just after water intake and there was gradual distension of abdomen, symptomatic treatment was done by the veterinarian of the unit during that period. On thorough clinical examination, animal was found dull and depressed, rectal temp was 100.8^{0}F, HR was 98/min, CRT was >3 sec, and >10% dehydration on skin tainting, there was moderate degree of abdominal distention with fluid thrill (Fig.1.), no any external injury was observed, vomiting was periodic after every 15-20 minutes with yellowish green in color. The radiographic examination in lateral recumbency reveals ground glass appearance of abdomen, on catheterization of urinary bladder only few drops of urine come out (Fig.2.). Therefore, on the basis history of anuria, abdominal distension, radiographic finings and urethral catherizattion findings the case was diagnosed as uroperitoneum due to rupture of urinary bladder with blunt trauma and treated accordingly.

Management

Abdominocentesis was done to remove the accumulated urine in the abdominal cavity with help of 21 g I/V catheter and connected with a drip set to remove urine (Fig.3), and simultaneously I/V fluid therapy consisting of NSS was given continuously to prevent development of shock, approximately 2 liters of urine was removed then abdomen became normal. Thereafter, surgical correction of ruptured urinary bladder was done by Midventral laparotomy ruptured bladder was closed in two layer inversion pattern with 2/0 vicryl. Postoperative management includes i/v fluid therapy with Hemaccel 10 ml/kg, DNS 10 ml/kg and inj RL 40 a daily for 3 days. Apart from fluid therapy Intacef 50 mg/kg i/v, Pantop 40mg i/v, Metrogyl 20mg/kg i/v, Perinorm 0.5mg/kg i/v, Neohepatax 1ml i/m and Polybione 1ml i/m was given for 3 days. Urobag was administered and fixed with Foleys catheter to monitor urine production daily for initial 3 days. Improvement in

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Fig. 1. Marked abdominal distension

Fig. 2. On catheterization, scanty urine output

Fig. 3. Ground glass appearance and distension of abdomen

Fig. 4. Radiograph of abdomen, on day 4th

Fig. 5. Removal of urine from peritoneal cavity (Uroperitoneum)

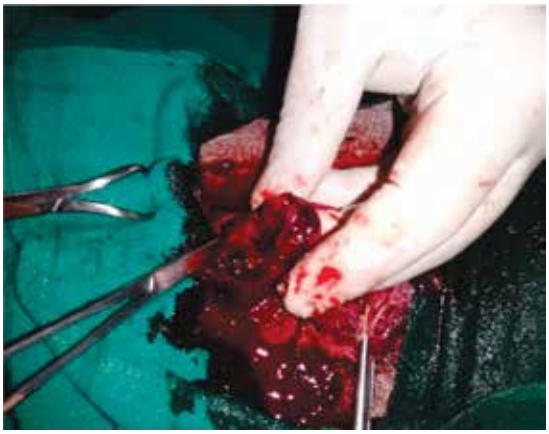

Fig. 6. Ruptured Urinary bladder

the condition of animal was observed on next morning, sufficient accumulation of urine was observed in the urobag, animal vomited only once in the night. After 2 days animal started taking water and liquid diet without any further vomiting, sufficient voiding of urine and defecated on 2nd day morning, since then condition of animal improved significantly and animal started taking normal solid food and water without any abnormality, on 4th day urobag and Foleys catheter was removed, animal started voiding urine normally without any discomfort. Thereafter, animal was maintained on Tab monocef@ 200mg orally twice daily, along with multivitamins and hepatoprotective drugs for next 7 days, after 10th day of surgery the skin sutures were removed.

Blunt trauma occurs when extensive external force exerted on the abdominal wall may have displaces

the bladder to an unbearable elastic (stretch) limits such that the bladder wall gets weakened and may lead to tears in the urinary bladder, characterized by high rate of associated intra-abdominal escape of urine and mortality (Dreitlein *et al*., 2001). In present case similar reasons might leads to uroperitoneum. Confirmatory diagnosis of urinary bladder rupture in present report was done by Abdominocentesis and radiography apart from history and clinical examinations. Abdominocentesis is necessary to definitively diagnose uroabdomen, although different imaging techniques such as abdominal radiography could also be helpful in diagnosing uroabdomen condition (Sura, 2011).

The clinical signs of urinary bladder injury are relatively nonspecific, presence or absence of hematuria, ability to void voluntarily, and presence of a palpable bladder do not predict urinary tract integrity (Kong *et al*., 2011). In present report also the signs are nonspecific that might be the reason for improper diagnosis by local veterinarian, Delayed signs are those of uremia and peritonitis, manifested in the form of abdominal distension, loss of condition, vomition and severe dehydration were noticed and it might have occurred due to other organ or system abnormalities (Rieser, 2005).

A peritoneal catheter can be placed to lavage and evacuate the urine, most of the time intraperitoneal bladder ruptures require surgical exploration and repair **(**McLoughlin, 2000). Lacerations are usually large in these cases, with the potential risk of peritonitis due to urine leakage (leading to uroperitoneum and subsequent uroperitonitis), in this case similar observations were noticed during the course of study. Management of intra-peritoneal bladder rupture by exploration and primary bladder closure requires intensive post-operative management otherwise failure may take place (Suad *et al*., 2011), but in present study, prompt reporting, early and accurate diagnosis and prompt surgical and therapeutic management might leads to successful outcome.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 107-108

Successful therapy of acute trypanosomiasis in a Doberman dog

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Abstract

A case of a trypanosomiasis of a Doberman pincher male dog of 3 year age was presented with hide bound condition and opacity in both eyes. Recording of vitals revealed fever and pale mucous membrane. The case was diagnosed by wet blood film examination which revealed moving trypanosome. The dog was treated with injection Quinapyramine. On re examination of blood sample after 12 days of treatment, were found negative for trypanosome.

Keywords: Trypanosomosis, Corneal opacity, Quinapyramine

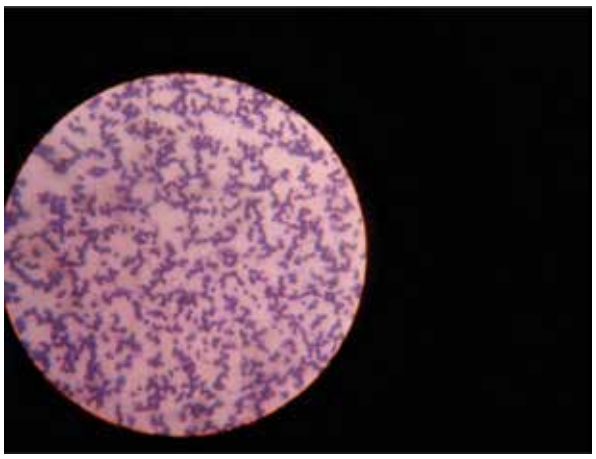
Trypanosomosis is a most widely prevalent haemoprotozoan disease which affects a wide variety of domestic as well as wild animals which causes severe anaemia of the animal. The disease is transmitted by biting flies including tabanus, tse tse fly stomoxys, culicoides etc. (Green 2006). In dogs an acute and fatal type is commonly seen and death possibly occurs in 2-4 weeks (Soulsby, 1982).

Clinical History and Observations

A 3 year old Doberman pincher male dog was brought to the Teaching Veterinary Clinical Complex, Parbhani with emaciated condition. The owner reported that the dog was suffering from opacity of both eyes. On thorough clinical examination the dog was found severely dehydrated (+++), dry and rough hair coat, corneal opacity of both eyes and eruption of the scrotum. Vital parameters were 103.4^0F rectal temperature, respiration rate 25/min, heart rate 70/min and pale mucous membrane.

Blood sample was collected for haemato biochemical examination. In wet blood film moving trypansoma was observed. Further a thin blood smear was stained with Giemsa stain showed large no. of trypomastigotes of trypanosome organism (Ramesh *et al,* 2016). In haematological examination blood parameters were Hb 7.34g/Dl, PCV 20.17%, RBC 3.14 x 10^6/micro L TLC 72.70/micro L, Neutraphil 24%, Lymphocyte 64%, Eosinophil 1%, Basophil 0%, which showed neutropenia and relative lymphocytosis (Aquino *et al,* 2002) with severe anaemia . Blood glucose level was also estimated which showed hypoglycaemia (28mg/dl). On the basis of clinical symptoms and wet blood film, blood smear examination and haemato biochemical analysis the case was diagnosed as Trypanosomiosis.

The dog was treated with single dose of Quinapyramine (Triquin S) as specific drug @3.33mg SC. Supportive therapy includes injection Dextrose 25% @5ml/kg IV followed by injection Dextrose 5% until correction of dehydration. Oral haematinic was given for 4 weeks and parental Iron injection was given twice in a week. Vitamin B complex and liver extract was given prenatally for 7 days daily. The dog was



under clinical evaluation for 12 consecutive days.

It causes bilateral corneal opacity of the eye. There was positive response to the therapy with improvement in general condition with the appetite and disappearance of the corneal opacity. Haemoglobin and blood glucose level was also come to normal range after 12 days evaluation. The dog was able to walk and complete recovery was observed after two weeks of treatment. From the present case we can interfere that single dose of quinapyramine is found effective in trypanosomiasis in dogs.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 109-112

Diagnosis and management of a rare case of splenic abscess with local peritonitis in a Murrah Buffalo

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Abstract

Splenic abscess is a rare disease in large animal practice with few reports in dogs and human medicine. In bovines, it has been mostly reported following penetrating foreign bodies. A 5 year old, 9 month pregnant Murrah buffalo was presented to large animal clinics of the university with the history of fever, pain, anorexia, lethargy and reduced fecal output for two days with previous history of diarrhea. On per rectal examination, loose feces were present in rectum and rumen was doughy. Laboratory tests reflected marked neutrophilia with moderate left shift. Radiographic examination revealed a potential foreign body in the reticulum. Ultrasound examination of the abdomen revealed splenic abscess of 8.7 x 12.5 cm at 8th intercostal space (ICS) midway on the left side along with local perireticular reaction and reticulophrenic adhesions. Therapy included drainage of abscess followed by medical management with antibiotics and fluid therapy. It was concluded that a high degree of precision in ultrasonography is helpful in early diagnosis and drainage of splenic abscess resulting into complete recover in two weeks in large animals.

Keywords: Ultrasonography, Splenic abscess, Buffalo, Foreign body

Foreign body syndrome or hardware disease in cattle and buffaloes is still a challenge in veterinary practice all over the world (Aref and Abdel-Hakiem, 2013; El-Ashker *et al*., 2013; Nugusu *et al*., 2013; Abu-Seida and Al-Abbadi, 2014; Mostafa *et al*., 2015). Various serious complications of foreign body syndrome are traumatic reticuloperitonitis (local and diffuse), traumatic pericarditis, reticular abscess, diaphragmatic hernia, hepatic abscess, vagal indigestion, splenic abscess, rupture of left gastro-epiploic artery, pleurisy, traumatic pneumonia and mediastinal abscess (Abouelnasr *et al*., 2012; Nugusu *et al*., 2013). However, Splenic abscess is a rare sequel to this disease in bovines. Although, prophylactic and therapeutic use of magnet has reduced its incidence to a great extent but the incidence of this disease is still high in all developing countries due to bad managemental and feeding practices, resulting into devastating economic losses (Semieka, 2010). The disease was recorded in 25% of the examined buffaloes in Egypt (Aref and Abdel-Hakiem, 2013) and in 87% of dairy buffaloes and 93% of buffaloes over 2 years of age in India (Sharma and Kumar, 2006). Various diagnostic methods such as metal detector, radiography and ultrasonography were used for diagnosis of this syndrome and its complications in bovines (Braun *et al*., 1993; Abdelaal *et al*., 2009; Athar *et al*., 2010). Ultrasonography is a non-invasive and advanced technique in veterinary practice which has been found very useful in diagnosing and treating space occupying lesions of visceral organs like spleen, liver, lungs etc. In the present case, a mid shaft splenic abscess has been drained using ultrasonography which helped in speedy recovery in a case of localized reticuloperitonitis.

This report may be the first report in current veterinary literature on diagnosis of splenic abscess and its follow up after treatment.

Clinical History, Diagnosis and Management

A seven-year-old Murrah buffalo was presented to Teaching Veterinary Hospital of the university with the history of anorexia, reduced fecal output and fever. Animal was 9 month pregnant and there was no history of pain. Clinical examination revealed rectal temperature 103.2˚f, mucus membrane congested, heart rate 70 beats per minute, ruminal motility of 3 per 3 minutes and respiration rate of 40 per minute. Auscultation of heart and lungs revealed normal heart and lung sounds. On rectal examination, rumen was doughy and there was presence of loose faces in rectum. Complete blood examination of the animal was carried out which revealed marked neutrophilia with moderate toxic changes in the neutrophils and left shift (Hemoglobin-10.1, Total Leucocyte Count-10040,

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Neutophils-84%, Lymphocyte-16%.

Radiographic examination of chest and reticulum showed a potential foreign body in the reticular region and diaphragmatic line was not clear in the ventral one third segment. Ultrasonography of left 6th to 8th intercostals space revealed localized peritonitis along with a splenic abscess of 8.7x12.5 cm size visible at 8th Intercostal Space (ICS) (Fig-1). Local reticulophrenic adhesions were evident on the left side with severe peri-reticular reaction. The ultrasound examination of other abdominal organs like liver, intestines and kidneys showed normal echotexture and ecogenecity.

Under local anesthesia, ultrasound guided drainage of pus was undertaken from the splenic abscess through 8th ICS on left side and the site was lavaged with saline solution (Fig-2). Fortified procaine penicillin was injected into the abscess after drainage. Magnet was also placed into the reticulum using balling gun to prevent further piercing of foreign body. Post drainage, ultrasound was again performed and it was revealed that there is drastic decrease in the size of pocket of abscess i.e. 5.12x2.92 cm. Animal was kept on fluids (NSS 10 litres daily for 3 days) and antimicrobials (Metrogyl @ 10mg/kg iv bid for 3 days), along with fortified procaine penicillin(@ 20,000 IU/kg bid) and enrofloxacin (@ 5 mg/kg im bid) for 5 days along with supportive therapy including liver tonics.

After drainage and medical treatment there was marked improvement in the condition and animal started taking feed and water normally within one week**.** No history of fever was recorded afterwards, animal was passing normal faeces. Animal parturited normally and there was no decrease in milk yield as compared to previous parity. Blood examination after one week of treatment showed marked improvement in condition.

Discussion

Splenic abscess cases have been rarely reported in animals and human beings. Out of 22% cases of foreign body in Iraqi buffaloes, 3 cases (0.8%) were suffering from splenic abscess (Abu-Seida and Al-Abbadi, 2015). In human beings, reported frequency of splenic abscess is as low as 0.14-0.7% (Alonso *et al.,* 1990).However, traumatic reticuloperitonitis (TRP) is a common problem among the cases presented in veterinary hospitals in India and other Asian countries. Most of the cases in bovines occur as a sequel to ingestion of foreign bodies along with feed and fodderAl-Abbadi *et al.* (2014) observed that the type of disease due to foreign body depends on the site of penetration of the object and size of the foreign body. Rare incidence of splenic abscess might be due to the fact that the foreign body mostly penetrate the cranio-ventral end of reticulum where the spleen usually ends except the cases of splenomegaly.

Common signs of indigestion are reduced appetite, ruminal atony, passing scanty faeces (Radosttitis *et al.,* 2007; Abdelaal and Floeck, 2015). On the basis of clinical signs like anorexia, ruminal atony, reduced fecal output and no history of pain, it was assumed that the animal might be suffering from gastrointestinal problem. Abdelaal *et al.* (2009) also observed signs of pain are less common in abdominal diseases in buffaloes as compared to thoracic diseases. In such obscure cases, radiography followed by ultrasonography is very valuable in diagnosis. Presence of foreign body was diagnosed with the help of radiography which was unable to diagnose the splenic abscess. The management of foreign body was done by placing the magnet in reticulum and ultrasonography helped to rule out there was no loss in the diaphragmatic line in this case. The case would have been treated for TRP, had the Ultrasonography not done. So, the sonography in this case diagnosed the splenic abscess in addition to localized peritonitis, the sequels of foreign body syndrome. In addition, the precision of sonography helped in drainage of splenic abscess which has been rarely attempted in veterinary practice till date. Chaing *et al.* (2003) reported that the non specific clinical presentation of splenic abscess and vague symptoms of the disease make the diagnostic imaging tools (x-ray and ultrasonography) very useful (Chun *et al.,* 1980). Moreover, ultrasound plays an important role in the diagnosis of spleenic abscess in human patients due to its sensitivity, accuracy, precision, safety, low cost and ease of repeatability (Ralls *et al.,* 1982; Chou *et al.,* 1992) but in animals no report regarding precision of ultrasound for diagnosis of spleenic abscess was there so for.

Hematological examination in the present study revealed the presence of suppurative inflammation in the body and it was confirmed by the presence of foreign body in the reticulum and presence of spleenic abscess on Ultrasonography. Studies in humans have revealed that splenectomy along with antimicrobial

Fig: 1: Ultrasound image at 8th intercostals space showing a 12.5 cm x 8.70 cm pocket of abscess in spleen

Fig. 2: Ultrasound image at 8th ICS after drainage of abscess

therapy resulted in complete recovery but is few cases it has been reported that ultrasound guided abscess drainage is the other treatment option (Tung *et al.,* 2006; Sangchan *et al.,* 2003). In our case splenectomy was not done due to high cost of surgery and owner unwillingness. Moreover, it was never tried in bovines till date. So ultrasound guided drainage of abscess along with aggressive antimicrobial therapy and management of foreign body by placing magnet in the reticulum helped in the complete recovery of the animal. It has been concluded that the Ultrasonography must be undertaken in cattle and buffaloes found positive for foreign body on radiography. In addition, the ultrasound guided drainage can be tried safely in buffaloes having splenic abscess followed by antimicrobial therapy.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 113-114

A report on clinical diagnosis and management of grain engorgement in a buffalo

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Abstract

A 2.5 year old *Murrah* buffalo was presented to the Veterinary Clinical Complex of university with the history of accidental consumption of excess oatmeal a day before the animal brought to the clinics. Animal was in lateral recumbency and unresponsive, and had sunken eyes and weak pulse. The study reports successful clinical management of grain engorgement in buffalo mainly with intravenous hypertonic sodium bicarbonate solution and Vitamin B1.

Keywords: Grain engorgement, Buffalo, Sodium bicarbonate

Excessive feeding of rapidly fermentable carbohydrates by ruminants, commonly referred to as "grain overload," is the classic situation which leads to clinical rumen acidosis (Snyder and Credille, 2017). The condition has also been named rumen overload, toxic indigestion, grain engorgement, grain overload and lactic acidosis (Garry, 2002). There is change in the rumen microflora from predominantly Gram-negative to Gram-positive lactic acid-producing bacteria, which leads to a rapid production of lactic acid. Ruminal fluid pH below 5.0 is the criteria for acute ruminal acidosis which occurs when excessive levels of organic acids accumulate in the rumen (Nagaraja and Titgemeyer, 2007). Clinical signs include distended rumen and its atony (Van Metre *et al*., 2000), anorexia, watery and foul smelling diarrhoea, hypothermia in advanced cases, dehydration, ataxia, tachycardia, and tachypnea with shallow respirations (Underwood, 1992). This syndrome is responsible for heavy morbidity and mortality in affected buffaloes. Acidic rumen pH causes alteration of the microbial population, leading to reduction in thiamine biosynthesis (Rahman *et al.*, 2014). Animals that survive may develop laminitis, liver abscesses, hypocalcemia and thiamine deficiency (Garry, 2002). Quantity and quality of carbohydrate rich feed determine the severity and clinical outcome (Gentile *et al*., 2004). The prognosis depends on the duration and severity of clinical signs. Diagnosis is mainly based on the history of exposure to offending feedstuffs, clinical signs and rumen fluid analysis (Snyder and Credille, 2017). Specific therapy is necessary and is focused on correction of plasma volume deficits, local and systemic acid-base disturbances, restoration of a normal rumen microenvironment, and management of potential secondary complications.

Clinical History and Diagnosis

A 2.5 year old Murrah buffalo was presented at the Veterinary Clinical Complex of the university with a history of excessive consumption of oat meal. While recording anamnesis it was found that buffalo was lying acutely ill for the last two days, and showed loss of defecation. On clinical examination it was observed that animal was recumbent, depressed, and dehydrated with sunken eyes, congested mucous membranes, weak but rapid pulse (84/min), and subnormal (95.1° F) body temperature. Ruminal fluid was collected and analysed for physical and microscopic examination. The results revealed absence of protozoal motility, low pH (4.8), milky grey colour, fetid smell and thin and watery consistency of ruminal fluid. On percussion of the rumen, fluid splashing sound were audible through left paralumbar fossa.

Diagnosis of acute carbohydrate engorgement was made on the basis of history, clinical presentation and rumen fluid analysis. Treatment was initiated immediately with 5% sodium bicarbonate followed by hypertonic saline solution along with thiamine, fortified procaine penicillin, multivitamin injection and rumen buffer orally. Initially, 5 litre of 5% sodium bicarbonate was given intravenously to counteract systemic acidosis and later isotonic sodium bicarbonate (1.3%) as fluid therapy was continued to counteract dehydration and to expand plasma volume. Fortified procaine penicillin at the dose rate of 22,000 U/kg/day was given im for 5 days to minimize the chance of bacterial rumenitis. Thiamine @10 mg/kg intramuscularly, was given to assist the

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metabolism of L-lactate produced by lactobacilli bacteria. Thiamine administration could be beneficial in alleviating thiamine deficiency caused by ruminal acidosis. Rumen buffer (Buffzone) was given orally to stabilize and restore the rumen microenvironment alongwith 10ml multivitamin (belamyl) injected intramuscularly. Animal recovered completely after giving this treatment for 5 days. This clinical case draws attention towards clinical diagnosis, therapeutic management and potential danger of grain overload that readily lead to the onset of ruminal acidosis.

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Therapeutic management of severe anaemia in a Labrador bitch

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Case History and Observations

A five year old female Labrador dog was brought to the Teaching Veterinary Clinical Complex (TVCC), Veterinary College Anjora, Durg, Chattisgarh, with complaint of severe weakness, lethargy and complete loss of appetite. The dog was earlier treated with antibiotics, anti-inflammatory drug, and cortisones by a local veterinarian. The dog did not respond to the treatment rather there was gradual deterioration in the condition.

A thorough investigation of the case was started. Detailed clinical examination revealed that the dog was having rectal temperature 99^0F, pale conjunctival mucous membranes, tachycardia (HR 140/min) and tachypnoea (RR 56/min). Blood was collected for complete blood count and blood smear was prepared for detecting presence of blood protozoa, if any. The CBC revealed severe anaemia (Hb 2.9 gm%, TEC-1.40 millions/cumm., TLC-3200/cumm., lymphocytes-81%, neutrophils 12%, eosinophils-03%, monocytes 04%, platelet count-80,000/cumm. The blood smear was negative for protozaol parasites.

Treatment and Discussion

Since the blood smears were negative for any protozoal parasitic infection and the animal was anorectic and anaemic, supportive treatment with D5-350 IV, IV multivitamin preparations @ 3ml along with haematinics @ 3 ml IM on alternate days was started for next 3 days. Oral multivitamin and multimineral preparations @ 1½ tsp BD was prescribed daily for 15 days. The dog showed only mild response by the end of the 1st week and the Hb increased to 4.4 gm %.

But there was no appreciable improvement. Looking to the severity of anaemia and condition ofthe dog, it was decided to perform blood transfusion. A healthy Labrador dog was used as donor after cross matching and detail blood investigation to rule out any infection. 150ml of whole blood (WB) from donor dog was collected safely in a blood collecting bag with Acid-citrate-dextrose (ACD) preservative.

Fresh WB contains red blood cells (RBC), leukocytes and plasma proteins including clotting factors and is a good source of functional platelets. (Tsuchiya, et al., 2003). Whole Blood transfusion is indicated when the patient is anemic, has a blood volume loss of more than 50%, or when the patient requires multiple components of blood (eg, RBC, clotting factors, platelets) (Lanevschi*, et al*., 2001). It is used primarily for managing acute, severe haemorrhage from trauma, surgery or coagulopathies (Prittie 2003). The blood volume to be transfused (VT) depends on the severity of anaemia, availability of blood products, body mass and donor PCV. The amount of WB to be administer can be calculated using formulae (Day, et al., 2012).

Out of the total eight blood groups in canines(commonly known as "dog erythrocyte antigen i.e. DEA), DEA 4 is a high frequency antigen, occurring in 98–100% of dogs whereas other blood types occur with low to moderate frequency (Giger et al. 1995; Iazbik et al. 2010). DEA 1.1 is highly antigenic and should be determined in all donor and recipient dogs before transfusion (Tocci& Ewing 2009).

The transfusion administration rate depends on the cardiovascular and hydration status and severity of anaemia. Transfusion-associated circulatory overload (TACO) and nonhemolytic febrile reactions (a temperature increase of 1 to 2 degree celcius within 1-2 hours of transfusion) are the most common reactions seen in dogs and cats. (Narick, et al., 2012 and Pandey and Vyas, 2012).In relatively stable patients that do not have active severe blood loss, the rate should be slow initially (0.25 mL/kg for 30 minutes) to allow identification of transfusion incompatibilities or reactions and can be increased thereafter, typically to 2–10 mL/ kghour (Harrell and Kristensen 1995). The maximum transfusion rate recommended for euvolaemicanaemic animals, to avoid volume overload, is 10–20 mL/ kg/ hour.

The recipient dog was given 2 ml dexona IM prior to blood transfusion, to avoid any reaction. The

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blood was slowly transfused to the recipient. The dog was observed for about half an hour for any anaphylactic reaction. The dog did not show any transfusion reaction.

Jutkowitz, et al., 2002 reported transfusion reactions in 6 dogs out of 15 dogs receiving a massive blood transfusion which were evaluated for transfusion volume, underlying disease process or injury, benefits and complications of transfusion, and outcome. Results suggested that massive transfusion is possible and potentially successful in dogs with development of some predictable changes in electrolyte concentrations and platelet count (low ionized calcium concentrations and thrombocytopenia).

After two days, the Hb increased to 6.5 gm%. The dog showed good active response and had started consuming food. Chronic anaemia is one of the major cause of anorexia. Iron deficiency in dogs and cats is caused by chronic blood loss. In stable patients, oral iron preparations are preferred over parenteral iron administration in small animals due to its low cost and higher safety (Giger, 2005). Ferrous salt preparations @ 5 mg elemental iron per kg bwt. OD for 10 days was included along with other multivitamin, multimineral preparations orally. By end of 3rd week dog recovered completely.

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Indian J. Vet. Med. Vol. 38, No. 1&2, 2018 pp. 117-120

Dorsal displacement of soft palate in two cattle calves

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Abstract

The present paper reports dorsal displacement of the soft palate (DDSP) in two calves presented with a common history of regurgitation immediately after drinking of milk or water through nostrils followed by coughing. There was normocytic hypochromic anaemia and neutrophilia in both the calves. Radiography of chest showed mild to moderate bronchial pattern in both the calves. Endoscopy with fiber optic endoscope in both calves revealed dorsal displacement of the soft palate along with atonous lower esophageal sphincter and food particles in esophagus. Epiglottis was not visible in both the calves. On the basis of endoscopy findings, the cases were diagnosed as dorsal displacement of the soft palate. This is believed to be the first report of DDSP in cattle calves. The calves were treated with bethanecol along with metaclopramide and combination of amoxycillin and gentamicin along with flunixin to cover up the secondary bacterial infection due to aspiration. Complete recovery was conveyed in one calf after five days.

Keywords: Soft palate Displacement, Calf, Regurgitation, Endoscopy

Palatal dysfunction is a form of dynamic upper respiratory tract obstruction and comprises dorsal displacement of the soft palate (DDSP) and palatal instability (Lane *et al.,* 2006). Dorsal displacement of the soft palate occurs when the caudal border of the soft palate becomes displaced to a position above the epiglottis resulting in obstruction of the rima glottidis (Franklin *et al.,* 2004; Lane *et al.,* 2006). Palatal instability mayor may not progress to DDSP during exercise (Lane *et al.,* 2006). This condition is one of the common physical causes of dynamic respiratory problems in horses and has been rarely reported in cattle. Due to the dynamic and intermittent nature of DDSP, obtaining a definitive diagnosis is often considered to be challenging. Furthermore the etiopathogenesis of the condition is incompletely understood and as a result numerous treatment options have been described. However, the efficacy of treatments remains controversial and there is little consensus about how best to treat this condition. Treadmill endoscopy has routinely been considered to be the 'gold standard' in equines for diagnosis of this condition (Barakzai, 2007). DDSP during the resting examination when the endoscope is withdrawn from the trachea is a sufficient criterion to establish a diagnosis (Parente and Martin, 1995; Woodie *et al.,* 2005). In the present study, DDSP was diagnosed in two cattle calves by endoscopy.

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Case History and Observations

Two cattle calves, aged 1½ months and 3 months, were presented to Large Animal Clinics of the university with a common history of regurgitation immediately after drinking of milk or water through nostrils followed by coughing. Both the calves were unable to deglute milk since birth. Clinical examination revealed normal body temperature, heart rates and respiration rates in both the calves however mucous membranes were pale in both the calves. In addition, the first calf had tick infestation. Rumen motility in second calf was 2 per 3 minutes.

Blood samples (2 ml) were collected aseptically from jugular vein in EDTA coated vials. Immediately after collection whole blood was used for determination of Haemoglobin (Hb) and total leukocytes count (TLC) by ADVIA Haematology System. The blood smears were prepared and evaluated for Differential Leukocytes Count (DLC) after staining with Leishman stain. The blood smears were also evaluated for the presence of hemoprotozoa in first calf. Further, both the calves were subjected to left lateral chest radiography. Fiber optic endoscopy was performed in both the calves without sedation.

Hematological analysis revealed absolute neutrophilia, lymphopenia, relative eosinophilia and normocytic hypochromic anaemia in first calf. Second calf had absolute neutrophilia and mild normocytic

hypochromic anaemia (Table 1). The blood smear examination was negative for any hemoprotozoa in the first calf.

Table 1 Haematological alterations in affected calves

Parameters		Calf 1	Calf 2
Hb (g%)		5.3	8.7
TLC (cu mm)		10000	11350
DLC	Lymphocytes (%)	20	36
	Neutrophils (%)	64	62
	Eosionophils (%)	16	2
Interpretation		Absolute neutrophilia Lymphopenia, relative eosinophilia	Absolute neutrophilia

Radiography: Radiography of chest in first calf showed mild bronchial pattern in caudal lung lobe (Fig. 1). Further, there was a 5 cm column of radio-opaque material in abomasum. Second calf had mild to moderate interstitial pattern and diffused moderate bronchial pattern (Fig. 2) and oesophagus was normal in both calves.

Endoscopic findings: Epiglottis was not visible, though upper portion of larynx and prominent corniculate process of the arytenoid cartilages were seen in both the cases (Fig. 3 and 4). Dorsal displacement of the soft palate was diagnosed in both the cases along with atonous lower esophageal sphincter and food particles in esophagus (Fig. 5 and 6). Based on these findings, both the cases were diagnosed as dorsal displacement of the soft palate. *Treatment* : The calves were treated with bethanecol @ 0.5 mg/kg bwt per os along with metaclopramide for 5 days. The combination of amoxycillin @ 10 mg per kg bwt bd and gentamicin @ 4 mg per kg bwt od aiming broad spectrum coverage along with flunixin meglumine were adminstered for 5 days to cover up the secondary bacterial infection due to possible aspiration secondry to regurgitation. The complete recovery was conveyed in first calf after five days. The second calf did not show any improvement.

Discussion

Dorsal displacement of the soft palate (DDSP) is a performance-limiting condition of the upper respiratory tract frequently observed in horses (Kelly *et al*., 2013). During DDSP, the caudal free margin of the soft palate moves dorsal to the epiglottis, creating a functional obstruction within the airway. The cross-sectional area of the pharynx is reduced, and airflow resistance and turbulence are increased. The present paper is believed to be the first report of DDSP in cattle calves. This condition has been commonly reported in horses (Lane *et al.,* 2006; Barakzai, 2007). However, Anderson *et al.* (1994) performed tracheoscopy in 13 cattle and reported dorsal displacement of the soft palate in seven cattle. The cause of DDSP is unknown in cattle and is probably related to a number of factors including the size and shape of the larynx and pharynx and the neuromuscular control of the pharyngeal stabilising muscles. The pathogenesis of this condition is also unknown, but may involve epiglottic hypoplasia, malformation, or neuromuscular dysfunction (Kelly *et al*., 2013). In these cases, neuromuscular cause is a likely contributing factor to the condition. In horses, during exercise the soft palate can move dorsal to lie on top of the epiglottis, thereby displacing and preventing the seal between the oral and nasal cavity from remaining intact. Holcombe *et al*. (1999) investigated the patho-physiology of DDSP in horses and suggested that retropharyngeal lymphadenopathy may cause neural dysfunction and thereby be involved in the pathogenesis of clinical DDSP in horses as the pharyngeal branch of the vagus nerve is in close proximity to the retropharyngeal lymph node chain. However, rarely a clinical case has been reported so far in calves. Anderson *et al.* (1994) diagnosed dorsal displacement of the soft palate by means of endoscopy and radiography in a 10-month-old Chianina/Angus bull and an 11-month-old Limousin bull. Both bulls had the primary complaint of exercise intolerance, dyspnea on excitation and respiratory noise that was audible in all phases of respiration. The former bull was treated with anti-inflammatory medication and rest. The respiratory noise resolved over a 4-month period. Sternothyroideus and sternohyoideus myectomy was performed in the later bull. Immediate postoperative improvement was noticed clinically and endoscopically. In the present cases, bethanecol was given as it has been shown to induce a significant concentration dependent increase in contractility traits of smooth muscle preparations from the oesophageal groove of calves *in vitro* (Barahona *et al.,* 1997), and as it has effect on atonous esophagus. Thus, bethanecol was believed to produce a desired response and did in first calf.

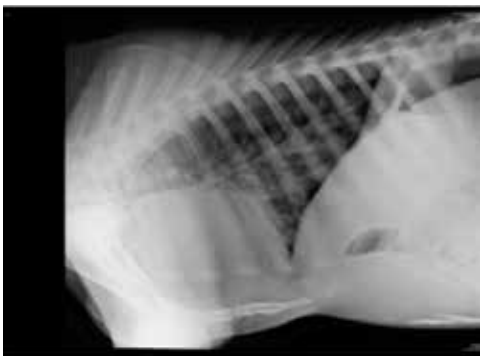

Fig. 1. Lateral radiograph of chest showing Moderate bronchial pattern and normal oesophagus in first calf

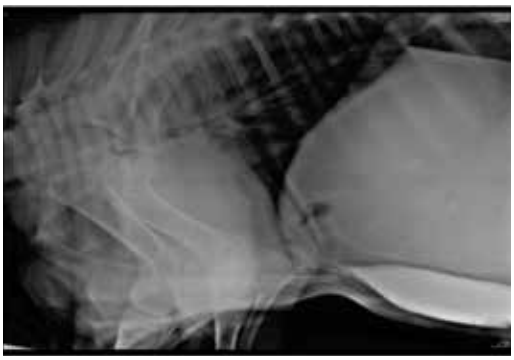

Fig. 2. Lateral radiograph of chest showing Moderate bronchial pattern in caudal lung lobes

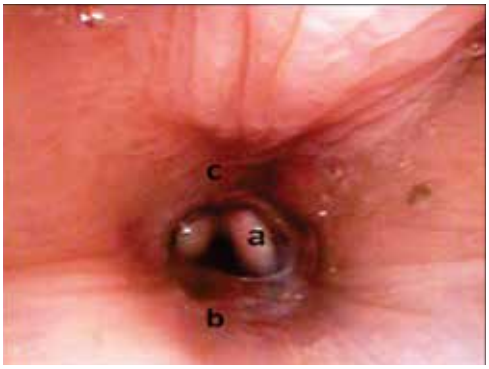

Fig 3. Endoscopic findings in Calf 1. Dorsal displacement of the soft palate a) Arytenoid cartilage b) Soft palate c) Dorsal phaynx

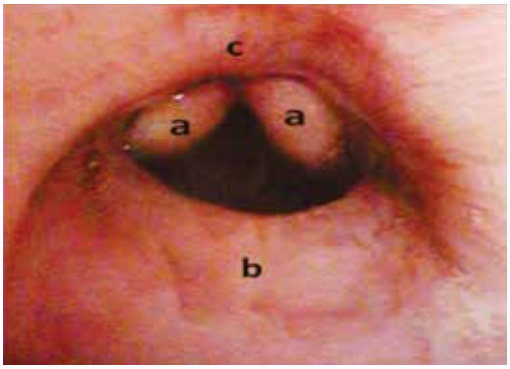

Fig. 4. Endoscopic findings in Calf 2. Dorsal displacement of the soft palate a) Arytenoid cartilage b) Soft palate c) Dorsal phaynx

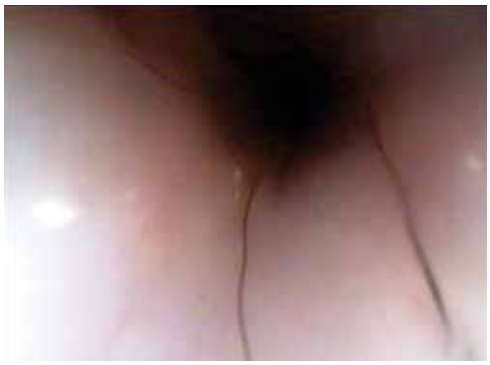

Fig. 5. Endocopic visualization of atonous esophagus

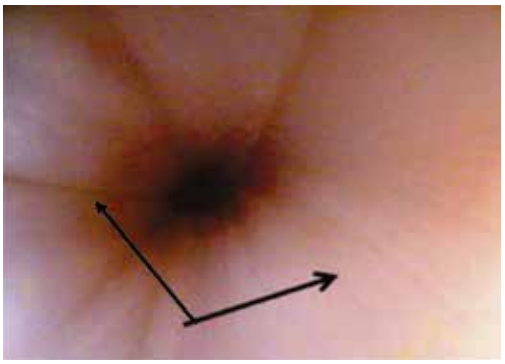

Fig. 6. Longitudinal folds of esophagus (solid arrow)

The DDSP mainly occurs in athletic horses during high-intensity exercise. In the present study, DDSP was confirmed in calves by means of endoscopy. In such cases where regurgitation is the primary complaint, combination of bethanechol and metaclopramide can be tried for good response. Although rare, DDSP should be considered while evaluating cattle with regurgitation.

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Successful therapeutic management of carbofuran poisoning in a Pitbull Dog

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Abstract

An eight months old Pitbull dog was presented with the history of accidental ingestion of Carbofuran pesticide. The animal showed clinical signs of abnormal behavior, shivering, lacrimation, respiration distress, vomiting, diarrheoa, abdominal pain and excessive salivation. Diagnosis of carbofuran poisoning was done on the basis of history and examination of the pack of pesticide that was ingested by dog and clinical signs. The dog was successfully treated with Atropine, fluid therapy and other supportive therapy.

Keywords: Atropine, Carbofuran poisoning, Dog

Accidental and malicious carbamate poisoning in animals is an emergency faced by clinicians, worldwide. Extensive use of carbamate pesticides in agricultural lands has resulted in its easy accessibility to the masses and that is thought to be an important reason for the rise of carbamate intoxication in animals in recent years (Arnot *et al.,* 2011). Carbofuran is one of the most toxic carbamate pesticides that act by inhibiting acetylcholinesterase activity in nervous tissues (Wang *et al.,* 2007). Carbofuran poisoning is an emergency and clinicians need to be able to promptly diagnose it and start with effective treatment immediately to ensure a favorable prognosis. This case report presents diagnosis and successful therapeutic management of carbofuran toxicity in a dog.

Case History and Observations

An eight month old pure bred male Pitbull dog was presented in critical condition to Teaching Veterinary Hospital of the Guru Angad Dev Veterinary and Animal Sciences University with the history of consumption of pesticide (Brand name, SUMO) and within half an hour of ingestion there was onset of shivering, abnormal behavior, lacrimation, diarrhea, abdominal pain and excessive salivation. Clinical examination revealed hyperthermia, respiratory distress, bradycardia, congested mucus membrane, hyperesthesia, excessive thick salivation, severe dehydration, restlessness and lacrimation, foul smelling watery diarrhea and vomiting.

Complete blood examination revealed Hemoglobin 13.5gm%, Total Leukocyte Count (TLC)-16650/µl, DLC- Neutophils-72%, Lymphocytes-24%, Eosinophils-4%. Blood biochemistry revealed ALT- 24 U/L, BUN-12 mg/dl, Creatinine-0.9 mg/dl, glucose-154 mg/dl and calcium-11.5 mg%. Diagnosis of carbofuran poisoning was done on the basis of history and examination of the pack of pesticide that was consumed by dog and clinical signs.

Dog was immediately put on oxygen therapy followed by administration of Injection Atropine Sulfate @ 0.5 mg/kg iv, Inj. Normal Saline Solution 1000 ml iv, Injection Ascorbic Acid- 10ml iv, Injection Ranitidine @ 2mg/kg im, Injection Dexamethasone @ 0.5mg/kg iv, Injection Vetalgin- 2ml im, Injection B Complex- 2ml im. Once the animal regained consciousness, oral medication with activated charcoal @ 1gm/kg diluted in water was done. Animal was kept under close monitoring for 8 hours after clinical recovery of clinical signs and animal was discharged thereafter. Owner was advised to give multivitamins and liver tonics orally for 7 days and regular follow ups at weekly intervals for 4 weeks.

Discussion

Carbofuran poisoning is an emergency and patients may die within minutes after ingestion due to respiratory failure (Siqueira *et al.,* 2015). Carbofuran belong to the group of acylating (carbamylating) acetylcholinesterase (AChE) inhibitors which inhibit AChE in the enzyme active site and results in hyperstimulation of cholinergic receptors due to accumulation of acetylcholine in the gap junction. Clinical signs of carbofuran poisoning are categorized into three syndromes; muscarinic (vomiting, diarrhea, salivation, lacrimation, myosis, dyspnea, bradycardia),

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Nicotinic (muscle tremors and twitching, paresis progressing to paralysis) and central (depression, behavioural or postural changes, hyperactivity, seizures) as described by Arnot *et al.* (2011). In contrast to organophosphate toxicity, the carbamate-induced inhibition of AChE is rapidly reversible with hydrolysis of the carbamylated complex. Atropine sulphate is the drug of choice in carbamate pesticide poisoning and reverses bronchospasm, bardycardia and circulatory depression (Leibson and Lifshitz, 2008; Jokanovi, 2009). Atropine also lowers the cerebral glucose threshold thus preventing the chances of brain damage (Pazdernik *et al.,* 1986; McDonough *et al.,* 1987). In cases with carbamate poisoning dose of atropine required to counteract toxicity is 10 times more than the recommended pre-anaesthetic dose i.e, 0.2-0.5mg/kg (Firth, 2000) and is repeated every 15-30 minutes, until the clinical signs like bronchospasm, excessive bronchial secretion and bradycardia are alleviated (Jokanovi, 2009; Waseem *et al.,* 2010). Intravenous fluid therapy is given to prevent severe dehydration due to ongoing loses in the form of vomiting, diarrhea and salivation. Oral medication and feeding is avoided to prevent chances of aspiration (Leibson and Lifshitz, 2008; Rosman *et al.,* 2009). Activated charcoal is used as an adsorbent to bind the carbamate toxicants present within the gastrointestinal tract to prevent further absorption. This is highly porous thus provides a large surface area for absorption, however, it should be noted that activated charcoal is contraindicated in patients with seizures or having weak swallowing response as they increase the risk of aspiration. In these cases naso-oesophageal tubing can be done to administer activated charcoal which also prevents initial absorption as well as re-circulating toxins via entero-hepatic circulation (Jokanovi, 2009; Plumb, 2008). Some patients may require Diazepam (benzodiazepine) to counter seizures (Leibson and Lifshitz, 2008; Roberts and Aaron, 2007), reduce anxiety and to induce muscle relaxation. It can be administered @ 0.5–1 mg/kg IV to dogs that are seizuring (Jokanovi, 2009).

Potential complications of the disease are pancreatitis and Organophosphate-induced delayed polyneuropathy (Arnot *et al.,* 2011) so in these cases time to time follow up is required to evaluate delayed toxicity.

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Diagnosis and Therapeutic Management of *Hepatozoon canis infection in Labrador* Retriever dogs

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Abstract

Eight Labrador Retriever dogs, aged 6 months, from 11th Battalion Sitapur, were referred to Referral Veterinary Polyclinic (RVP), Indian Veterinary Research Institute (IVRI) with the history of anorexia, pyrexia, vomiting and heavy tick infestation. Clinical examination revealed icteric and anemic signs. Complete blood count examination revealed anemic changes and presence of *H. canis.* Postmortem examination showed yellowish tinged fluid in the abdominal cavity. Histopathologically, liver and kidney sections revealed severe fatty degeneration.

Keywords: Canine, Hemoparasitic disease, *Hepatozoon canis,*

Clinical History and Observations

Eight Labrador Retriever dogs, aged 6 months, from 11th Battalion Sitapur, were referred to Referral Veterinary Polyclinic (RVP), Indian Veterinary Research Institute (IVRI) with the history of anorexia, pyrexia, vomiting and heavy tick infestation. Clinical examination revealed pale to icteric conjunctival mucous membrane, tachycardia, palpable popliteal lymph node. One dog died at the time of clinical examination. Blood samples were collected from the remaining dogs for complete blood count and haemoparasite disease diagnosis and the carcass of dead dog was referred to division of pathology (IVRI) for PM examination. Thin blood smears were stained with Giemsa stain and examined under microscope to ascertain hemoparasite infection. Three dogs (3/8) were found positive for *H. canis.* Anemic and leukocytosis changes were also noticed in all three dogs**.** Postmortem examination revealed edematous and haemorrhagic hind limbs, yellowish tinged fluid in the abdominal cavity, yellowish and friable liver and kidney (Fig. 2). Histopathologically, the liver section revealed severe fatty degeneration of hepatocytes with infiltration of few inflammatory cells (Fig. 3). The kidney section also revealed severe degeneration of tubular epithelial cells. The spleen, liver and brain collected on ice were negative for other viral infections such as infectious canine hepatitis, parvoviral infection and rabies by PCR and (Fluorescent Antibody test) FAT respectively. Similarly, liver samples were also negative for any toxicants by HPLC**.**

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The dogs were treated with Imidocarb dipropionate (Imicarb, SAVAVET) @ 5 mg/kg SC, Tab. Doxycycline (Doxypet, SAVAVET) @ 5 mg/Kg bid PO for 21 days. They were also treated with Inj. Neohepatex (Biological E Ltd) 1ml IM, Inj. Hemaceel (Piramal HC) 100 ml IV, Inj. Dexamethasone (Dexona, Zydus AHL) 0.4 ml IV, Inj. Ascorbic acid 2 ml IV for 7 days. It was also advised to give Tab Famotidine (Facid, INTAS) 150 mg PO bid for 21 days along with Silymarin syrup (Livsure, Visham lifecare) (1 tsp bid PO) and Fipronil (Fiprofort, SAVAVET) for topical application to get rid of ticks. All four animals were found to be healthy after 21 days of treatment and blood reports were negative for any hemoparasite.

Discussion

Canine hepatozoonosis is widely spread in South Europe (Kontos *et al*., 1991; Hervas *et al*., 1995), Africa (Ezekolli *et al*., 1983), Asia (Murata *et al*., 1991; Rajamanickam *et al*., 1995), South America

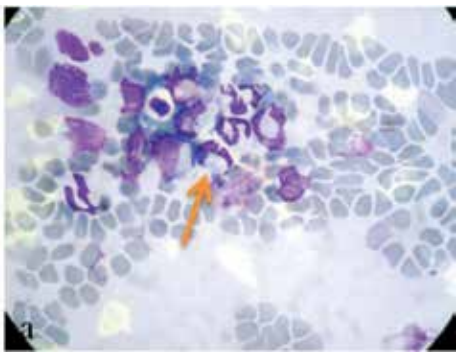

Fig. 1: Stained blood smear showing H. canis (a) (Giemsa stain, 100x)

Fig. 2: PM lesion showing free fluid in abdomen, yellowish, friable liver and kidney and edematous and hemorrhagic hind limbs

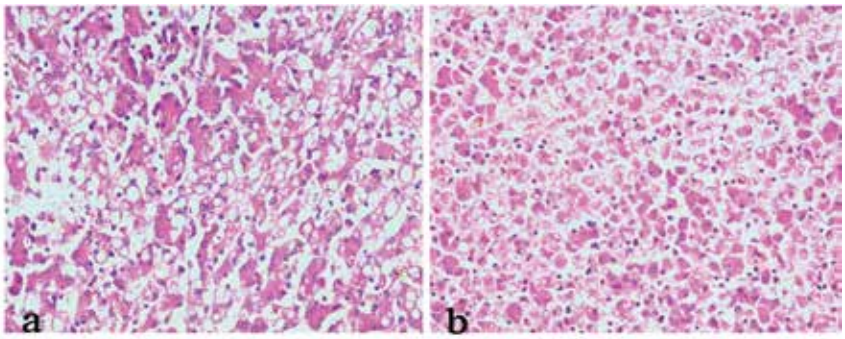

Fig. 3: Fatty degeneration of hepatocyte (a) and degeneration of kidney tubular epithelial cell (b) (H & E stain, 20 x)

(O'Dwyer *et al.,* 2001). Its distribution is directly related to population of *R. sanguineus* (Craig, 1990). Seroprevalence of *H. canis* was found to be 36% in Portugal, 17.6% in Nigeria, 2.5 % in India, 2.3 % in Israel, 2.1% in Thailand (Gevrey, 1993). The incidence of haemoparasite disease in our study was in accordance with the earlier (Rao *et al*., 1986; Varshney *et al*., 2003) observations, wherein authors reported frequent infections at summer in Andhra Pradesh and Uttar Pradesh respectively. Microscopic examination of blood smear is frequently used for diagnosis of hepatozoonosis. But, PCR was considered the most sensitive detection method than stained blood smear examination (Otranto *et al*., 2011). Similarly, nested polymerase chain reaction had high sensitivity (47.7 %) in detecting the acute cases followed by buffy coat (29.5 %) and blood smear examination (22.7 %) of canine monocytic chrlichiosis (Behera *et al*., 2015)

Based on the history, clinical observation and clinical pathology, these dogs were confirmed with hepatozoonosis. Imidocarb dipropionate @ 5 mg/kg SC or is the primary drug used for canine hepatozoonosis. To treat the possible coinfections transmitted by brown dog tick, often Imidocarb is combined with Doxycycline @ 5 mg/kg for 3-4 weeks. The clinical manifestation of dogs with *H. canis* is mostly dependent upon concurrent diseases. Level of lipid peroxides (LPO) in RBC hemolysate of infected dog were significantly increased ($p<0.05$) which indicates oxidative stress (Behera *et al*., 2011). Concurrent infections of *E. canis* and *B. gibsoni* have resulted in a fulminating attack of canine ehrlichiosis and canine babesiosis (Harikrishnan *et al*., 2005). The prevention of *H. canis* infection is based upon the effective tick control program. Regular cleaning and combing of dogs would prevent them tick infestation.

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Meter	m	Centimeter	cm	Microlitre	µl
Counts per minute	cpm	Milligram	mg	Cubic centimeter	cm^3
Millilitre	ml	Degree centigrade	°C	Minute(s)	min
Degree Fahrenheit	°F	Once a day	od	Decilitre	dl
Parts per million	ppm	Gram	g	Percent	%
Hour(s)	hr	Picogram	pg	Inch	in
Revolution per min	rpm	Intramuscular	im	Seconds(s)	sec
Intraperitoneal	ip	Square centimeter	cm^2	Intravenous	iv
Subcutaneous	sc	Kilo calories	kcal	Thrice a day	tid
Kilogram	Kg	Year(s)	yr	Twice a day	bid
Volts	V				

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1. Place of publication	:	Department of Veterinary Medicine College of Veterinary Science Guru Angad Dev Veterinary and Animal Sciences University Ludhiana-141004, Punjab
2. Periodicity of its publication	:	Bi-annual
3. Printer's Name	:	Mr. Kamal Chopra
Nationality	:	Indian
Address	:	Foil Printers 2051, Gobind Nagar, Ludhiana-141 001, Punjab
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Dated: 29 January, 2019 **Swaran Singh**

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The Editorial Team is highly thankful to esteemed ISVM members and veterinary fraternity for contributing overwhelmingly, as a result the pending issues of "Indian Journal of Veterinary Medicine" have been published within a short span of 4-5 months.

ISVM is grateful to the our senior and experienced members Dr JP Varshney, Dr S. Prathaban and Dr NK Sood for their valuable review articles on Veterinary Homeopathy, Contrast Enhanced Ultrasound (CEUS) and Chronic Renal Failure.

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