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Rhodococcus equi on horse farms and factors influencing its prevalence and outcomes

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SUMMARY

This literature review examines the prevalence of *Rhodococcus equi* (*R. equi*) on horse farms, highlighting the multifaceted factors influencing its presence and impact. *R. equi* remains a critical pathogen in equine health due to its pervasive environmental presence and significant impact on foal morbidity and mortality. Its prevalence is shaped by a combination of environmental conditions, immunological vulnerabilities in foals and farm specific practices.

Advancements in diagnostic methods, such as PCR and thoracic ultrasonography, have improved the detection of clinical and subclinical cases of *R. equi* infection. However, limitations persist in achieving definitive diagnoses. Reliance on outdated biomarkers and blanket treatment protocols underscore the necessity for more precise diagnostic strategies

Treatment strategies have evolved over the years, with macrolide-rifampin combinations significantly improving survival rates. However, the emergence of MDR (multidrug resistant) *R. equi* strains poses a grave threat, emphasising the urgency for more judicious antimicrobial use and alternative treatments.

Preventative measures such as hyper-immune plasma and gallium maltoate have shown varying degrees of success. The absence of an effective vaccine remains a significant barrier to mitigating the prevalence of *R. equi* on horse farms. While promising vaccine strategies are in development, they must address the unique immunological challenges in foals, such as their immature cytokine responses and the potential interference of maternal antibodies.

The review emphasises that reducing prevalence of *R. equi* disease on horse farms requires a multidisciplinary approach. This includes refining management practises such as enhancing paddock conditions and ensuring a well ventilated, manure free stables.

The prevalence of *R. equi* is a man-made challenge, driven in part by the indiscriminate treatment of subclinically affected foals. Reconsidering these approaches can reduce reliance on antimicrobials and curb the spread of resistance.

ÖSSZEFOGLALÁS

Ez az irodalmi áttekintés a *Rhodococcus equi* (*R. equi*) lóállományokban való előfordulását vizsgálja, kiemelve azokat a sokrétű tényezőket, amelyek jelenlétét és hatását befolyásolják. A *R. equi* az állategészségügy egyik fontos kórokozója, mivel környezetben széleskörűen elterjedt és a csikók megbetegedésére és halálozására jelentős hatással van. Előfordulását a környezeti feltételek, a csikók immunológiai sérülékenysége és a farmra jellemző gyakorlatok együttesen alakítják.

A diagnosztikai módszerek fejlődése, például a PCR és a mellkasi ultrahangvizsgálat, javította az *R. equi* fertőzés klinikai és szubklinikai eseteinek kimutatását. Azonban a biztos diagnózis felállítása továbbra is korlátokba ütközik. Az elavult biomarkerekre és az általános kezelési protokollokra való támaszkodás rámutat arra, hogy szükség van pontosabb diagnosztikai stratégiákra.

A kezelési stratégiák az évek során fejlődtek, és a makrolid-rifampin kombinációk jelentősen javították a túlélési arányokat. Azonban a multidrog-rezisztens (MDR) *R. equi* törzsek megjelenése súlyos fenyegetést jelent, hangsúlyozva az antimikrobiális szerek körültekintőbb alkalmazásának és az alternatív kezelések kidolgozásának sürgősségét.

A megelőző intézkedések, például a hiperimmun plazma és a gallium-maltolát, változó mértékben bizonyultak sikeresnek. Az *R. equi* lóállományokban való előfordulásának mérséklésében továbbra is jelentős akadályt jelent a hatékony vakcina hiánya. Bár ígéretes vakcinák fejlesztése folyamatban van, ezeknek meg kell birkózniuk a csikók egyedi immunológiai kihívásaival, például az éretlen citokinválaszokkal és az anyai antitestek lehetséges zavaró hatásával.

A tanulmány hangsúlyozza, hogy az *R. equi* betegség előfordulásának csökkentése a lóállományokban multidiszciplináris megközelítést igényel. Ez magában foglalja a gazdálkodási gyakorlatok finomítását, például a kifutók körülményeinek javítását, valamint jól szellőző, trágyamentes istállók biztosítását.

Az MDR *R. equi* elterjedése emberi tényezőkre vezethető vissza, amely részben a szubklinikai esetek válogatás nélküli kezeléséből ered. Ezeknek a megközelítéseknek az újragondolása csökkentheti az antimikrobiális szerekre való támaszkodást, és visszaszoríthatja a rezisztencia terjedését.

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ABBREVIATIONS

AMR – Antimicrobial resistance

AMRG - Antimicrobial resistance genes

BALF – Bronchoalveolar lavage fluid

CFU – Colony Forming Unit

HIP – Hyper Immune Plasma

HIV – Human Immunodeficiency virus

IFN – Interferon

Ig – Immunoglobulin

kDa – Kilo Dalton

MDR – Multi Drug Resistance

MIC - Minimum inhibitory concentration

PCR – Polymerase Chain Reaction

qPCR – Quantitative Polymerase Chain Reaction

R. equi – *Rhodococcus equi*

TBA - Tracheobronchial aspirate

Vap – Virulence associated protein

1. INTRODUCTION

Rhodococcus (R.) equi is a well-recognized bacterium on horse farms. It is a major cause of clinical disease in young foals, which can lead to decreased performance in their future careers, and in severe cases, death. Additionally, the zoonotic potential of *R. equi* makes it a noteworthy bacterium from a public health perspective.

Despite its impact, there are currently no licensed vaccines against *R. equi* on today's markets, necessitating a heavy reliance on antimicrobials for disease control. However, the increasing prevalence of multidrug resistant strains of *R. equi* has raised concerns about the sustainability of this approach and highlighted the need for a deeper understanding of the factors influencing the bacterium's prevalence and clinical outcomes on horse farms.

This literature review aims to provide a comprehensive overview of the factors influencing the prevalence of *R. equi* on horse farms and their outcomes.

The main objective of this review is to examine existing findings on topics such as epidemiology, environmental and management practises as well as examine the impact of antimicrobial treatment and lack of preventative treatment.

This review will evaluate the current literature to get a more informed perspective on the factors influencing the prevalence of *R. equi* and outcomes on horse farms in the world today.

2. LITERATURE REVIEW

2.1. DEFINITION

Rhodococcus equi (*R. equi*) formerly known as *Corynebacterium equi,* was initially described by Magnusson in 1923 as a causative agent of purulent bronchopneumonia in young foals in Sweden [1]. However, German botanist Wilhem Friedrich Zopf first used the name *Rhodococcus* ('red coccus') in 1891 while classifying pigment producing fungal and bacterial organisms [2]. Today *R. equi* has worldwide distribution and can be found in the environment as well as in manure of healthy herbivores [3]. It can be defined, as an opportunistic pathogen is a significant cause of pneumonia in 3 week to 6-month-old foals as well as other immunocompromised hosts [4]

R. equi is a gram-positive, non-motile, obligate aerobic asporogenous bacterium. *R. equi* grows optimally at 30°C but can grow at temperatures from 10°C – 40°C [5]. Colonies form on solid media in less than 48 hours and appear round, smooth and mucoid. The characteristic salmon pink colour, from which the bacterium got its name, does not appear until the culture is older, at around day 4-7 [6]. Its morphology varies from bacillary to coccoid, depending on growth conditions. Furthermore, *R. equi* is catalase positive, mostly urease positive and oxidase negative. The bacterium is surrounded by an antigenically variable, thick and lamellar polysaccharide capsule with at least twenty-seven different serotypes having been described [5].

The bacterium is a member of the order Actinomycetales, suborder Corynebacterineae, family Nocardiaceae and genus *Rhodococcus* [7]. There is a possibility that *R. equi* might undergo a taxonomic reclassification in the future due to advances in genetic and phylogenetic analysis. *Prescotella equi* and *Rhodococcus hoagii* have been proposed [8], however for the purpose of this literature review, we will use the term *Rhodococcus equi (R. equi)*

2.2. Virulence and Plasmids

Virulent strains of *R. equi* carry a virulence plasmid and express a highly immunogenic surface protein called virulence associated protein A (VapA). This protein is essential for the bacterium's' intracellular survival inside macrophages. Strains lacking the VapA gene cannot successfully replicate inside the macrophages and therefore considered avirulent to foals. [9]. Most clinically affected foals carry VapA positive strains so clinical samples should be tested for the presence of the VapA gene to confirm virulence [10].

To date, three host-associated virulence plasmid types of *R. equi* have been identified as follows: the circular pVapA and pVapB, related to equine and porcine isolates in 1991 and 1995, respectively [11], and a recently described linear pVapN (N indicating negative A, B) plasmid associated with bovine and caprine strains in 2015 [12].

The discovery or the virulence associated 15 to 17 kDa protein antigen A (VapA) and the pVapA plasmid in 1991 significantly advanced the understanding of the pathogenesis, diagnosis, and molecular epidemiology of *R. equi* on horse farms [13]. Epidemiological studies have now shown 14 distinct pVapA subtypes and their geographical preferences [11].

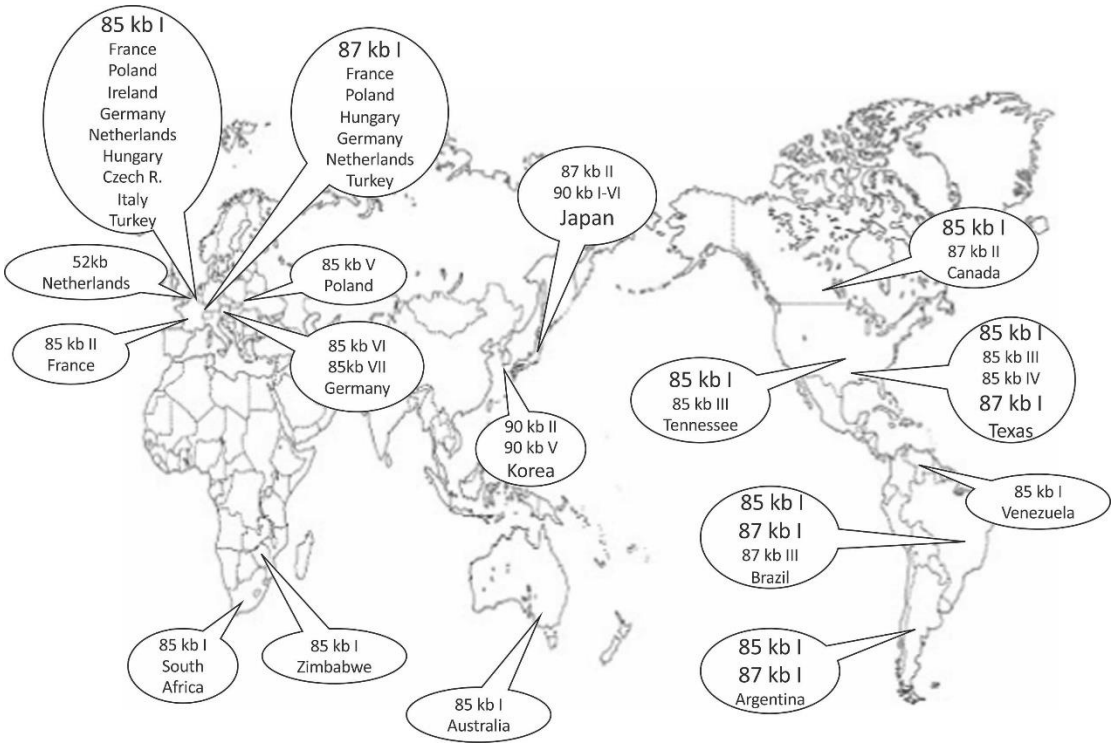

Figure 1. Distribution of pVAPA plasmid subtypes in the world [11]

The smallest plasmid, a 52-kb variant, was found in an isolate from the Netherlands. The most common plasmid type, the 85-kb type I , has been found in isolates from various countries in Europe like Netherlands, France, Germany, Hungary, Poland Czech Republic, Italy, Turkey, Ireland. Furthermore in North and South America, Africa (South Africa and Zimbabwe) and Australia [11]. Molecular studies have shown that the distribution patterns of plasmid subtypes are influenced by the migration of horses, underlining the role of plasmid movement in the global prevalence of *R. equi* on horse farms [11, 14, 15].

2.3. Multi-host pathogen

R. equi is zoonotic with the first documented human infection reported in 1967. A stockyard worker, responsible for cleaning animal pens developed subcutaneous abscesses but responded well to treatment with erythromycin with no reoccurrence of infection [16]. Additionally, *R. equi* has been isolated as part of the resident microbiota of a healthy nasal cavity in human, where it can potentially become an opportunistic pathogen in immunocompromised patients [17].

Furthermore, *R. equi* has been isolated from various sources, including contaminated wounds, abscesses and pyogranulomatous lesions of muscles, bones, body organs like liver, spleen, lymph nodes, eyes trachea, vagina, joints and lungs of infected foals, cats, dogs, goats, cattle including immunocompromised humans and many more wild animal species like monkeys and wild boars [15,18].

R. equi is also regularly isolated in the retropharyngeal, bronchial and mediastinal lymph nodes of cattle. An abattoir survey in Ireland conducted between 1997 and 1998 determined that 4% of bacteriologically examined tuberculosis like lymph node granulomas in cattle contained *R. equi*. The prevalence in the total population of 3.3 million cattle examined post mortem was 0.008%. Interestingly there was a lower prevalence of *R. equi* induced lesions in older cattle. *R. equi* granulomas were present in a significantly higher proportion of the lesions detected in steers and heifers compared to cows, displaying perhaps older cattle have a greater resistance to infection and have the potential to overcome the infection thus leading to regression of lesions in lymph nodes [18].

Since the first description of human *R. equi* infection in 1968, over 300 cases have been reported [16, 19]. Most patients who become infected with *R. equi* are immunocompromised, such as those infected with human immunodeficiency virus (HIV), recipients of organ transplantation, and cancer patients [15]. The most common manifestation of the disease is pneumonia occurring in around 80% of human cases [20]. Other clinical manifestations include pyrexia, diarrhoea, abscesses in the thyroid gland, brain, meninges and peritoneum, lymphadenitis, pericarditis, bone and joint inflammation [21]. The mortality rate among immunocompetent patients is approximately 11%, it rises to 50-55% among HIV infected patients and 20-25 % among non-HIV infected immunocompromised patients [21].

Possible routes of *R. equi* transmission to humans include exposure to contaminated manure, especially from foals and soil as well as inhalation and inoculation into a wound or mucous

membrane. Horizontal transmission between humans has been scarcely reported [22]. Proven links with horses are not always apparent and some reports and case series have failed to identify any such associations. For example, there have been reports of food borne transmission from pigs, which can contribute to the probability of disease development in human patients. Undercooked swine, cattle and horse carcasses should be acknowledged as a potential source of human infection, though not the primary route. This risk is particularly relevant in countries where the consumption of undercooked or raw beef or horsemeat is a traditional practise [22].

In a study done in 2007, no VapB (pig-type) plasmid were found in equine isolates, no VapA (horse-type) plasmid was identified among bovine isolates, and no VapN (bovine-type) plasmid was found among porcine isolates. By contrast, the three-plasmid types were common among *R. equi* strains from humans (11%, 55%, and 34% of plasmid-positive isolates, respectively), a host in which the infection is opportunistic and associated with immunosuppression [23]. These findings suggest that host immunity influences the selection of specific plasmid types, where plasmid type determines *R. equi* host tropism in naturally susceptible species.

2.4. Pathogenesis

R. equi primarily exists as an environmental saprotroph thriving in soil, its main reservoir. The bacteria uses manure as a growth substrate, explaining its abundance in farming habitats. Moreover, *R. equi* can also multiply in the intestine contributing to its dissemination in the environment through the faecal oral cycle [24].

Possible routes of transmission of *R. equi* are inhalation, inoculation of wound and mucous membrane or transcutaneous infection or contamination of wound, ingestion or food borne transmission, and dissemination to distant sites by haematogenous route. In foals, the predominant route of transmission is inhalational [25]. Inversely in cats and dogs, most common route of transmission is transcutaneous infection of wounds [15].

The pathogenesis of the disease commences with transmission of the bacterium through inhalation of *R. equi* contaminated dust. *R. equi* is taken up by the lung macrophages, which serve as the primary site for its survival and replication [25]. Within alveolar macrophages *R. equi* evades phagosome-lysosome fusion allowing unregulated multiplication of the bacterium inside the macrophages [4]. This process leads to macrophage death through necrosis,

triggering a strongly pro-inflammatory response [26]. The cascade of pro-inflammatory signals ultimately leads to abscess formation and tissue destruction [27]

This in turn leads to the bacterial colonization in the lungs and the development of pneumonia [25, 27]. The incubation period is around 18 days, with clinical signs appearing after a week. There are marked seasonal and individual variations in response to infection. Experimental infections with intra bronchial challenge show varying incubation times, ranging from 9 days with a substantial inoculum to about 2-4 weeks with a lower inoculum [28, 29].

2.5. Clinical signs

R. equi infection can result in a diverse range of clinical manifestations, encompassing both pulmonary and extrapulmonary disorders. Usually foals first manifest clinical signs of *R .equi* pneumonia between 3 and 24 weeks of life, with most foals showing clinical signs before 16 weeks of age. Bronchopneumonia is the most common manifestation of rhodococcal disease that affects foals. [4]. Clinical signs are fever, lethargy, cough as it progresses, anorexia, tachycardia, tachypnoea, flared nostrils, increased effort and abdominal excursion during respiration. Nasal discharge can be observed in fewer cases. [30].

Examples of extrapulmonary disorders described in the literature are polysynovitis, abdominal abscesses, diarrhoea, pyogranulomatous typhlocolitis, abdominal lymhadenitis and uveitis. A wide array of extrapulmonary disorders can occur either alone or concomitant with pneumonia. Reus et al., found that of 150 foals aged between 3 weeks and 6 months of age, that had already a diagnosis of *R. equi* infection, 74% had at least one extrapulmonary disorder along with *R. equi* pneumonia or ill thrift [31]. Some extrapulmonary disorders for example intra-abdominal abscesses markedly worsen the prognosis for survival, and can develop in the face of successful management of pneumonia. Survival of foals with extrapulmonary disorders is around 43% compared to foals without extrapulmonary disorders [4, 31].

The diverse range of clinical signs can be attributed to the various methods of bacterial transmission. In foals, inhalation is the primary route although transcutaneous transmission can also occur. Additionally, the bacterium’s ability to disseminate hematogenously contributes to the complexity of the disease [15]. Acknowledging the diverse manifestations of the *R. equi* induced disease is essential for accurate diagnosis disease and assessing the prevalence of *R. equi* on horse farms.

2.6. Diagnosis

A presumptuous diagnosis of rhodococcosis is based on clinical signs, history of disease on farm, X-ray and haematology. However, for a more definitive answer, the use of culture and fluid analysis of tracheobronchial aspirate (TBA) in cases of pneumonia or by culture of a sample obtained from an extrapulomnary site can be used [10]. In recent times, vets often choose the direction of presumptive diagnosis in farms with a history of the disease *in lieu* of obtaining a definitive diagnosis with culture. Their reasoning being the health risk for foals, time and physical effort for the procedure, and costs for TBA and associated cytological and microbiological testing, is too high [30].

2.6.1. Bacterial Culture and PCR

Bacteriological culture or amplification of the Vap A gene by polymerase chain reaction (PCR) from a TBA are the only acceptable ways of establishing a diagnosis [30]. Bacterial culturing can take up to 72 hours and in reality, most vets just opt for antimicrobials that are broad-spectrum during the waiting period.

Real-time quantitative polymerase chain reaction (qPCR) is another diagnostic tool for *R. equi* infection. It detects *R. equi* by amplifying the vapA gene in from TBA and from faeces. While effective, qPCR has limitations; it does not identify other bacterial species present that could be causing the infection.

Interpretation of qPCR on faecal samples results is complicated by the ecology of *R. equi*, as affected and unaffected foals shed it, thus there is a potential for false positive results. The specificity of faecal testing is further complicated by the presence of foals with subclinical *R. equi* pneumonia that will not develop disease but may shed virulent *R. equi* in faeces [32]. Additionally, it does not preserve live *R. equi*, which is necessary for conducting antimicrobial susceptibility tests or for genotyping or phenotyping of strains for clinical or epidemiological analysis.

2.6.2. Thoracic Ultrasonography

The use of ultrasound to screen foals before weaning has improved the ability to identify foals with subclinical pulmonary rhodococcosis in equine practice [33]. Often large stud farms especially thoroughbred studs across the USA, Ireland and Germany have adapted their breeding programme to include a mandatory ultrasound screening of young foals. The areas of the lung affected by *R. equi* are often hypoechoic lacking the normal air echo at the surface.

Additionally, abscesses can be identified by their cavitated appearance and the loss of normal pulmonary structure within the affected area [33].

Lesions detected on ultrasonography can vary in size and many studies describe different methods of grading these pulmonary abscesses. Slovis, for example uses the grading scale where the severity of pneumonia is determined not by the total number of lesions visible, but by the highest grade observed.

Grade	Description
Grade 0	No evidence of pulmonary consolidation. Pleural irregularities appear as vertical hyperechoic lines and are described as reverberation artefacts
Grade 1	Lesions are <1 cm in diameter/depth
Grade 2	Lesions are 1.0-2.0 cm in size
Grade 3	Lesions are 2.0 – 3.0 cm in size
Grade 4	Lesions are 3.0-4.0 cm in size
Grade 5	Lesions are 4.0-5.0 cm in size
Grade 6	Lesions are 5.0 – 6.0 cm in size
Grade 7	Lesions are 6.0 -7.0 cm in size
Grade 8	Lesions are 7.0 – 9.0 cm in size. If pleural effusion is present if lesser grades of consolidation or abcessation are present
Grade 9	Lesions are 9.0 – 11.0 cm in size
Grade 10	The entire lung is affected

Table 1: Grading of pulmonary abscesses by Slovis [32]

2.6.3. Serological Testing

Serological method, such as agar gel immune diffusion (AGID) and ELISA tests are not reliable for diagnosing *R. equi* infection due to their low sensitivity or specificity or both. The detection of antibodies through these tests only indicates exposure, subclinical infection or passive transfer of antibodies. It does not necessarily indicate infection leading to clinical disease [34].

2.6.4 Other Diagnostic Methods

Laboratory findings associated with *R. equi* pneumonia often include elevated white blood cell count, fibrinogen levels and serum amyloid A [35]. However, these markers alone are insufficient for a definitive diagnosis and primarily serve as indicators of subclinical disease. Their main role is to prompt further diagnostic investigation to confirm the presence of *R. equi* pneumonia or rule out other underlying conditions.

Accurate and timely diagnosis of *R. equi* infection is of the utmost importance as it enables appropriate and prompt treatment, reducing the severity of the disease, additionally clarifying whether it is a case of a clinical vs subclinical infection.

3. EPIDEMIOLOGY OF *R. EQUI* IN HORSE FARMS

R. equi can be cultured from the environment of virtually all horse farms, the clinical disease is endemic and devastating in some farms, sporadic on others, and unrecognised on most. On farms where the disease is endemic, costs associated with veterinary care, long-term therapy and mortality of some foals may be very high [36]. A study in 1998 even linked infection as a foal to decreased racing performance as an adult [37].

There is considerable variation in reports on annual incidence on farms where the disease is endemic. One study in America describes that incidence of disease is believed to be 10-20% in foals from birth until weaning [30], while in Australia; it is estimated that *R. equi* affects 1 to 10% of foals each year. Mortalities ranged from less than 1%, to occasional outbreaks occur reaching 20%. Clinical disease had a higher prevalence in New South Wales with mainly thoroughbred racing foals affected [38]. Furthermore, in Poland, clinical pulmonary rhodococcosis affects approximately 25% of the population of foals, while subclinical pulmonary rhodococcosis appears to be present in approximately 70% of foals [39]. The prevalence and incidence of *R. equi* pneumonia are typically seasonal, peaking in late spring and summer months when the number of susceptible hosts is high corresponding with a risk of aerosol challenge from the environment or stable mates [40].

Interestingly, virulent *R. equi* isolates from soil and faeces of foals on a farms with endemic *R. equi* infections was significantly higher than that of farms with no history of the disease. The critical factor is whether the *R. equi* to which they are exposed is virulent or avirulent, as this significantly influences the risk of disease development. The proportion of virulent *R. equi*, those which contain pVapA, may range from <2% to 23% of all isolates [41]. Differences in the prevalence of the disease might reflect in variation in environmental conditions such as temperature, dust and management practices and differences in the number of virulent isolates in the environment [13, 42]. Soil concentration of *R. equi* at horse breeding farms is usually between 10^2 to 10^5 colony forming units (CFU) per gram of soil irrespective of farm history of pneumonia [43]

Mares are not a clinically important source of *R. equi* for their foals. One hundred and seventy one mares and foals from a farm in Kentucky found that dams of foals with *R. equi* associated pneumonia did not shed more *R. equi* in faeces than dams of unaffected foals. *R. equi* infection in foals is not associated with comparatively greater faecal shedding by their dams.

However detection of virulent *R. equi* in faeces of all mares at least at one time point suggests that mares can be an important source of *R. equi* for the surrounding environment [44].

Identifying the environment and management factors that drive these variations is key to mitigating the prevalence and impact of *R. equi* on horse farms.

4. ECOLOGY & MANAGEMENT PRACTICES

The ecology and management practises surrounding *R. equi* on horse farms is highly influential on both the prevalence of the bacterium and its clinical outcomes. Some of those ecological factors and management practises include soil and air concentrations, faeces and stable bedding as well diet.

4.1.Soil and Air Concentrations

A study conducted on Australian thoroughbred farms in 2006 investigated the relationship between the ecology of virulent *R. equi* and the epidemiology of *R. equi* pneumonia. It revealed the prevalence of *R. equi* pneumonia correlated with the airborne burden of virulent *R. equi*, while no such association was found with the soil burden of virulent *R. equi* [40]. Furthermore, a study in Kentucky concluded that variations in soil concentration of virulent *R. equi* or the proportion of virulent isolates in the soil do not predict or correlate with the incidence of foals with pneumonia attributable to *R. equi* [45].

A study conducted on three breeding farms in Ireland, endemic with *R. equi* and situated in a temperate climate, highlighted that air in the stables of endemically affected farms may be a major source of virulent *R. equi* for infected foals. Air samples were collected sequentially over the 2003 foaling season from the paddocks and stables on three Irish horse-breeding farms affected by *R. equi* pneumonia [40]. The odds of detecting airborne virulent *R. equi* in stables was found to be 17.3 times greater than in paddocks. Confined foals in stables face heightened exposure to dust and aerosolized bedding material contaminated with virulent *R. equi*, originating from mare and foal faeces. Conversely, airborne virulent *R. equi* concentrations was lower in paddocks, reducing the likelihood of direct contamination from heavily soiled areas [40]. Additionally, foals housed in stalls are more likely to be exposed to airborne virulent *R. equi* compared to those in paddocks, particularly earlier in the foaling season (January and February) compared to later months (May and June) [41].

Therefore, implementing management strategies to enhance air hygiene in stables or reducing the duration which foals are stabled may effectively mitigate the prevalence and severity of *R. equi* pneumonia on farms in temperate climates where stabling of mares and foals is necessary.

More recently, a 2013 study conducted on horse farms in Texas, which is characterised as a subtropical or desert climate confirmed these findings. The study exhibited that concentrations of virulent *R. equi* in air samples from stalls housing foals that developed *R.*

equi pneumonia were significantly higher than those from foals that did not develop pneumonia [46]. They attributed this to high density of mares and foals in the affected barn leading to an increase in airborne concentrations of *R. equi* and acknowledged that it was plausible that strategies like controlling ventilation to ensure good air hygiene might reduce the risk of infection.

4.2.Ecology

Environmental conditions play a crucial role in the development of *R. equi* pneumonia in foals. Exposure of foals to airborne virulent *R. equi* during the first 2 weeks after birth is significantly associated with the disease's onset [46]. The concentration of *R. equi* in the air is higher during dry, windy conditions compared to damp, calm weather conditions [21]. Warm ambient temperature, low pasture height and dry soil contribute to elevated airborne *R. equi* levels. Areas with less grass cover and low soil moisture have higher concentrations of airborne *R. equi*, increasing the risk for foals spending more time in these environments [42]. These findings suggest that land management practices such as maintaining soil moisture and ensuring full grass cover could potentially help reduce the prevalence of *R. equi* on horse farms; however, this has yet to be conclusively proven.

4.3.Faeces and stable bedding

Growth of *R. equi* is enhanced by volatile fatty acids in horse faeces, which support bacterial growth and survival. Optimal conditions for bacterial growth include a temperature of approximately 30 degrees Celsius, aerobic environment and a pH 7.5 - 8. Faecal enriched soil promotes better bacterial growth compared to soil alone [47].

Housing foals in stalls with dirt floors increases the risk for development of *R. equi* pneumonia [47]. Potentially fragile bedding materials, such as matting, shredded paper or cardboard, may be less likely to generate dust contaminated with virulent *R. equi* and therefore reduce the prevalence of *R. equi* pneumonia [42]. However, another study suggested there was no significant differences in the bedding used in the stalls between affected and unaffected farms [48]. The use of cardboard litter stables has been advocated in the management of horses with respiratory diseases based on its capacity to reduce significantly the concentrations of airborne fungal and bacteria spores [49]. Nevertheless, establishment of good hygiene and manure disposal protocols on horse farms can aid in the reduction of *R. equi* prevalence on horse farms.

4.4.Management

Chaffin investigated whether specific farm management and preventative health practices contribute to the development of *R. equi* pneumonia in foals on farms. Personnel on affected farms were observed to be more involved in attending foal births, testing foals for adequate or passive immunity, administering plasma or other treatments to foals to supplement serum immunoglobulin (Ig) concentrations, providing hyperimmune plasma prophylactically to foals, vaccinating mares and foals against *Streptococcus equi* infection and using multiple anthelmintic. Interestingly, several recommended management practises appeared to correlate with an increasing risk of foals developing of *R. equi* pneumonia. The study did remark it is plausible that farms with better management practices observe foals more closely and are therefore, more likely to detect foals with *R. equi* pneumonia [47].

4.5.Birth Month

Birth month may be risk factor for developing *R. equi* pneumonia in foals. The incidence of *R. equi* pneumonia increases with the increase in outside temperature, possibly due to intensified management of equine breeding farms and climate change. A study in Japan revealed that the rate and density of tracheal colonization of *R. equi* in foals varied by birth month, gradually increasing with the rise in outside temperature. The highest percentage of *R. equi* positive foals, based on clinical signs and microbiological culture from transtracheal aspiration, were those born in March and April. It is noted that the foals born in February and early March were scarcely exposed to soil-dwelling *R. equi*, since the surface of paddocks or fields had been covered with snow or ice from December to the end of March [50]. This suggests that a certain period during the foaling season may be positively correlated with the development of *R. equi* pneumonia, highlighting the impact of climate change even in veterinary and equine breeding fields.

4.6.Diet

Another interesting hypothesis suggests a correlation between dietary management and the severity of some bacterial infections. Preliminary studies have revealed that a high-protein and high-carbohydrate diet may lead to the development or exacerbation of clinical signs in strangles, a well-known equine disease [51]. This paper describes the relationship between an excessive diet and its influence on the production of cell wall polysaccharides, which are one of the major pathogenicity factors in many bacteria, including *Streptococcus*, *Pasteurella* and *Clostridium* but more importantly *Rhodococcus*. It was found that dietary ingredients

enhanced *Streptococcus equi* capsular polysaccharides, which protect bacteria from the immune system and phagocytosis. Since the equine gastrointestinal tract and gut microbiota are adapted to a diet very high in fibre, an over nutritious diet may trigger intestinal dysbiosis and eventually diminish natural immunity.

It further raises the question of whether birth month as a risk factor for the development of *R. equi* in foals is connected to diet, given that later months in spring bring grasses with higher protein content. A diet rich in protein content can potentially lead to over nutrition of foals, exacerbating clinical signs of bacterial diseases. However, further in-depth research is needed to investigate this possibility.

5. IMMUNITY

Horses' individual immunity can significantly influence the prevalence of *R. equi* on horse farms. Resistance to disease caused by *R. equi* varies from horse to horse. Some papers allude to immunological differences in different age groups as a potential contributing factor to varied prevalence on horse farms. Newborn foals are immunologically immature and exhibit various immune system deficiencies compared to older horses. Pneumonia caused by *R. equi* appears to be restricted to foals. Essentially horses older than 1 year are rarely affected, and when mature horses are affected, there is usually an accompanying immunodeficiency [30].

5.1.Importance of Passive Transfer

The epitheliochorial structure of the equine placenta prevents transfer of maternal immunoglobulins to the foetus. Thus, humoral protection of the newborn foal depends on the absorption of pre-formed antibodies from the colostrum immediately after birth. The highest absorptive capacity of the neonatal gut immunoglobulins is around 6-8 hours, declining afterwards and ending by 24 to 36 hours of age [52]. Failure of the passive transfer via colostrum leads to increased susceptibility of the foal to infection from environmental pathogens such as *R. equi*. Even with sufficient IgG, foals are susceptible to certain pathogens that rarely affect adult horses such as *R. equi*. The reasons for this phenomenon remain to be determined; nonetheless, certain immunological differences between foals and adults may provide insight.

Immunity to intracellular pathogens like *R. equi* is primarily driven by adaptive T lymphocyte (T cells) responses [53]. T cells are responsible for the production of cytokines like CD4+ T helper type 1 cells. Th1 immune responses are characterised by their product of interferon (IFN)-γ. Newborn foals have a considerable inability to express the IFN-γ gene and produce the IFN-γ compared to adult horses [54]. This was in the case of both circulating lymphocytes and pulmonary lymphocytes. However, the ability of foal lymphocytes to produce IFN- γ steadily increases as they age, starting from 1 week of age and is reaching adult levels as early as 3 months of age. It is potentially possible that this deficiency has led to the increased susceptibility of young foals to *R. equi* infection. Thus explaining the high prevalence of pneumonia *R. equi* infection among foals [54].

In addition to the passively acquired antibodies obtained through colostrum, foals also depend on other components of the innate immune system to provide further protection against *R. equi* infection.

5.2.Opsonisation

Innate immune responses are crucial for protection against *R .equi* in neonates as there is an initial delay until adaptive immune responses will be fully developed. Phagocytic function is imperative until more complete acquired immunity develops [55]. Once inhaled *R. equi* is taken up by macrophages in the lungs and its inherent ability to modify phagosome-lysosome fusion allows for its uncontrolled replication [4]. One such immune process to counteract this, which allows foals to resist the infection, is opsonisation. Opsonisation is an immune process that uses opsonins to tag foreign pathogens for elimination [56]. It seems that oponising IgG antibodies have a potential role in facilitating the phagocytosis of *R. equi* and supressing intracellular growth by enhancing bacterial elimination [55]. Highlighting the importance of *R. equi* antibodies in preventing infection.

The aim of understanding the equine immunity against *R. equi* will help in the development of immunoprophylaxis. Such methods including vaccines and the use of hyper-immune plasma (HIP) can hopefully reduce the prevalence of *R. equi* on horse farms.

6. TREATMENT

Pneumonia caused by *R. equi* is a slowly progressive disease that leads to significant economic losses due to high costs of prolonged treatment as well as reduced performance in racing as adults [37].

Control and early detection of disease using thoracic ultrasonography and starting the foal on a treatment of antimicrobials prior to development of clinical signs appears to decrease mortality due to *R. equi* pneumonia on farms with endemic disease [35].

Since .*R. equi* is a facultative intracellular pathogen that can survive and replicate in macrophages [4], effective antibiotics must have strong cell-penetrating abilities and high lipophilicity. Selecting lipid-soluble antibiotics with good intracellular penetration is considered essential for the successful treatment of *R. equi* pneumonia in foals.

Since the 1980s, the standard treatment for foals with *R. equi* pneumonia has included oral administration of erythromycin and rifampin. These antibiotics work synergistically and achieve excellent penetration into macrophages, substantially reducing mortality in foals affected by *R. equi*. Prior to the adoption of the erythromycin-rifampin combination as a treatment protocol, the prognosis of *R. equi* infected foals was poor with survival rates around the 20%. Following its introduction, studies like that of Hillidge have reported survival rates as high as 88% [35].

Erythromycin remains the drug of choice for *R. equi* pneumonia, however; newer macrolides like clarithromycin and azithromycin offer a greater bioavailability after oral administration and achieve higher concentrations in phagocytic cells and tissues. A retrospective study by Giguere further found that clarithromycin-rifampin combination is more effective than either azithromycin-rifampin or erythromycin-rifampin combinations for the treatment of pneumonia caused by *R. equi* in foals. The belief being clarithromycin is likely to achieve high concentrations in pulmonary epithelial lining fluid and alveolar macrophages. Furthermore, the combination of azithromycin-rifampin does not provide any advantages over traditional therapy with erythromycin- rifampin [57].

6.1.Adverse Effects of Treatment

The erythromycin-rifampin has shown remarkable results significantly increasing survival rates in foals with pneumonia. However, erythromycin, requires frequent dosing and can lead to serious side effects, including diarrhoea and hyperthermia which can be life threatening.

Additionally, there has been reports of mares developing severe enterocolitis after inadvertently ingesting erythromycin-contaminated manure from treated foals [58]

Stieler et al. found that all commonly used macrolides for treating *R. equi* pneumonia, i.e. erythromycin, azithromycin and clarithromycin, could induce anhidrosis in foals [59]. This was demonstrated using a quantitative intradermal terbutaline sweat test, which showed suppression of sweating in healthy foals given erythromycin. Additionally, co-administration of erythromycin and rifampicin also suppressed normal sweating, but rifampin alone did not have this effect [60]. Foals treated with any of these antibiotics should be considered at risk for hyperthermia. The mechanism of macrolide-induced anhidrosis remains to be further investigated.

6.2.Monotherapy

Rifampicin is a highly lipophilic bactericidal drug that works by inhibiting bacterial DNA-dependant RNA polymerase, making it effective against intracellular organisms like *R. equi* [35]. Dosage recommendations in literature range from 15-25 mg of erythromycin / kg of body weight PO, every 6-12 hours with 3-10 mg of rifampin /kg body weight PO every 8-12 hours [61]. The main drawback of rifampin is that it decreases the bioavailability of macrolides. Co-administration of rifampin to foals lowers concentrations of macrolides in plasma and bronchoalveloar lavage fluid (BALF) by inhibiting intestinal absorption of macrolides [61].

The question arises: why not rifampin alone as a monotherapy? Especially given its impact bioavailability and the adverse effects associated with macrolides. Burton addressed this by comparing the in vivo efficacy of clarithromycin and rifampin, both individually and in combination using a mouse model of *R. equi* infection. The results showed that the combination of clarithromycin with rifampin resulted in a significantly lower number of *R. equi* CFU in the organs of mice than treatment with either drug alone or placebo. There was no significant difference in the CFU counts between mice treated with clarithromycin monotherapy, rifampin monotherapy or placebo, highlighting the enhanced effectiveness of the combination over monotherapy [62].

6.3.Further investigations

Tulathromycin, a popular, antimicrobial agent in farm practise, is commonly used to treat respiratory diseases in swine and cattle. It has shown effectiveness in treating mild to moderate bronchopneumonia in foals, though it is less effective than the azithromycin -

rifampin combination. Studies observed clinical recovery with tulathromycin, assessed through physical examinations and thoracic ultrasonography. However, the study also noted that resolution of ultrasonographic lesions occurred fasted in foals treated with azithromycin-rifampin compared to those treated with tulathromycin [63].

Gamithromycin has also been investigated as a potential new treatment alternative to existing antimicrobial agents due to its ability to accumulate in lung tissue and phagocytic cells. The availability of a long-acting antimicrobial agent that maintains therapeutic concentrations at the site of infection would result in less frequent administrations. Intramuscular administration of gamithromycin to foals at a dosage of 6mg/kg has been demonstrated to provide concentrations above the minimum inhibitory concentration (MIC) required to inhibit the growth of 90% of organisms of *R. equi* in BALF fluid cells and neutrophils for about 7 days suggesting that weekly administration of gamithromycin might be feasible [64]. Less frequent administration of antimicrobials could enhance client compliance and ensure more prudent use of antimicrobials. However, studies indicate gamithromycin is non-inferior to azithromycin- rifampin combination for treating mild bronchopneumonia in foals. Furthermore, it was associated with a higher frequency of adverse effects including colic and hind limb lameness, noted only in gamithromycin treated foals [65].

Although numerous antimicrobials are active against *R. equi* in vitro, many prove to be ineffective when used in vivo. This discrepancy can be attributed to a lack of comprehensive field trials and notable differences in vitro and in vivo drug activity.

7. ANTIMICROBIAL RESISTANCE

7.1.Prudent use of antimicrobials

Historically, detection of pulmonary abscess on thoracic ultrasonography called for immediate use of antimicrobials. The rationale being to prevent fatalities. While effective [35] this approach is costly and raises concerns about the potential to promote antimicrobial resistance (AMR). There is reason to believe that widespread and sometimes unnecessary use of antimicrobials has contributed to increased prevalence of *R. equi* on certain horse farms.

A pivotal 2011 study by Venner highlighted that many foals with pulmonary abscess recover without antimicrobial intervention [36]. Specifically foals with mild subclinical ultrasonographic pulmonary lesions associated with *R. equi* recover without therapy, and administration of antimicrobial agents to these sub-clinically affected foals showed no faster resolution of lesions relative to administration of a placebo [36]. Subsequent research reinforced these findings, indicating that most foals with subclinical pulmonary abscesses smaller than 10 cm in diameter recover without antimicrobial treatment, and treating these foals offered no clear advantage over administration of a placebo [66].

Minimising the number of foals treated with antibiotics is crucial for mitigating the development of AMR; however, it is equally essential to ensure that this reduction does not lead to increased morbidity and mortality rates among foals with *R. equi* pneumonia. A retrospective study from Arnold-Lehna examined this conundrum, by changing their policy to treat foals with *R. equi* pneumonia in the later course of disease, to see if they could decrease antimicrobial usage without increasing mortality rate. In the period form 2008- 2011, every foal with pulmonary abscess was treated and in the 2012-2016 period, the protocol was changed so that only foals with lager lesions were treated. They noted that from all foals that developed *R. equi* pneumonia, 81.5 % received antibiotic treatment in 2008-2011 period compared to 50.9% in 2012-2016. Ultimately, the percentage of foals that died from *R. equi* pneumonia did not differ significantly between the two periods [67].

7.2.Emergence of resistant isolates

In recent times, more and more reports are emerging of bacterial isolates with multidrug resistance. *R. equi* is no exception. The increasing prevalence of multidrug resistant (MDR) *R. equi* is concerning. A study in 2018, which cultured *R. equi* from soil samples in horse farms in Kentucky, found 76 out of 100 farms yielded antimicrobial resistant *R. equi* [68]. The

constant exposure, starting from birth through the most susceptible periods of a foal's life, potentially increases prevalence of MDR *R. equi* infections in foals.

The impact on the emergence of MDR *R. equi* on treatment outcomes. one study found the survival proportion of foals infected with resistant *R. equi* isolates (2/8 [25.0%]) was significantly less, compared with the survival proportion in foals that received the same antimicrobial treatment from which antimicrobial-susceptible isolates were cultured (55/79 [69.6%]) [69] suggesting MDR *R. equi* isolates seem to be associated with a poorer prognosis in foals.

With the rising frequency of macrolide-rifampin resistant *R. equi* isolates in pneumonic foals over the last decade [69], now more than ever it has become important to ensure prudent use of antimicrobials Recently it has been demonstrated that the combination of macrolide plus rifampin had a lower mutant prevention concentration than any of the macrolides alone[70]. Rifampin should not be used as monotherapy as it increases the development of resistance. Employing a combination of two different drug classes helps to minimise reduces the likelihood of *R. equi* drug resistance, furthermore no known mechanisms of cross-resistance between macrolides and rifampin [68].

*7.3.*International spread of multidrug resistant *R. equi*

Phylogenetic analysis has aided in research, displaying there is increasing prevalence of MDR *R. equi* since it was first documented in 2002. The increasing prevalence of macrolide-rifampin resistant *R. equi* is largely attributed to the emergence of MDR 2287 clone, which likely occurred due to the use of antimicrobials as a prophylactic therapy [71]. Macrolide resistance in *R. equi* is caused by acquisition of the macrolide resistance gene *erm*(46) [72]. Rifampicin resistance results from the substitution of a limited number of highly conserved amino acids in the RNA polymerase β subunit encoded by the *rpoB* gene [73].

Due to international horse trade, horse movements will likely spread these MDR isolates internationally. The MDR-*R. equi* 2287 has been detected outside the USA, appearing Ireland in both 2016 and 2021 [74]. While in the USA, the MDR- *R. equi* 2287 has continued to spread [71]. The 5-year gap between detection has been hypothesized to result from stricter European regulations on prophylactic use of antibiotics in Ireland as opposed to the widespread use of antimicrobials prophylactically in the USA [74]. A recent paper showed that antimicrobial residues excreted in the environment following antimicrobial treatment enhance resistant microbial communities in the environment and maintenance of

antimicrobial resistance genes (AMRGs) [75]. The study essentially showed that the presence of macrolide residues was associated with higher prevalence of *R. equi* carrying AMRGS. The prevalence of MDR *R. equi* will be expected to increase due to constant exposure of foals to this resistant pathogen in their environment from birth. With 80% of antimicrobial products sold worldwide intended for use in animals, there is strong evidence the animal industry including the equine industry play a role in the facilitation of AMR [76].

8. PREVENTION

8.1.Hyperimmune plasma

The use of HIP serum as a method of chemoprophylaxis to reduce the prevalence of *R. equi* on farms, as well as its possible ability to ameliorate the disease, has been investigated. The HIP provides recipient foals with a broad-spectrum of specific anti *R. equi* antibodies, and perhaps other immune modulators to enhance the humoral immunity in their fight against *R. equi* infection [77].

The method involves harvesting plasma from donor horses previously inoculated with the bacteria. The blood collected from these hyperimmunised donors is sedimented by gravity at 4 degrees Celsius for 24 hours, followed by aseptic removal of the plasma. Any contaminating erythrocytes are removed by centrifugation. Finally, the purified plasma is tested for bacterial contamination and stored at -20 degrees. [77].

In a 1991 study in the United States, passive immunization with hyperimmune plasma, effectively decreased prevalence of the disease in foals from endemically affected farms [78]. Since then its effectiveness has been debated in literature. Evidence suggesting there is often spontaneously regression without the need for antimicrobials in subclinical and mild *R. equi* pneumonia cases. [36]. It remains possible that the foals would have recovered regardless of the administration of HIP.

A study by Giguere looked at the efficacy of commercially available HIP with the purpose of prevention of pneumonia in foals caused by *R. equi*. The study consisted of three groups. The first being those treated with HIP, second those non-treated as a control and those with failure of passive transfer of immunity and treated with HIP as third group. The HIP was administered intravenously which had been commercially made from donor horses who had been previously immunised with *R. equi* antigens. The antibody titre was evaluated using enzyme-linked immunoabsorbent assay. They identified foals with failure of passive transfer, by taking serum and evaluating the IgG concentration. Foals were given 950 millilitres HIP between 1 and 10 days of age and again between days 30 and 50 of age. They evaluated the results using TBA from foals showing clinical signs and cultured them. The difference in incidence of pneumonia, those not treated with HIP was 30% and foals treated with HIP was 19.1%. The 13 foals with failure of passive transfer that received HIP and developed. Difference in incidence of pneumonia due to *R. equi* between foals who had successful passive transfer and received HIP and those which did not, was not significant [79].

A study on thoroughbred farms in Australia looked at whether HIP would reduce the prevalence of *R. equi* pneumonia in foals born in 1992 breeding season. They found that there was no significant difference in the number of transfused foals developing *R. equi* pneumonia compared with untreated foal. Interestingly noted in the Begg paper their previous experience that no foal born in the first 6 weeks of the foaling season developed pneumonia caused by *R. equi*, so therefore in their study they did not consider them suitable candidates and were not included in the study [38].

In 2016 Sanz found while infection with *R. equi* was not prevented by HIP administration, the severity and development of clinical signs were decreased by its use [80]. On the other hand, there has been consequences of the use of commercial HIP. In 2006, an outbreak of Equine Infectious Anaemia in Ireland occurred due to iatrogenic transmission of the virus with contaminated HIP [81]. At the time the product was unregulated which led to the outbreak, since then the Health Products and Regulatory Authority has been controlling the product.

8.2.Gallium maltoate

Gallium maltoate is being studied as a chemoprophylatic agent to decrease to prevalence of *R. equi* on horse farms. It is a semi metal resembling ferric iron and can inactivate some iron dependant enzyme reactions in certain bacteria including *R. equi*. It provides high bioavailability following oral administration, when administered to neonatal foals intra-gastrically; it is safe and absorbed readily, achieving high serum concentrations. [82].

However, its effectiveness is still uncertain .A study involving 483 foals raised on farms with histories of *R. equi* infections, found there to be no significant differences in the cumulative incidence of *R. equi* pneumonia between foals receiving a placebo and those who received gallium maltoate for their first two weeks of life [83]. In a 2015 study, they found it to be equally as effective as standard antimicrobial therapy for the subclinical form of pneumonia [84].

8.3.Vaccine

The absence of effective preventative measures has led to a heavy reliance on antibiotics for managing *R. equi*. In recent years, treatment strategies for *R. equi* pneumonia have been revised focusing on minimizing the number of foals treated and limiting the emergence of antimicrobial resistant strains of *R. equi* [68]. Consequently, this has ignited an increasing interest in developing protective *R. equi* vaccines to combat the disease.

Currently there is no licensed vaccine available for *R. equi*. The development of a vaccine *R. equi* is dependent upon stimulating an effective immune response. The challenge is the age of which foals are infected, elimination of *R. equi* from the environment completely is not possible due to its ubiquitous nature; therefore, foals are exposed to the avirulent or virulent *R. equi* from the day of birth. A successful vaccine would need to be effective in eliciting immune response in foals to environmental challenge and the presence of maternal antibodies, which can interfere with the development of immunological memory [27].

The more traditional or historic vaccine approaches like live attenuated and killed vaccines have currently proven ineffective in consistently providing protection against *R. equi* infection. Conversely, modern vaccine strategies such as mRNA, subunit and electron beam inactivated bacterial vaccines have shown mixed results in eliciting immune protection for foals [27].

Electron beam inactivated vaccine is one of the more modern form of vaccinations. In its simplest form, it is an inactivated vaccine of electron beam irradiatated *R. equi*. The irradiation inactivates the replication *R. equi* while maintaining out cell wall integrity, allowing antigen recognition to occur and elicit an immune response. In one trial, the vaccine was administered enterally with a nasogastric tube to foals on day 2, 9, 16 and 23 of life. They assessed mucosal humoral immune response by evaluating the *R. equi* specific Igs in BAL fluid and *R. equi* specific IgA in nasopharyngeal swabs, moreover *R. equi* specific IgA and IgG isotopes were evaluated in serum concentrations to validate systemic humoral responses. Cell mediated immune responses were gaged by using an equine interferon γ (IFN-γ) enzyme linked immunosorbent assay test.

The trial was successful in generating cell- mediated and upper respiratory immune response that mirror the immune responses from maternal antibodies passively transferred [85]. It did not however examine to efficacy of the vaccine. In 2016, Rocha et al. investigated its efficacy, to examine whether electron beam inactivated *R. equi* could protect foals against developing pneumonia after challenge with virulent *R. equi*. The results unfortunately yielded an answer of no. The vaccination did not elicit a strong enough immune response from the foal to be effective [86].

The search continues for discovering an effective preventative method to *R. equi* infection. Finding an effective vaccine would be monumental in case of reducing prevalence of *R. equi* and would be a welcome sight for equine breeders worldwide. Continued advancements in

preventative measures remain crucial for controlling the prevalence of *R. equi* and improving foal outcomes on affected horse farms.

9. CONCLUSION

This thesis has explored the multifaceted factors influencing the prevalence of *R. equi* on horse farms and its associated outcomes. Through a comprehensive review of existing literature, key topics such as epidemiology, environmental and management practises as well as treatment and preventative strategies, were analysed.

The biological attributes of *R. equi* – its zoonotic, intracellular, and ubiquitous soil dwelling nature- influence its persistence and prevalence. This thesis has highlighted the bacterium's ability to exploit the immunological vulnerabilities of foals, making it a significant challenge for equine health management.

The thesis brings to light the ever-growing challenge of AMR and its influence on the bacterium's prevalence on horse farms. These findings emphasise the importance of using antimicrobials therapeutically rather than prophylactically, opting for combination therapies over monotherapy and withholding antimicrobial treatment in cases where spontaneous regression of smaller lesions is possible. Such an approach minimises the risk of resistance, while avoiding the side effects of traditional therapies, such as diarrhoea and anhidrosis.

Human interventions, such as unlicensed use of hyperimmune plasma, flawed diagnostic methods, intensive farm practises and ill guided antimicrobial use, have shown to exacerbate the prevalence of *R. equi.* Addressing these human factors, alongside targeted farm management practises, is essential for reducing the bacterium's impact.

Implementing evidence based practises such as ensuring adequate passive transfer, maintaining manure free stables, providing adequate paddock conditions and promoting proper ventilation for stabled horses can significantly lower morbidity and reduce the prevalence of *R. equi.*

The development of an effective vaccine that overcomes the immunological challenges in foals would represent a monumental breakthrough. Such advancements could mitigate the financial loss and performance decline in the equine industry, particularly in racing and breeding.

In conclusion, with continued research and collaboration across the equine industry, it is possible to address the influential factors driving the prevalence of *R. equi*. By doing so, we can reduce morbidity and mortality, improve foal health, and ensure a more sustainable future for horse farms worldwide.

10.FIGURE SOURCES

FIGURE 1: Takai S, Suzuki Y, Sasaki Y, et al. Short review: Geographical distribution of equine-associated pVAPA plasmids in *Rhodococcus equi* in the world. Vet Microbiol. 2023;287:109919. doi:10.1016/j.vetmic.2023.109919

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