Rhodococcus equi infection in foals - an update
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Rhodococcus is a genus of facultative intracellular, aerobic, non-motile, nonsporulating gram-positive bacilli or coccobacilli bacteria. This genus closely resembles to mycobacterium and corynebacterium. Most of species of genus rhodococcus are non-pathogenic and a few species are pathogenic, *Rhodococcus equi* is a pathogenic species. *R. equi* is a worldwide disease of foals and a big concern for equine industry (Muscatello, 2012). There are various reports of incidences of *R. equi* infection in foals from India (Dedar et al., 2017; Javed et al., 2017). Foals between 3 weeks to 5 months age are most susceptible and most of the cases occur in foals below 6 months of age (Dawson et al., 2010). Foals up to 9 months of age can be found affected with *R. equi* (Dedar et al., 2017). It can also cause pneumonia in immunocompromised patients and occupationally exposed persons (Yamshchikov et al., 2010). It is a soil dwelling actinomycete, found more in the soil contaminated by equine faeces, especially foal’s dung. *R. equi* causes pulmonary and extrapulmonary pyogranulomatous infection in foals and susceptible persons (Dedar et al., 2017). Virulent strains possess 80-90 Kb plasmid encoding virulence associated proteins including virulence associated protein A (vap A) (Dawson et al., 2010). Environmental temperature, pH and availability of nutrients are reported as regulatory factors for the expression of virulence gene. Optimum expression of vap A gene occurs in mild acidic conditions and environmental temperature of 37°C (Dedar et al., 2017; Ren and Prescott, 2003). It develops pink colonies on Muller Hinton agar those later converts into yellow colonies. *R. equi* can be found in areas where no horses are reared. At present, *R. equi* is the major cause of the foal’s mortality worldwide (Chaffin et al., 2003).

Epidemiology

Prevalence of *R. equi* has been reported from various countries like Argentina, Australia, Canada, France, Hungary, Japan, Ireland etc. (Ocampo-Sosa et al., 2007). Infections have also been reported from Thailand (Asoh et al., 2003), Korea (Kim et al., 2008), USA (Weinstock and Brown, 2002; Burton et al., 2013), Denmark (Gudeta et al., 2014), Brazil (Gressler et al., 2014) and China (Liu et al., 2014). Infection has been described in almost every continent and organism has been widespread in animal dung, manures, soils of grazing field etc. High temperature favors the organism load in air during windy days. Organism has also been isolated from terrestrial and aquatic animals like crocodiles, several avian species, arthropods (Prescott, 1991) and immunocompromised humans like AIDS patient (Weinstock and Brown, 2002). Major route of transmission in both animal and human population is exposure to contaminated soil (Cisek et al., 2014). Horse manure is very good medium for the growth of *R. equi* (Prescott, 1991). Infection may be acquired by either inhalation or ingestion of contaminated material, traumatic inoculation or sometime due to super infection of wounds. Horses and foals act as natural host but infection may be disseminated in other animals like sheep, goat, cattle, cats, dogs, and wild birds etc. (Cisek et al., 2014). Avirulent strain of *R. equi* can be isolated from the feces and environment of almost every stud farm while virulent strain is more commonly isolated from the feces and environment of farm with endemic disease history thus suggesting that foals bred on a farm with endemic disease are exposed more frequently to virulent *R. equi* infection. Continuous shedding of organism in feces is an important mechanism responsible for progressive development of disease at farm (Takai, 1997).

Clinical Signs

Although characteristic clinical signs may play an important role in the diagnosis of *R. equi* infection but these are not always present in foal until disease progresses in advance stage. Hence, the early confirmation of disease requires cumulative information of previous history of disease at farm, clinical signs, haematobiochemical profile, result of diagnostic imaging (Pulmonary ultrasound and radiography), microbiological culture of airways (Franklin, 2016), cytological examination, molecular and serological tests (Giguere et al., 2011 and Javed et al., 2017). Both pulmonary and extrapulmonary symptoms are common with *R. equi* infection however, usually the respiratory system is affected (Dedar et al., 2017).
Pulmonary symptoms

Pulmonary symptoms are relatively common and consistent in foals affected with *R. equi* and characterized by subacute to chronic bronchopneumonia, pyogranulomatous pneumonia, and pulmonary abscessation which can be detected either by ultrasonography or thoracic radiography. Horses have very large respiratory reserve capacity, so generally they show clinical signs related to respiratory system only after affection of a large area of lungs. Similarly, in *R. equi* infection foals show respiratory signs in later stage of the pneumonia. Initially decreased exercise is the only clinical sign. A mild nasal discharge may also be observed.

Extra pulmonary symptoms

These include fever, polysynovitis, uveitis, enterocolitis, abdominal abscess, and osteomyelitis (Giguere et al., 2011). In clinical practice clinical cases of cough and diarrhea which are not responding to general treatment in the foals of age group between 1 to 6 months should be suspected for *Rhodococcus equi*.

Diagnosis

Major constraint in the diagnosis of *R. equi* is to differentiate the lower respiratory tract infection caused by *R. equi* from that caused by other bacterial pathogens. Diagnosis can be made by appropriate history (epidemiological data), clinical signs (bronchopneumonia and enteritis), cytological examination (G+ve coccobacilli), bacteriological culture (pink colonies on Muller Hinton agar), hemato-biochemical examination (neutrophilic leukocytosis, fibrinogen concentration, RBC etc.), biochemical characteristics (catalase-positive, mostly urease-positive and oxidase-negative), serological (ELISA, AGID etc), molecular (PCR, qPCR) test (Bargen & Haas, 2009) and characteristic necropsy findings. Disease history with high incidence at farm requires less diagnostic evaluation for early confirmation of disease especially subclinical infection or carrier foal (Giguere et al., 2011).

Serum hemato-biochemical markers

Serum concentration of fibrinogen and total leukocyte count are important haematobiochemical parameters for the screening of infection in conjunction with positive ultrasonographic and radiographic findings i.e. pulmonary abscessation (Giguere et al., 2011). Increase in white blood cell count with increased neutrophil percentage is the most consistent finding in the clinical cases of *R. equi*. In later stages, decrease in red blood cell count and haemoglobin is also observed (Dedar et al., 2017). TLC>20,000 cells/µL, and serum fibrinogen>700 mg/dL, with the evidence of lung abscessation is more likely associated with pneumonia caused by *R. equi* (Leclere et al., 2011). However, considerable overlap exists in distribution these parameters should not be used as diagnostic as well as prognostic markers (Lavoie et al., 1994).

Bacterial culture and Cytology

Bacteriological culture of tracheo-bronchial aspirate (TBA) and molecular diagnosis by PCR technique are the most important and only acceptable methods for the diagnosis of *R. equi* infection (Muller and Madigan, 1992). Cytological examination of bronchoalveolar lavage fluid (BALF) of foals can be used to differentiate the pneumonia caused by *R. equi* from that caused by other bacteria owing to more nucleated cells and neutrophils with lesser number of macrophages in case of *R. equi* infection than other bacterial infection associated with foal pneumonia (Valentina et al., 2019). It is a quite easy procedure and can be performed in field. Moreover, the result of BALF cytology can be obtained earlier while waiting for culture report.

Serological and Molecular Assays

Different serological and molecular tests with variable diagnostic accuracy (Table 1) are important in early detection of infection. Serological tests such as ELISA, agar gel immunodiffusion (AGID), synergistic hemolysis inhibition, radial immunodiffusion enzyme assay etc has poor sensitivity and specificity and the presence of antibodies does not necessarily indicates the infection. There is no current recommendation for using serological test as a diagnostic method for *R. equi* infection (Phumoonna et al., 2006). However, due to simplicity and high sensitivity, the radial immunodiffusion enzyme assay (RIDEA) can be an ideal test for routine use where ELISA is not available (Takai et al., 1990). Molecular diagnosis is based on the amplification of 16 S rRNA, vapA and vapB gene by using PCR technique. Diagnostic accuracy (sensitivity and specificity) of PCR test depends on the type of sample used (Pusterla et al., 2007) (Table 1). False negative results are more common when test is conducted on faecal material or nasal swab (Machiels et al., 2000) which may be due to the presence of inhibitory substances in the faeces that can interfere
with nucleic acid extraction or amplification. Recently, a more advance technique known as quantitative PCR (qPCR) has been introduced for rapid detection and quantification of vapA positive strain of R. equi which can detect as small concentration of organism as 20 cfu/ml of trachea-bronchial aspirates (Radostits et al., 2016). It has good sensitivity (94%) and specificity (72%) as compared to cultural examination of tracheobronchial aspirate which is also time consuming and complicated but offers the advantage of detecting other pathogenic microbes present, and permits in vitro susceptibility testing of recovered pathogens. Hence, it is recommended to perform PCR amplification of vapA gene in conjunction with bacterial culture. It can be used as alternative to tracheobronchial aspiration for the diagnosis of R. equi in suspected foals but results should be applied on other population cautiously because of concentration of R. equi vary with geographical location and managemental practice (Shaw et al., 2015). Gross postmortem findings include large abscesses with creamy and cheesy pus in lungs. In the cases of GIT involvement, usually a single large abscess is found (Dedar et al., 2017). On histopathological examination, neutrophilic infiltration is observed.

### Virulence gene Identification

Virulence of Rhodococcus equi is plasmid regulated (vapA and vapB gene family) and arrests phagosome maturation, assists in survival and replication within macrophage and causes host cell necrosis. The antigens and plasmids, associated with virulence of R. equi, can act as epidemiological biomarkers for the population.
diagnosis and Immunoprophylaxis of virulent R. equi (Takai et al., 1994). VapA-B type plasmid is associated with most of the infection in equines (Ocampo-Sosa, 2007). All virulent Indian isolates are having 99-100% similarities in vapA sequence analysis based on both nucleotide as well as amino acid showing that this gene is highly conserved among all available isolates (Duquesne et al., 2010). Most of the isolates from pneumatic foal have been identified to encode 80-90 kb plasmid mediated seven virulence associated proteins (vap), which are closely related to each other. These proteins are designated as VapA, VapC, VapD, VapE, VapF, VapG and VapH (Byrne et al., 2001 and Takai et al., 2000). Although plasmid mediated virulence factors are more important in pathogenicity of R. equi, there are many chromosomal mediated factors (aceA, NarG, htrA, PepD, ChoE etc) which cannot be ignored. Chromosome and plasmid gene acts in very cooperative fashion for the development of pathogenicity and virulence but presence of plasmid mediated vapA gene exacerbates virulence potential (Bargen and Albert, 2009). In our previous study, we have targeted Rhodococcus equi specific virulence associated genes Vap A gene 550 base pair (bp) and Vap C gene 700 base pair (bp) nucleotide sequence present on the virulent plasmid for amplification (Chhabra et al., 2015; Kumar et al., 2020). Nasal or faecal swab samples are placed in Rhodococcus equi specific CAZ-NB medium and incubated at 37°C for 72 hours under aerobic conditions for culture of Rhodococcus equi. After 2-3 days of incubation, DNA of cultured sample can be isolated by using commercially available bacterial genomic DNA isolation Kits. In one of our study, we have used Forward primer: 5’ACAAGACGTTTCTAAGGGCG3’ and Reverse Primer: 5’TTGTGCCAGCTACCAGAGGCC3’ for identification of pathogenic Vap A gene and Forward primer: 5’GGTGCGTCCATCCAAATCGA3’ and Reverse Primer: 5’GGTCAGGCTATCACCCTGT3’ for the identification of pathogenic Vap C gene (Kumar et al., 2020). After DNA extraction, PCR run at the annealing temperature of 51.7°C. Then gel electrophoresis of PCR products was carried out in 1% agarose gel stained with ethidium bromide dye. Gel electrophoresis should run the gel for one and half hour to complete separation of DNA bands. This procedure showed the DNA band of Vap A & C genes of 550 bp and 700 bp respectively in UV illuminator. If in above process the band observed in gel is of very low intensity, then the previously cultured fecal sample was inoculated in CAZ-NB supplemented Mueller Hinton Agar and incubated for 5-7 days. After 2-3 days of incubation on Mueller Hinton Agar, yellow colonies were observed. These yellow colonies of R. equi turned light pink after an incubation period of 6-7 days (Salmon pink). Then these specific colonies of R. equi were inoculated in the Mueller Hinton broth and incubated for 2-3 days. After 2-3 days of incubation samples showed a specific clump formation of the bacterial growth at the bottom of the culture tube which was visible after gentle shaking of culture tube. These yellow and salmon pink colonies and clump formation were specific for R equi positive samples (Badsiwal et al., 2015). In another study (Takai et al., 1991) plasmid content of ten different strains of R. equi (ATCC 6939, ATCC 33701, ATCC 33702, ATCC 33703, ATCC 33704, ATCC 33705, ATCC 33706, ATCC 33707, CE220, and Ll) were examined to assess the association of presence of plasmid with expression of 15-17 kDa antigen. Isolation of plasmid was done by modified alkaline analysis method (Birnboim and Doly, 1979). The bacteria were incubated at 37°C for 2 h in a buffer (containing 0.05 M Tris hydrochloride, 0.01 M EDTA, 0.05 M NaCl and 20% (wt/vol) sucrose and 5 mg of lysozyme per ml). Cellular lysis occurred in 3.0% (wt/vol) SDS in 0.05 M Tris hydrochloride buffer (pH 12.6) at 55°C for two hour. Precipitation of chromosomal DNA occurred with 5 M potassium acetate-acetate buffer (pH 4.8) when centrifuged at 10,000 x g for 15 min. The partially purified DNA was subjected to electrophoretic analysis for detection of plasmids. For restriction enzyme digestions, the plasmid DNA prepared by large-scale isolation was purified by means of cesium chloride-ethidium bromide density gradient centrifugation (Singer and Finnerty, 1988). Javed et al. (2017) also targeted the identification of virulence associated vapA gene which is plasmid regulated. They extracted DNA from purified bacterial colony by snap and chill method and stored at -20°C till further use. PCR was carried out in 25 μl reaction volume using 0.2 ml with final concentration of 3.50 mM MgCl2, 0.2 mM concentrations of each dNTPs and 2.5 μl of ×10 PCR buffer (Muscatello et al., 1997). Whole amplification process comprised of denaturation at 94°C for up to 2 min, followed by 40 cycles, each consisting of initial denaturation at 94°C for 1.5 min, annealing at 57°C for 1 min and extension at 72°C for 2 min followed by final extension at 72°C for 10 minutes. Forward and reverse primers used in this study were 5’-GACTCTTCACAAAGACGGT-3’ and 5’-TAGCGTGTTGCTGAGC-3' respectively.
Thus, we can say that both plasmid and chromosome can contribute to virulence of R. equi but the presence of plasmid associated Vap gene makes the organism more virulence. Different plasmid and chromosome mediated virulence factors are given in Table 2. qPCR is a rapid, sensitive, specific and reliable method for identification and amplification of gene sequence related to virulence of R. equi making the epidemiological studies more easy and accurate and facilitates early diagnosis of infection.

**Prevention and control**

**Early detection and treatment**

Horses have very large respiratory reserves, so the foals do not show significant clinical signs during early stage of infection. Majority of foals show clinical signs of respiratory distress only after extensive damage in the lungs. Control of R. equi depends on early detection of disease and antimicrobial treatment (Venner et al., 2012). For timely diagnosis of disease at farm, many practices can be opted out, such as culture of Rhodococcus equi from faecal sample or nasal swabs and detection of Vap A gene by using PCR. Ultrasonic screening of the foals and identification of the foals with lesion in lungs is also practiced at many farms. Blood examination and identification of foals showing high total leukocyte count (> 14000/cub mm) with increased neutrophil percentage (>75%) is also practiced at many farms for early diagnosis of the disease. Many studies suggest that at most of endemic farms on ultrasonographic examination, 30 to 70 percent foals are found positive for pulmonary abscessation (Slovis et al, 2005; McCracken and Slovis 2005; Venner et al., 2009). While at endemic farms, clinical signs of pneumonia are reported only in 5 to 20% foals (Chaffin et al., 2003). It shows that most of the foals infected with Rhodococcus equi recover without treatment. So mass treatment of visible healthy foals having lung abscessation with antibiotics is not required, even no significant difference is found in recovery from small lung abscesses between control and antibiotic treated foals (Venner et al., 2013).

**Therapeutics**

There are number of drugs and drug combinations which can be used successfully for the therapeutics and prophylaxis of R. equi infection. Some important drugs like erythromycin, clarithromycin, azithromycin, rifampin, ceftiofur, gentamicin, enrofloxacin, vancomycin, imipenem, or doxycycline (Giguere et. al., 2015) have been evaluated for their anti R. equi activity either in vivo or in vitro trial. Usually combination of rifampicin and macrolide antibiotics (erythromycin or azithromycin) is used for the treatment. Rifampicin @10 mg/kg body weight orally with azithromycin @10 mg/Kg orally for 40 days is successful (Giguere, 2017). Most foals with subclinical ultrasonographic pulmonary lesions recover without treatment and antibiotic administration to these foals is useless (Giguere, 2017). In severe cases,
Azithromycin was used parentally for initial 7 days. All the cases showed successful recovery except the cases where treatment was started very late. Erythromycin @ 25 mg/kg body weight can also be used as an alternative to azithromycin. Microflora of the colon and caecum of horse are very sensitive to the broad spectrum antibiotics. So, continuous treatment with antibiotic for 40 days may disturb it and cause diarrhea or colitis. If diarrhea is noticed during the treatment then treatment can be halted for 3 to 4 days and can be reinstated after recovery from the diarrhea. Dose of the medication should be counted carefully. Overdose of rifampicin is very risky and may cause colitis or typhicoculitis. Colitis has also been reported and observed by authors in the mares, whose foals were under treatment of rifampicin and macrolide. Exact cause of this colitis in mares is not ascertained, but it might be due to eating of foal’s faeces by the mares (John, 2016). Apart from conventional antimicrobial therapy, some nanoparticle-based drug (Liposomal gentamicin formulation), antimalarial drug (Chloroquine) and heavy metal (Gallium) have been successfully evaluated against R. equi infection. Liposomal gentamicin (LG), at the dose rate of 6.6 mg/kg IV is effective in the treatment of R. equi infection (Cohen et. al., 2016). Due to emergence of macrolide resistant strain of R. equi (Giguere et. al., 2010) and adverse effects of macrolides (Giguere et. al., 2011 and Stratton-Phelps et. al., 2000), LG can be a good alternative for the treatment of R. equi infection in foals. Chloroquine (CQ) is an antiprotozoal drug, widely used for treatment of malaria in human beings, decreases the intracellular replication of R. equi due to unavailability of iron resulting into death of pathogen (Gressler et. al., 2016). Use of Gallium as an antimicrobial agent for the chemoprophylaxis and therapeutic intervention in R. equi is also another latest approach but needs further research (Harrington et. al., 2006). Basis for the use of gallium as antimicrobial agent against R. equi is its ability to interfere iron metabolism and competitive binding to transferrin which results into acquisition of gallium instead of iron by bacteria, hence failure of normal redox cycle and inactivation of various intracellular enzymes (Bernstein, 1998) resulting in death of parasite. Usually subcutaneous or oral route is preferred to minimize the nephrotoxicity which is an important side effect of gallium and common with rapid intravenous injection.

**Vaccination**

Development of vaccine against intracellular pathogen like R. equi is challenging. Vaccine is not available against R. equi because both antibodies and cellular immune mediators provide protection against R. equi. A conserved surface antigen poly-N-acetyl glucosamine (PNAG) found on surface of several bacteria including R. equi can provide immunity in the foals and prevent development of pneumonia in 90% of foals (Benteley et. al., 2018). PNAG also provides immunity against several other bacteria. R. equi specific hyperimmune plasma is used to protect foals from clinical rhodococcal pneumonia (Sanz et. al., 2016). Aluminium adsorbed, inactivated bivalent (containing 10⁹ CFU/ml of R. equi serotype 1, and 1.5 × 10⁷ PFU/ml of equine herpesvirus-2) or monovalent (containing R. equi only) vaccine has been evaluated on pregnant mare (6 and 2 weeks prior to foaling) and newly born foals (twice at 3 and 5 week age) for their efficacy and found that vaccination of foal is more advantageous than pregnant mare (Varga et. al., 1997).

**Managemental Practices**

**Dung disposal**

Dung of the foals is most important source for Rhodococcus equi, so foal dung must be removed as soon as possible from the barn. Composting significantly reduces R. equi concentration in the dung (Huber, 2018). Aerosol infection via dust particle is the main source of infection. It is also observed that mostly aerosol infection occurs just after foaling. Studies carried out in western countries suggest that foals are more prone to R. equi infection in summer months because environment temperature in summer remains around 30°C, which is favourable temperature for growth of R. equi, while in India, it was found that foals born in rainy months were more prone to R. equi infection in summer months because environment temperature in summer remains around 30°C, which is favourable temperature for growth of R. equi, while in India, it was found that foals born in rainy months were more prone to R. equi infection, than the foals born in summer months (April, May, June). This is because very high temperature of summer (>40°C) do not favour the growth of R. equi. While after beginning of monsoon, temperature falls and increased humidity also favors the growth of R. equi. So, it can be said that, breeding should be planned in such a way that foaling should be avoided in rainy season.

**Antibiotic resistance**

Combination of rifampicin and macrolide antibiotics such as erythromycin and azithromycin is the only available treatment against R. equi in foals. Increase in the prevalence of macrolide and rifampicin resistant
isolates of *Rhodococcus equi* since 2007 is a matter of concern (Huber et al., 2019). There are many reports of resistant strains of *R. equi* (Burton et al., 2013; Giguère et al., 2010). Now strategy to treat subclinical infections by antibiotics is being changing and only limited to clinically affected foals (Lehna, 2019). Positive aspect of antibiotic resistant strains of *Rhodococcus equi* is that their fitness to grow is decreased. On repeated sub culturing, their resistance to rifampicin and macrolide can be lost (Willingham- Lane et al., 2019). Alternatively, plant compounds are also being reported with promising efficacy against *R. equi* (Kumar et al., 2020)

Farm management practices

Combined prophylactic measures including improved husbandry practices, decreased environmental and aerosol contamination levels, boosting innate immunity, use of hyperimmune plasma can be used to control the incidence of *R. equi* at a farm (Dawson et al., 2010). High density of foals at equine farm is one of the most important risk factors (Chaffin et al., 2003), that’s why most cases of *R. equi* in foals are encountered at big and organized farms. *Rhodococcus equi* infection is an aerosol infection. Probability of presence of *R. equi* in air with dust particles is more in the hot summer months, because of increased amount of dust in the environment due to fast winds. So, the foals born in summer months are more susceptible (Takai et al., 1987; Prescott et al., 1989, Prescott et al., 1993, Badsiwal et al., 2019). Though *R. equi* is a pathogen of foals but it can be shed in faeces by the many adult animals also.

Conclusion

*Rhodococcus equi* infection is one of the economically important diseases in large equine population causing widespread mortality in new born foals. Early detection and treatment of infection by rational use of antibiotics is pivotal to manage the clinical incidence in large organized stud farms. Good animal husbandry practices along with appropriate Immunoprophylaxis is the key to prevent the new infection and reduction in mortality. Adjustment of breeding schedule in such a way to avoid foaling in rainy season is another effective animal husbandry practice to prevent clinical disease and mortality in foals.

References


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