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# Prevalence of *Rhodococcus equi* in foals of west northern Rajasthan by Vap A and Vap C genes specific polymerase chain reaction

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**Abstract**
*Rhodococcus equi* infection is considered as the most common cause of severe pneumonia in foals and has a substantial impact on equine breeding farms because of its high prevalence and case fatality rate. The objective of the study was to identify prevalence of *Rhodococcus equi* in foals of west north Rajasthan. Fecal swab samples were collected and cultured on *Rhodococcus equi* specific CAZ-NB media and DNA sample was extracted. 100 foals screened from west north Rajasthan state, out of which 18 foals were found positive based on PCR results for the *Rhodococcus equi* infection as these samples showed the amplification of Vap A and Vap C genes. The prevalence of *Rhodococcus equi* in female foals was 26 per cent while the prevalence of *Rhodococcus equi* in male foals was 9 per cent.

**Keywords:** Pneumonia, *Rhodococcus equi*, Vap A, Vap C

## 1. Introduction
Equines are susceptible to a variety of infectious diseases spread through common feeding, watering utensils and tick vectors. Chances of the spread of infectious as well as vector borne diseases are more in draught equine population of urban areas where the contact among affected and healthy animals is frequent and inevitable [21]. *Rhodococcus equi* is a Gram positive facultative intracellular coccobacillus in the order *Actinomycetales* and is considered as the most common cause of pyogranulomatous pneumonia in foals [8, 11, 15, 19, 20, 22, 23]. *Rhodococcus equi* infection is considered as the most common cause of severe pneumonia in foals and has a substantial impact on equine breeding farms because of its high prevalence and case fatality rate.

*Rhodococcus equi* infections have also been associated with extrapulmonary disorders of foals, including enterocolitis, lymphadenitis, nonseptic polysynovitis and osteomyelitis [5,6] and diarrhoea in some cases [10]. Predominant routes of *Rhodococcus equi* infection are the respiratory and alimentary tracts [1, 9, 12, 16, 17, 19, 20, 22]. However, despite considerable advances in the understanding of *Rhodococcus equi* foal pneumonia in the past few years, efforts to control and prevent infection remain elusive. *Rhodococcus equi* is largely a soil inhabitant that survives and multiplies in the gastrointestinal tract, and it is found in the faecal matter of grazing animals [2]. It is often described as a coprophilic soil-associated actinomycete [3]. Pathogenicity island of *Rhodococcus equi* found within the plasmid contains seven Vap genes. Discovery of more Vap genes has taken this number to nine Vap genes Vap A, -B, -C, -D, -E, -F, -G, -H and -I.

The presence or absence of the Vap A gene can be used as a marker to show the presence or absence of the Vap A encoding virulence plasmid. Virulence plasmid negative strains of *R. equi* do not express Vap A gene and are therefore incapable of causing disease [13]. Additional genes carried on the virulent plasmid, Vap C, -D, and –E are tandemly arranged downstream of Vap A. These new genes are members of a gene family and encode proteins that are approximately 50% homologous to Vap A and each other. Vap C, -D, and -E are found only in *Rhodococcus equi* strains that express Vap A and are highly conserved in Vap A-positive isolates from both horses and humans [4].

Keeping the aforesaid facts in view, the present investigation was planned to study the prevalence of *Rhodococcus equi* infection in apparently healthy foals based on Vap A and Vap C genes by a specific polymerase chain reaction.

## 2. Materials and Methods

One hundred foals (below one year of age) irrespective of sex were screened for faecal shedding of *Rhodococcus equi*. These foals were screened from different stud farms located in west northern districts of Rajasthan. Information regarding owner's particulars, animals details, number of horses reared by the owner, age of foal, gender of foals, floor of foaling pan, type of soil, pH of soil, moisture of soil, preventive measures like soil change, firing of pan, application of lime on floor of pan, bedding material etc., frequency of removing foal faeces in a day, distance of dumping of faeces from pan and clinical signs of *Rhodococcus equi* pneumonia if any were recorded.

Faecal swab samples were collected (In triplicate) directly from the rectum of foals using sterile swabs from various stud farms. Faecal swab samples placed in *Rhodococcus equi* specific CAZ-NB Medium were incubated at 37 °C for 72 hours under aerobic conditions for the culture of *Rhodococcus equi*. DNA was extracted using commercially available DNA-Sure Blood Mini Kit NP-61107 Genetix Biotech Asia Pvt. Ltd (New Delhi). DNA quantification was carried out by the spectrophotometric method. *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 was targeted for amplification [7]. Agarose gel electrophoresis was carried out in a horizontal, submerged electrophoresis unit to check the integrity of DNA. Each DNA sample was mixed with a one-fifth volume of the gel loading buffer.

The PCR products were subjected to electrophoresis at 120 V/cm for 1 to 1.5 hr. (depending upon the length of the gel or till the dye migrated more than half of the length of the gel) in 1.5% agarose gel prepared in 1 x TBE buffer containing 0.5 ng/ml of ethidium bromide. The PCR products (10µl) were run along with 100 bp DNA ladder (Invitrogen) and amplicons were visualized under UVP Gel Doc Bio-imaging System.

PCR bands were designated on the based on their molecular size (length of DNA fragment amplified). The molecular size was estimated by comparing with molecular weight marker loaded simultaneously with each primer product in the gel. The distance run by amplified fragments from the well was translated to molecular size with reference molecular weight marker.

Previously cultured faecal samples were further streaked on CAZ-NB supplemented Mueller Hinton Agar and incubated for 6-7 days. After 2-3 days of incubation in Mueller Hinton Agar the yellow colonies of *R. equi* were observed (Fig: 1). These yellow colonies of *R. equi* PCR positive samples turned light pink after an incubation period of 6-7 days (salmon pink). Than these specific colonies of *R. equi* were inoculated in the Mueller Hinton broth and incubated it for 2-3 days.

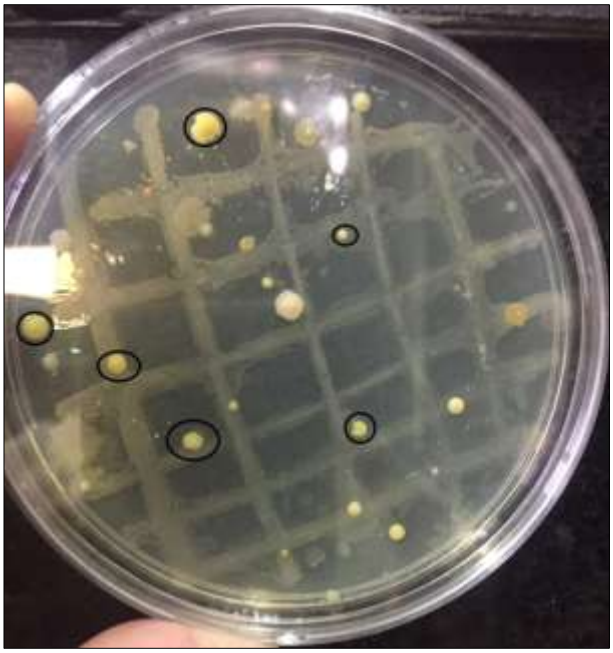

**Fig 1:** Yellow colour (In circles) colonies of *R. equi* after 2-3 days of incubation in CAZ-NB Supplemented Mueller Hinton agar.

After 2-3 days of incubation PCR positive samples showed a specific clump formation of the bacterial growth at the bottom of the culture tube which was the visible after gentle shaking of the culture tube. These yellow and salmon pink colonies and clump formation were specific in all *R. equi* positive samples. Then DNA was again isolated from these broths culture and PCR and gel electrophoresis were carried out. This time all the previously positive samples showed the high intensity DNA band of Vap A and C genes under Ultra Violet illuminator. This whole process of culturing the sample was thought to given the pure culture of *R. equi* by repeated culturing of bacteria in *R. equi* specific CAZ-NB medium.

The diagnosis was based on molecular typing of *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 of *Rhodococcus equi*.

## 3. Results and Discussions

Out of a total of 100 samples collected, 18 samples showed 550 and 700 bp bands on 1.5 per cent agarose gel for Vap A & Vap C genes (Fig: 3). In the present study prevalence of Vap A and Vap C bearing pathogenic *R. equi* was found to be 18 per cent. Among these 18 foals, 3 foals were found clinically ill (Table1, Fig: 2).

**Table 1:** Overall prevalence of *Rhodococcus equi* infection

| Particular | Number of foals | Per cent |
|---|---|---|
| Foals examined | 100 | - |
| Positive foals | 18 | 18 |
| Clinically ill foals | 3 | 3 |

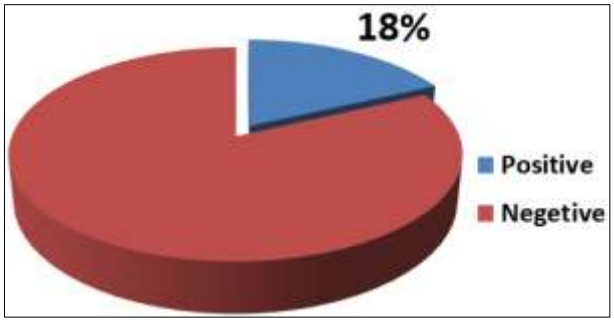


**Fig 2:** Prevalence of *R. equi* in foals

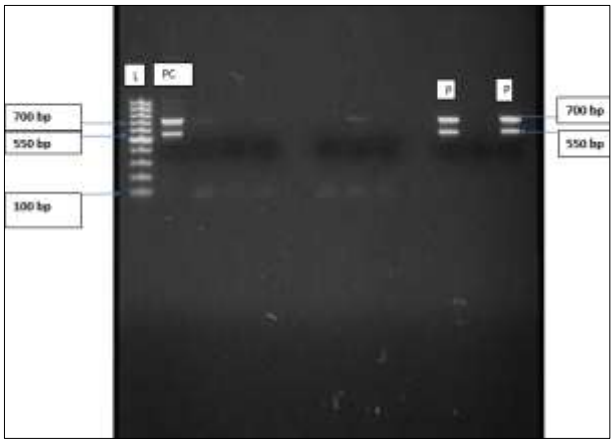


**Fig 3:** Amplification of 550 and 700 bp fragment of *R. equi* specific Vap A and Vap C genes, respectively, PC –Positive control, P – Positive sample, L –Ladder

Prevalence of *R. equi* in the present study was similar to the previous study by Kumar *et al*., (2014) [14] carried out in Bikaner district using swabs from the upper respiratory tract. This indicates that both the methods of sampling (faecal or upper respiratory tract swabs) are equally useful to study the prevalence of *R. equi* in foals. However, Kumar *et al*., (2014) [14] recorded a higher prevalence of *Rhodococcus equi* in horses owned by small scale owners (19.70%) as compared to that of the organized farm animals (15.71%). In the present study higher prevalence was observed in the organized farms having a large number of horses and having comparatively more number of foaling per year. Prevalence of *R. equi* in Rajasthan in the present study was found lower than Jammu & Kashmir, where the prevalence of *R. equi* in foals was found 26 per cent [18]. The disease is present worldwide and has highly variable patterns [20].

## 4. Conclusion

On the basis of above study it was concluded that the prevalence of R. equi infection in apparently healthy foals screened from some districts of Rajasthan state was 18% based on PCR of Vap A and Vap C genes. The result has a potential of substantial impact on equine breeding since this infection is considered to be one of the most common cause of severe pneumonia in foals.

## 5. Acknowledgement

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**6. Conflict of Interest:** The authors declare no conflict of interest.

## 7. References

1. Barton MD, Embury DH. Studies of the pathogenesis of *Rhodococcus equi* infection in foals. Australian Veterinary Journal. 1987;64:332-339.
2. Barton MD, Hughes KL. Ecology of *Rhodococcus equi*. Veterinary Microbiology. 1984;9(1):65-76.
3. Barton MD. The ecology and epidemiology of *Rhodococcus equi*. In Proceedings 6th International Conference on Equine Infectious Diseases. 1992;6:77-81.
4. Byrne BA, Prescott JF, Palmer GH, Takai S, Nicholson VM, Alperin DC, *et al.* Virulence plasmid of *Rhodococcus equi* contains inducible gene family encoding secreted proteins. Infection and Immunity. 2001;69:650-656.
5. Chaffin MK, Martens RJ. Extra pulmonary disorders associated with *Rhodococcus equi* pneumonia in foals: retrospective study of 61 cases (1988-1996). In Proceedings of Annual Convention of American Association of Equine Practitioner. 1997;43:79-80.
6. Chaffin MK, Honnas CM, Crabill MR, Schneiter HL, Brumbaugh GW, Briner RP. Cauda equina syndrome, diskospondylitis and a paravertebral abscess caused by *Rhodococcus equi* in a foal. Journal of the American Veterinary Medical Association. 1995;206:215-220.
7. Chhabra S, Khurana SK, Kapoor PK, Singha H, Singh Y, Khirbat R. Characterization of *Rhodococcus equi* isolates from foals with respiratory problems using a multiplex PCR for the Vap genes. Advances in Animal and Veterinary Science. 2015;3(1):28-32.
8. Cohen ND, Chaffin MK, Martens RJ. Control and prevention of *Rhodococcus equi* pneumonia in foals. Compendium on Continuing Education for the Practicing Veterinarian. 2000;22(11):1062-1070.
9. Dedar RK, Kumar L, Badsiwal D, Kumar S, Legha RA, Mehta SC, *et al*. Herbal disinfection against *Rhodococcus equi*. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases. 2020;41(1):19-30.
10. Dedar RK, Vaid RK, Anand T, Singh J, Virmani N, Khurana SK, *et al. Rhodococcus equi* diarrhoea and suppurative pneumonia in marwari filly: A case report. Veterinary Practitioner. 2017;18(2):245-248.
11. Giguère S, Prescott JF. Clinical manifestations, diagnosis, treatment, and prevention of *Rhodococcus equi* infections in foals. Veterinary Microbiology. 1997;56(3-4):313-334.
12. Hondalus MK. Pathogenesis and virulence of *Rhodococcus equi*. Veterinary Microbiology. 1997;56:257-268.
13. Jain S, Bloom BR, Hondalus MK. Deletion of Vap A encoding virulence associated protein A attenuates the intracellular actinomycete *Rhodococcus equi*. Molecular Microbiology. 2003;50:115-128.
14. Kumar K, Maherchandani S, Kashyap SK, Bohara DL, Vaid RK. Prevalence of *Rhodococcus equi* in equine environments in arid zone. Indian Journal of

Comparative Microbiology, Immunology and Infectious Diseases. 2014;35(1):34-38.
15. Kumar L, Sankhala LN, Dedar RK, Kant L, Badsiwal DK, Kumar S. Evaluation of in vitro antibacterial property of some plants of subtropical climate against *Rhodococcus equi*. Journal of Entomology and Zoology Studies. 2020;8(3):1590-1594.
16. Martens RJ, Fiske RA, Renshaw HW. Experimental subacute foal pneumonia induced by aerosol administration of *Corynebacterium equi*. Equine Veterinary Journal. 1982;14:111-116.
17. Martens RJ, Martens JG, Fiske RA. *Rhodococcus equi* foal pneumonia: pathogenesis and immune prophylaxis. In Proceedings of the Annual Convention of the American Association of Equine Practitioners. 1990;35:199-213.
18. Mir IA, Kumar B, Taku A, Bhardwaj RK, Bhat MA, Badroo GA. Prevalence and antibiogram study of *Rhodococcus equi* in equines of Jammu and Kashmir, India. Journal of equine science. 2015;26(1):21-24.
19. Prescott JF, Hoffman AM. *Rhodococcus equi*. Veterinary Clinics of North America, Equine Practice. 1993;9(2):375-384.
20. Prescott JF. *Rhodococcus equi:* An animal and human pathogen. Clinical Microbiology Review. 1991;4:20-34.
21. Radostits OM, Gay CC, Hinchcliff KW, Constable PD. A textbook of the diseases of cattle, horses, sheep, pigs and goats. Veterinary Medicine Ed.10; c2007. p. 896-920.
22. Takai S, Sasak Y, Tsubaki S. *Rhodococcus equi* infection in foals current concepts and implications for future research. Journal of Equine Science. 1995;6:105-119.
23. Takai S. Epidemiology of *Rhodococcus equi* infections, A review. Veterinary Microbiology. 1997;56:167-176.