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# Rhodococcus Equi Infection at a Stud Farm
# - a Retrospective Study -

Diploma Thesis

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submitted by
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## 1. Introduction

The genus *Rhodococcus,* class *Actinobacteria,* family *Nocardiaceae*, is a gram-positive, non-motile cocccoid rod. It is assigned to the group of partial acid-fast bacteria because its cellular wall contains mycolic acids, allowing them to be visible in Kinyoun stain in red color (Selbitz et al. 2015). *Rhodococcus* can be cultured in aerobic environment on blood-agar and forms white to salmon-colored mucous-humid colonies. It forms a Christie-Atkins-Munch-Peterson (CAMP)-like process of hemolysis in the presence of *Staphylococcus aureus* (*Staph. aureus*), due to the reactivity of the equi-factor and the alpha-hemolysine of *Staph. aureus*, which is important in the diagnostic of *R. equi* (Selbitz et al. 2015). Because of its importance in endemic foal pneumonia on stud farms all following information is focused on the species *Rhodococcus equi* (*R. equi*).

### 1.1. Ecology and epidemiology

*Rhodococcus equi* is a soil saphrophyt and ubiquitous in the environment but also widespread in the gastro-intestinal flora of grazing animals (Hughes and Sulaiman 1987). Foals initially get in contact with *R. equi* per oral ingestion and gut colonization within the first weeks of life. This is essential for immunity priming (Chirino-Trejo et al. 1987, Hooper-McGrevy et al. 2005). This leads to the importance of faecal shedding. Because of faecal shedding the environmental contamination increases and ensures the ongoing of *R. equi* faecal-oral life circle. As *R. equi* is an ubiquitous pathogen also non-infected horses play an important role in faecal shedding. In comparison to non-infected horses which do shed less than 2 x 103 colony forming units [cfu]/g faeces with 10 to 15 % violent strains, non-infected foals do shed 1 x 103 to 1 x 104 cfu/g faeces with an amount of 10 to 40 % violent strains. In clinically infected foals shedding is clearly increased to 1 x 106 to 108 cfu/g faeces and also the percentage of virulent strains increases to up to 80 % (Takai et al. 1994, Muscatello et al. 2007). This proves that faeces of infected foals are a potent source of contamination (Muscatello 2011). Because of *R. equi´s* resistance towards low pH values and oxidative stress it has a high tenacity in the environment (Selbitz et al. 2015), survives the passage through the gastro-intestinal tract and gets excreted again (Barton and Hughes 1984, Barton 1991).

Because of its importance for the foal pneumonia, the aerogenic pathway is the most relevant route of infection. Primarily important for infection is the fact that virulent strains can be inhaled, whereas soil bound organisms are irrelevant (Muscatello et al. 2007). There is a clear correlation between the amount of airborne pathogens and not environment soil concentration, and the prevalence for foal disease (Muscatello et al. 2006). There is evidence that *R. equi* is infectious as well as contagious, so it can be spread to other animals by infected ones. There is a higher concentration of virulent *R. equi* strains in samples collected from the respiratory tract than in the aerosols of contaminated environments (Muscatello et al. 2002, Muscatello et al. 2005). That is why the primary approach to prevent *R. equi* outbreaks is by improving environmental conditions. Starting with well-ventilated, dust free areas and correct management stable hygiene by preventing mud contaminated yards and boxes (Giguère and Prescott 1997). Decreasing density of foals, reducing herd size and the time foals are crowded in yards result in lowering aerosolization levels of *R. equi* (Muscatello et al. 2007).

Infections can occur sporadically and not at all but can have potential as an endemic disease in horse breeding farms (Takai et al. 1991, Chaffin et al. 2003, Muscatello et al. 2006). For those horse farms managing *R. equi*, a reduction of contamination is the key to lower clinical infections in foals.

The incubation time is suggested to follow a log-normal distribution and calculated to be 49 days and shorter, depending on the type and date of the infection (Sartwell 1950). Prevalence does not stick to a specific percentage, even not in endemically infected herds, and so does have variability from season to season (Chaffin et al. 2003, Muscatello et al. 2006). Morbidity is around 20 % whereas mortality can range from 5 to 100 % depending on the time of diagnosis and the initiation of treatment (Chaffin et al. 2003, Muscatello et al. 2006, Venner et al. 2009).

## 1.2. Pathogenesis and virulence

*Rhodococcus equi* is inhaled as an aerosol and adheres to the epithelial surface of the lower respiratory tract. As the bacteria appears to be an obligatory aerobic, facultative intracellular pathogen, it preferentially replicates in mononuclear cells like alveolar macrophages. Pursuing intracellular parasitism in suppression of microbicidal activity and resulting in destruction of the unspecific immunity, the bacteria can now cause a clinical disease (Yager 1987, Selbitz et al. 2015). But only specific strains of *R. equi*, which have an area in their pathogenicity island of the plasmid called virulence-associated-protein (vap) gene area, cause severe illness. Especially the vapA is relevant for virulence in the foal and progression to severe pneumonia (Selbitz et al. 2015).

Susceptibility depends on the individual immunity of the horse, but *R. equi* pneumonia can be seen as a disease usually occurring in foals up to three months of age (Zink et al. 1986, Horowitz et al. 2001, Muscatello et al. 2006). The onset often coincides with the time when maternally-derived immunity decreases and the foals immunity is not yet fully developed (Prescott 1991, Hooper-McGrevy et al. 2005). Both humoral and cell mediated immune responses are important for susceptibility to the disease. Two scenarios are possible: The T-helper cell-1 response will go for macrophage activation to clear rhodococcal infection, whereas T-helper cell-2 response is associated with developing further lung lesions (Kanaly et al. 1993, 1995, 1996). Unfortunately, some foals do lack a T-helper cell-1 response as a newborn and therefore have inherent T-helper cell-2 bias (Giguère et al. 1999). Consequently, the foal is not able to mount an effective T-helper cell-1 response and has a higher risk of developing *R. equi* pneumonia.

Considering previous information, an adult horse assuming to have an appropriate T-cell response is at a lower risk to be affected by the disease. Adult horses generally act as a vector and eliminator of the pathogen but in some cases *R. equi* is associated with genital-tract infections of the mare, fertility problems and abortion (Szeredi et al. 2006). Horses with acquired immunodeficiency can develop septicemia and lung abscesses (Freestone et al. 1987).

## 1.3. Pathology and clinical features

Pathologic reports in foals primarily show a chronic pyogranulomatous bronchopneumonia including abscesses in the lungs and regional lymph nodes (Zink et al. 1986). A less frequent manifestation is a gastro-intestinal tract form with ulcerative enterocolitis and typhlitis and suppurative inflammation of the mesenteric and colonic lymph nodes (Zink et al. 1986), potentially followed by adhesions in the peritoneum, peritonitis (Baldwin et al. 1992) and peritoneal effusion (Giguère and Prescott 1997).

Bacteremic spread out of affected lungs and the gastrointestinal tract, can result in septic polyarthritis and osteomyelitis (Giguère and Prescott 1997). Due to immune mediation, non-septic polyarthritis can appear as well (Sweeney et al. 1987). *Rhodococcus equi* should, therefore, always be on the list of differential diagnoses when orthopedic symptoms occur in foals from one to six months of age.

The acute or chronic outcome of the disease begins with mild lethargy and a decreased appetite. More specific but not pathognomonic is a rise in respiratory rate resulting in increased breathing effort with enhancement on abdominal support and mild to severe fever (Beech and Sweeney 1991). Intermitted cough and nasal discharge come along with most infections of the respiratory tract and are, therefore, inconsistent and not very sensitive. Some foals do develop a subacute form and end up in severe respiratory distress, high fever and even death, unless aggressive treatment is provided (Beech and Sweeney 1991). Symptoms associated with the abdominal form of the disease may include fever, depression, anorexia, weight loss, colic, and diarrhea (Zink et al. 1986).

## 1.4. Diagnostic investigations and prevention

Thorough diagnostics combined with early treatment is highly effective to minimize environmental contamination and the risk for other foals to become infected (Giguère and Prescott 1997, Muscatello et al. 2007). Daily temperature control, clinical examination, frequent white blood count and plasma fibrinogen control and, if possible, pulmonary ultrasonographic evaluation provide a good foundation for early detection of the disease (Prescott et al. 1989, Giguère and Prescott 1997).

Passive immunization is provided to the foal by the mother through colostral antibodies. To ensure sufficient immunity in the foal the use of hyperimmune plasma (HIP) from donors, vaccinated with autogenous bacteria, has been tested and turned out to be highly effective in reducing incidence and mortality due to *Rhodococcus equi* pneumonia (Martens et al. 1989, Madigan und Muller 1991, Muller and Madigan 1992).

Another method to minimize the risk of infection is to coordinate breeding so that mares will foal in winter. This should lead to foals not spending their most sensitive time on dusty paddocks as it is the case in summer and may decrease the incidence of the disease (Giguère and Prescott 1997). Nevertheless, bad housing situations in winter can also lead to a higher exposure and a higher risk of infection for the foals.

Clinical symptoms are an apparent indication but often occur late in progression and are not reliable for early detection (Beech and Sweeney 1991). Auscultation and percussion are important investigations which allow to detect affected lung areas, however, findings do not correlate with the severity of the disease (Falcon et al. 1985).

A wide range of laboratory tests can be done in foals with *R. equi* pneumonia but none of these tests distinguish between *R. equi* and pneumonia of other origins (Giguère and Prescott 1997). Neutrophilic leucocytosis with or without monocytosis is commonly seen. Hyperfibrinogenemia is a consistent laboratory result and normal levels of fibrinogen are rare (Falcon et al 1985, Sweeney et al. 1987). Blood gas analysis is important to monitor oxygenation and acid base status during therapy of foals with respiratory distress (Giguère and Prescott 1997). Because of maternal impact and widespread exposure there are currently no reliable serological tests available (Giguère and Prescott 1997). Seroconversion with paired samples can be used to detect a clinical outbreak (Giguère and Prescott 1997, Prescott et al. 1989).

When using thoracic radiography, the most common findings are prominent alveolar patterns characterized by ill-defined regional consolidation (Falcon et al. 1985). Thoracic ultrasonography is the most commonly used technique to diagnose *R. equi* pneumonia. In affected foals, pleural irregularities, lung consolidations and abscess areas can be detected (Ramirez et al. 2004). Lung consolidations are poorly defined, hypoechoic regions in contrast to pulmonary abscesses which appear are variable in size, well-defined, hypoechoic nodules that can be surrounded by an echogenic capsule (Ramirez et al. 2004). Sometimes B-Lines could be visualized but are not consistent within a *R. equi* infection and just indicate areas of cellular or fluid infiltration (Reimer 1990). The described lesions are not pathognomonic for *R. equi* pneumonia and neither could ultrasonography detect lesions deep within the lung tissue, that is where radiography is a good complement (Ramirez et al. 2004). Scintigraphy showed major pulmonary perfusion defects appearing through decrease or absence of radioisotope activity and, therefore, detects affected areas of the lungs (Martens et al. 1989).

Bacteriologic culture of tracheobronchial aspirate (TBA) or bronchoalveolarlavage (BAL) are reliable techniques for a definitive diagnosis (Giguère and Prescott 1997, Muller and Madigan 1992). *R. equi* can be isolated within 48 hours of culture of fresh specimen assumed that no antimicrobial therapy has been initiated. In contrast, polymerase chain reaction (PCR) testing of vapA genes from TBA samples is very sensitive and more rapid especially when the foal is treated with antimicrobial agents (Giguère and Prescott 1997). Cytology could be theoretically performed from both TBA and BAL. However, cytology taken by TBA is superior to that of BAL (Rossier et al. 1991) because TBA examination gives clear evidence of acute inflammation. In contrast to TBA or BAL, culture of nasal swab or faeces cannot be taken as evidence of infection as *R. equi* is known to be a ubiquitous organism (Giguère and Prescott 1997). Blood culture could also be performed as it is showing good results (Leadon et al. 1988).

## 1.5. Therapy

Because *Rhodococcus equi* is an intracellular pathogen, only lipophilic antimicrobial agents who penetrate abscess areas and invade macrophages should be considered as a treatment (Prescott 1991). Lipophilic agents effective against *R. equi* in vitro are makrolids especially tulathromycin and erythromycin, and rifampicin as an agent of the rifamycines (Giguère et al. 2012, Venner et al. 2013, Giguère 2017). A combination of azithromycin and rifampicin is highly effective in foals with bronchopneumonia associated with *R. equi* (Wetzig et al. 2020) but also the combination of erythromycin and rifampicin decreases the mortality in foals suffering from *R. equi* pneumonia (Hillidge 1987, Sweeney et al. 1987). Due to the combination of different types of antibiotics, a synergistic effect is induced and minimizes the risk of developing antimicrobial-resistant strains (Asoh et al. 2003, Giguère 2017). Especially due to treatment of subclinically infected foals, resistant strains against macrolide and rifampicin increase in environment as well as in the foals itself (Burton et al. 2013). In cases where rifampicin and erythromycin cannot be used, those strains are often sensitive to fluoroquinolone, oxazolidinone and glycopeptide antibiotics (Vázquez-Boland et al. 2013, Giguère 2017).

Treatment can be supported by application of non-steroidal-anti-inflammatory-drugs (NSAIDs) to reduce fever and improve attitude and appetite, but they should be used with awareness of potentially negative side effects (Giguère and Prescott 1997). In cases with severe dyspnoea or hypoxemia, oxygen therapy and other breathing-supporting measures should also be provided (Hoffman and Viel 1992, Giguère and Prescott 1997). Nursing care, provision of adequate nutrition, hydration and maintaining the foal in a cool well-ventilated environment are always important basic measures. The normal duration of therapy ranges from 4 to 9 weeks (Hillidge 1987).

## 1.6. Aim and hypothesis of the study

The aim of this thesis is to come clear about the correlation between the immunity of the mother and the progression of the foal's disease. The hypothesis to prove is whether foals of mares who already got infected by *Rhodococcus equi* when young, get sufficient immunity, so that the probability for the disease to get clinically concerning drops clearly. For the investigation, data of 2018, 2019 and 2020 were used.

## 2. Materials and methods

### 2.1. Brandenburg State Stud

The Brandenburg State Studs were founded in 1788 and have been home to brood mares, their foals and breeding stallions ever since. The data for following retrospective study was collected from foals kept on the stud.

At the Brandenburg State Stud in Neustadt (Dosse), Germany, more than 40 foals are born every year. In 2014 the stud had to compete with an acute outbreak of foal pneumonia, confirmed by tracheobronchial lavage to be caused by *R. equi.* In 2015, since the endemic had continued, a surveillance program was initialized including clinical examination, temperature and leucocyte count control and thoracic ultrasonographic screening.

### 2.2. Animals

The study is based on data collected in the years 2018, 2019 and 2020. A total of 56 Warmblood sport horse broodmares (aged 4-20 years) and 91 foals (female n=36; male n=55) were included in this study. All mares were born and raised at the stud and no horses were introduced into this herd at least during the last 10 years. Mares were kept in straw-bedded group stables with daily access to an outdoor sand-paddock. Mares were fed oats, mineral supplements and hay twice daily and water was available at all times. Two weeks before parturition the mares were brought to single boxes to be observed 24 hours per day. In all foals, plasma IgG concentration was checked 18-24 h after birth and was ≥800 mg/ dl (SNAP Foal IgG test, Idexx Kornwestheim, Germany). After parturition the mare and its foal stayed at the foaling unit for 5 to 10 days and were then brought back to their herd. Beginning in May housing was changed from group stables and sand-paddock to group stables at night and pasture during the day. The foals were weaned at approximately 6 months of age.

### 2.3. Surveillance program

A surveillance program was initiated in 2016 and runs from March until September. It includes daily clinical examination and control of body temperature as well as weekly leucocyte count. By suspicion, when breathing frequency above 40 per minute, temperature above 38,5 °C and leucocyte count more than 11.000 G/l, thoracic ultrasonographic examination was initiated. This surveillance program should help to detect early abnormalities, so therapeutic measures can be started preventing progression of the disease. Since 2016 data has been collected to investigate on further questions. For this study the years 2018 to 2020 were investigated.

The ultrasonographic examination of the thorax was performed with the foal in standing position. The coat was not clipped but soaked in 70 % ethanol. A 5 to 8.5 MHz curved area transducer (Mindray, Shenzhen, China) was used scanning the thorax starting from the 16. intercostal space from dorsal to ventral and from caudal to rostral. Pulmonary abscesses were characterized as variable-sized, well-defined, hypoechoic nodules or areas of consolidated lung tissue included ill-defined, hypoechoic regions. Both abscesses and consolidations were defined as morphologic abnormalities caused by *R. equi* (Ramirez et al. 2004) and were measured by diameter, for circular areas, or by length and width.

The leucocyte count was checked weekly in each foal from two months of age. The blood was taken from a jugular vein (Vacuette, Greiner, Kremsmünster, Austria) and analyzed in the laboratory (LADR GmbH Medizinisches Versorgungszentrum Neuruppin, Germany).

### 2.4. Therapy

If abscess size was more than 0.5 $cm^2$ and/or leucocyte count higher than 11.000 G/t, therapy was initiated. In mild to moderate cases, tulathromycin (1 ml/40kg, i. m., Draxxin, Zoetis Schweiz GmbH, Delémont, Switzerland) was administered once weekly whereas in severe cases, daily double antibiotic treatment with rifampicin (10 mg/kg KGW, p. o., Eremfat 300 mg Tbl., Riemner Pharma

GmbH, Greifswald, Germany) and azithromycin (10 mg/kg KGW, p. o., Azithromycin HEC 250 mg Tbl., HEC Pharm GmbH, Berlin, Germany) was switched to or already started with. Therapy was continued until regression of ultrasonographic lesions and improvement of clinical signs.

## 2.5. Data analysis

### 2.5.1. Weather data

Weather data such as temperature [°C], precipitation [$l/mm^2$] and humidity [%] were collected to verify the results depending on the climate conditions (www.wetterkontor.de; www.weatheronline.de; www.proplanta.de, www.timeanddate.de).

### 2.5.2. Data presentation and statistical analysis

Data was collected retrospectively from records of 2018 to 2020. First, the mares were classified according to their age and the number of previous foals (Table 1). All maiden mares were born after 2015 and did have *Rhodococcus equi* exposure or illness caused by *R. equi* as a foal. Then the foals were sorted by sex.

Because data was not normally distributed (Kolmogorov-Smirnov test), analysis was made by non-parametric statistical tests. Data of different study years were analyzed by Kruskal-Wallis test. In case of significant differences further analysis was made by Mann-Whitney U test. Mann-Whitney U test was also used for data analysis regarding mares` reproductive status (maiden vs. pluriparous mares) and foal sex (male vs. female). A p-value $<0.05$ was considered significant. All values given are means ±standard error of mean (SEM). Statistical analysis was made with the IBM-SPSS 26 statistics package (IBM, Armonk, NY, USA).

## 3. Results

In the years 2018, 2019 and 2020, 85.7, 82.8 and 91.2 % of the foals showed at least one abscess defined as morphological evidence abnormalities caused by of *R. equi* pneumonia. There are no significant differences in age, leucocyte number and abscess size of first detected lung abscesses between 2018, 2019 and 2020 (Figure 1 a-c). In contrast, duration of disease changed over the study period ($p<0.05$; Figure 1 d). In 2018, the duration of illness was significantly shorter compared to 2019 ($p<0.05$) and 2020 ($p<0.01$), but there was no difference in the duration of illness between the years 2019 and 2020.

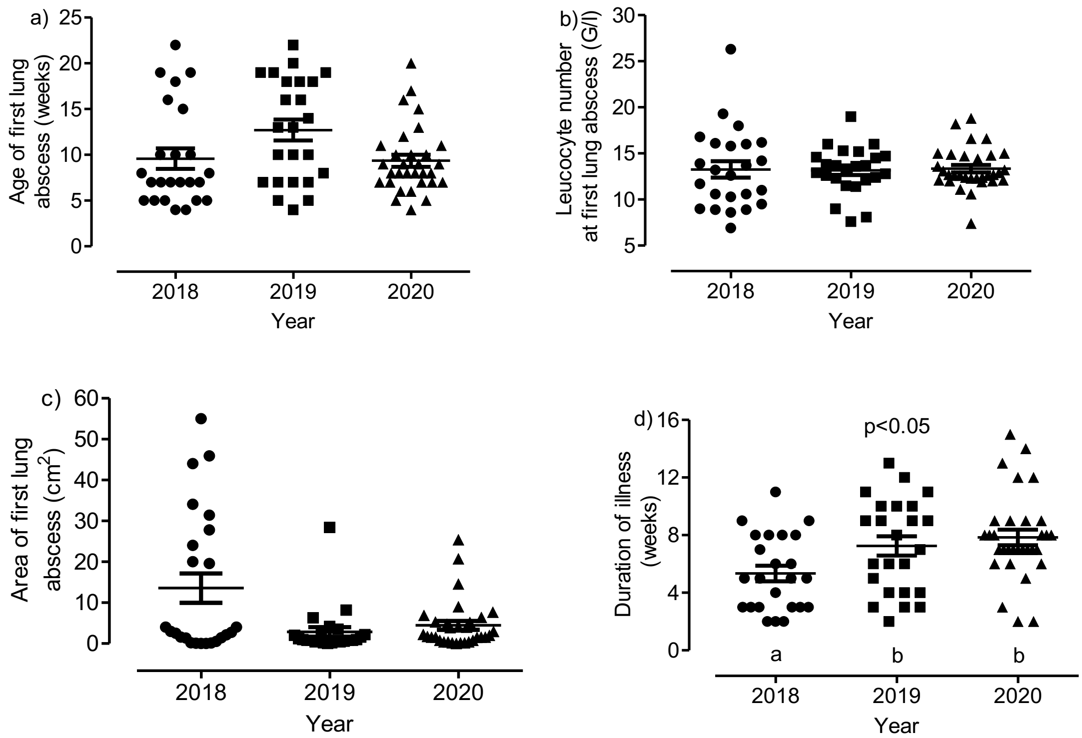


Figure 1: a) Foals age (weeks) b) leucocyte number (G/l) c) abscess size ($cm^2$) and d) duration of illness (weeks) at detection of first lung abscesses in three different years (2018, ●, n=24; 2019, ■, n=24; 2020, ▲, n=31). Significant differences are indicated in the figure. [a,b] Significant differences ($p<0,05$) between different study years.

Foals of maiden mares were significantly older (p<0.05; Figure 2 a) and the duration of illness (p<0.05; Figure 2 d) was significantly shorter compared to foals of pluriparous mares. Whereas the leucocyte number (Figure 2 b) and abscess size (Figure 2 c) at the time of the first lung abscess did not differ between foals of maiden and pluriparous mares.

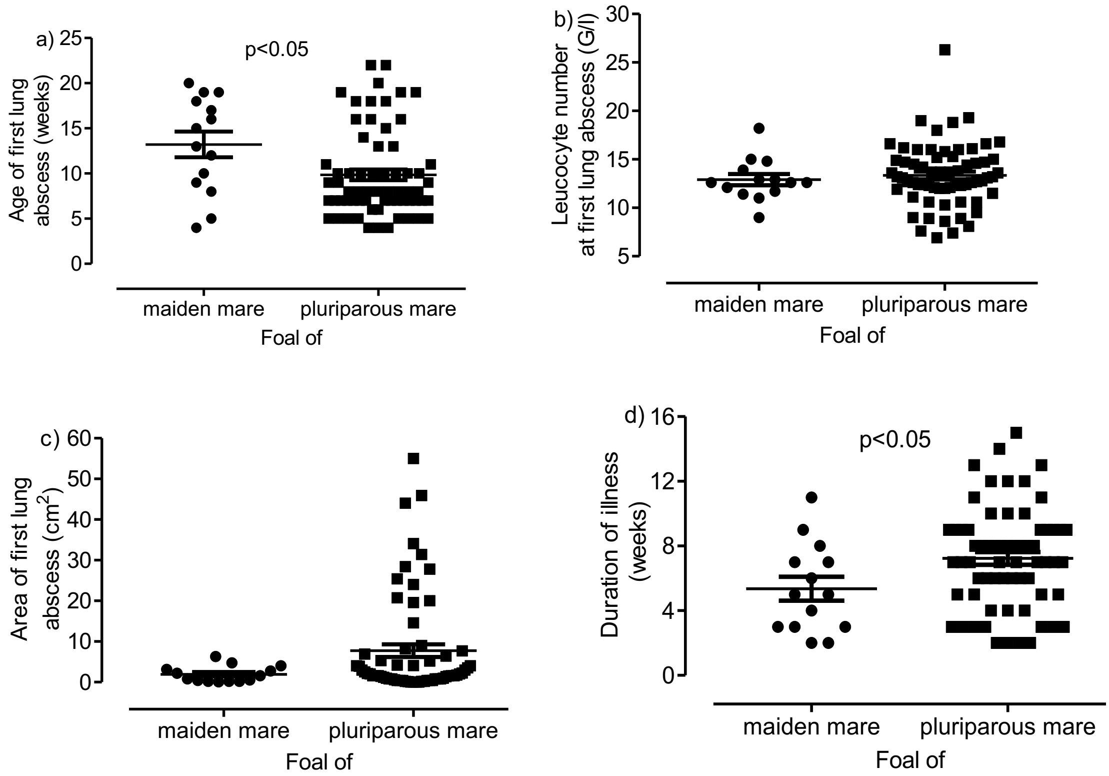


Figure 2: a) Foals age (weeks) b) leucocyte number (G/l) c) abscess size ($cm^2$) and d) duration of illness (weeks) of foals of maiden (●, n= 14) and pluriparous mares (■, n= 65) at the time of the first lung abscesses within the years 2018 to 2020. Significant differences are indicated in the figure.

Regarding sex of the foals, there were differences neither in age, leucocyte number and abscess size of first lung abscesses nor duration of illness between female and male foals (Figure 3 a-d).

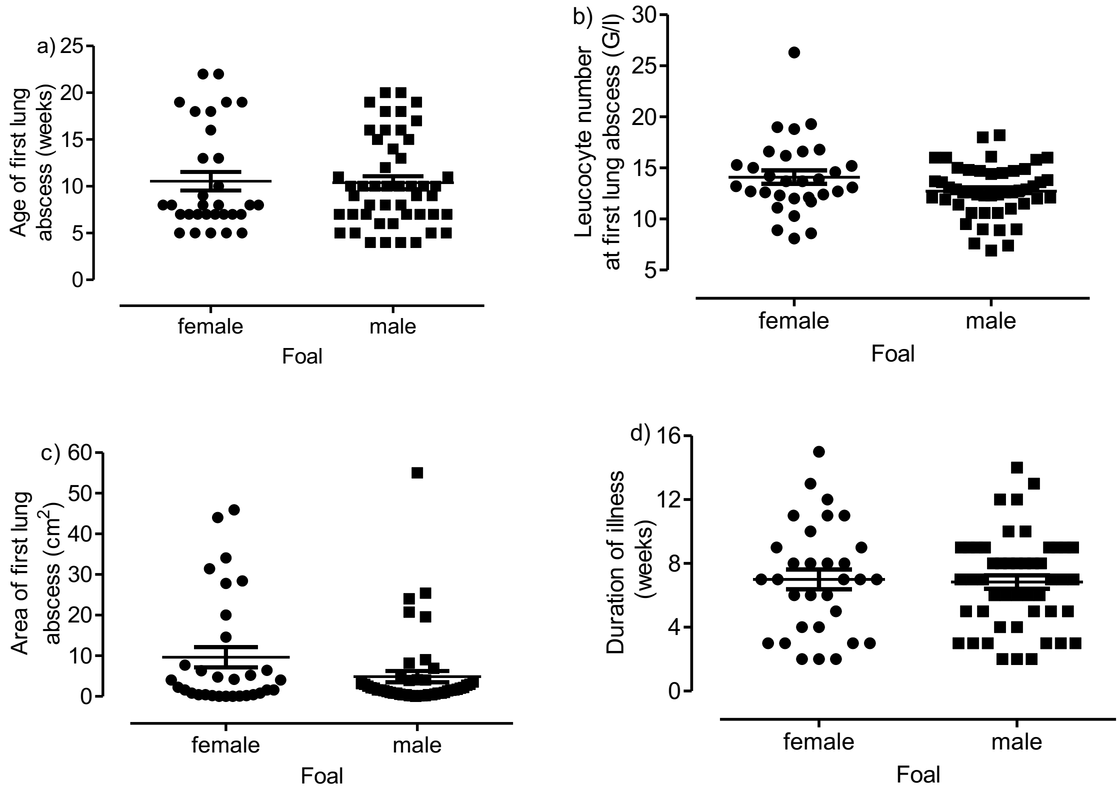


Figure 3: a) Foal age (weeks) b) leucocyte number (G/l) c) abscess size ($cm^2$) and d) duration of illness (weeks) of female (●, n=31) and male (■, n=18) foals at the time of detection of first lung abscesses within the years 2018 to 2020.

## 4. Discussion

The main results of this study indicate that foals of maiden mares, which had confirmed *Rhodococcus equi* exposure as foals, were older at first detection of lung abscesses and the duration of illness was shorter compared to foals from pluriparous mares. This supports the hypothesis that foals of maiden mares are better protected against *R. equi* infection than foals born to pluriparous mares.

Foals have to receive a sufficient amount of IgG from colostrum to reach adequate passive immunity (Jeffcott et al. 1974). This passive immunization is obligatory and failure of passive transfer can either lead to acute sepsis often followed by death of the foal or can be the reason for growth retardation and long-term orthopedic and respiratory tract problems (Palm et al. 2011). Particularly in the first weeks of life, foals develop very quickly and their own immune system is just beginning to mature. In general, younger foals are more susceptible and older foals are often more resistant against infections since both the innate and the acquired immune system develop with each month of life (Bernoco et al. 1987, Bernoco et al. 1994, Demmers et al. 2001). In contrast to this, as time goes on the passive immunity built up by maternal IgG is decreasing (Wilson et al. 2001). It has been shown that young foals are clearly more susceptible to *R. equi* infection than foals that are older (Horowitz et al. 2001). Most infections with *R. equi* occur at a very young age with incidence decreasing in older foals (Sanz et al. 2013) and infections becoming extremely rare in adult horses. Thus, a later occurrence of infection is often associated with a faster recovery, as was shown in the foals of maiden mares in the present study.

A high colostral IgG concentration and adequate transfer to the foal is essential for protection against opportunistic or pathogenic bacteria, such as *Streptococci*, *Staphylococci* and *Escherichia coli* (Robinson et al. 1993). In case of *R. equi*, mares with natural exposure reach a high IgG concentration in serum and colostrum. Furthermore, high IgG concentration in colostrum was correlated with a high IgG concentration in the foals´ serum (Triskatis 2004). The same study showed that older mares had a higher antibody concentration than younger mares. Also, the antibody concentration against *R. equi* in the serum of the foal correlated with the parity of the mares – the older the mare the higher the antibody concentration of the foal. However, no explicit distinction was made between pluriparous mares and maiden mares (Triskatis 2004).

As after natural exposure, the same was true for mares vaccinated against *R. equi*. Vaccinated mares had a higher colostral antibody concentration and their foals had higher serum IgG concentration after colostrum intake than non-immunised mares and their foals. But as all foals developed pneumonia there is no evidence that vaccination of the mares protected the foals against *R. equi* (Martens et al. 1991). In contrast, different studies show that passive immunization of foals with specific hyperimmune plasma against *R. equi* have protective effect on the foal and are protected better than foals of mares who were vaccinated with *R. equi* (Madigan et al. 1991).

There was, however, no correlation between the *R. equi* antibody content of the foal`s serum and the future health of the foal and neither the severity nor the timing of the disease were influenced by *R. equi* antibody titres (Triskati 2004). Although *R. equi* antibody titres of mares` and foals` serum were not determined in the present study, transferred immunity against *R. equi* infection apparently has an effect on age at onset and severity of infection but this impact might not be restricted to antibody concentration in the foal.

Other investigated parameters like study year or foal sex, were without effect on *R. equi* infection of the foals. Foal sex affects gestation length (Kuhl et al. 2015) and postnatal behavior (Wulf et al. 2016) which both might influence foal health, but with respect to *R. equi* infection foals sex did neither influence the onset nor the severity of infection.

Considering the three-year study period, there were no differences in the age of first occurrence or severity of *R. equi* infection in foals. Thus, it can be assumed that diagnostic procedures and foal management were largely constant over the three-year study period. In contrast to this, duration of illness was significantly longer in 2019 and 2020 compared to the year 2018. These differences are likely caused by weather and soil conditions and treatment as has been described previously (Muscatello et al. 2006). Due to the retrospective character of this study, information regarding weather conditions (temperature, precipitation and humidity) could only be collected from national weather information services and was not recorded directly on the stud. Therefore, weather conditions are presented in the supplemental material but were not considered for statistical analysis. Foal treatment was always performed by the same veterinarian, but changes in therapeutic intervention over time cannot be ruled out completely.

Limitations of this retrospective study are data availability and comparability. Therefore, providing the same frame conditions is important, that is why the same investigators always carried out the examinations according to the same criteria, thus increasing comparability between the years. Besides this, the missing data of weather and soil conditions should be mentioned. However, there are no differences between the study years.

The main result of this study is an impact of the reproductive status of the mare on the foals´ health. Further investigations should include a higher number of maiden mares. Also including horses from different studs would be important as *R. equi* outbreaks do vary among regions and farms. Furthermore, the colostrum of maiden mares and pluriparous mares should be compared as the composition of colostrum changes due to birth order (Barreto et al. 2020). It could be suggested that the significant factors might not be only antibodies but also other substances which increase the health or actively protect the gastro-intestinal system of the foals.

## 5. Conclusion

Foals of maiden mares with a documented exposition to *Rhodococcus equi* at foal age seem to be affected later and less severe by *R. equi* infection than foals of older mares. It seems that immunity against *R. equi* infection is transferred from the mare to the foal but this is most probably not restricted to the amount of antibodies.

## 6. Summary

*Rhodococcus equi* is an endemic bacterial microorganism on most horse farms and is particularly dangerous for foals. In order to further develop the prevention and management of infectious diseases, it is essential to know the underlying mechanism of the immunity against *R. equi*. In this retrospective study it was investigated whether the onset and course of disease in foals of maiden mares with known *R. equi* exposure differs from that of foals of pluriparous mares. For this purpose, data from mares and foals were collected and analyzed over a three year period. A significant difference was found between foals of maiden mares which grow up in a *R. equi*-affected population as foals and foals of pluriparous mares. The results showed that foals of maiden mares were affected by *R. equi* pneumonia later in life and had a shorter, weaker progression of disease. Further research should address for the different disease course of foals from maiden mares and from pluriparous mares.

## 7. Zusammenfassung

*Rhodococcus equi* ist in den meisten Pferdebeständen ein endemischer Keim, welcher besonders für Fohlen gefährlich ist. Um die Gestütsmedizin auf dem Gebiet der Prävention und des Managements von Infektionskrankheiten weiterzuentwickeln ist es essentiell zu wissen worauf die Immunität gegen *R. equi* beruht. Im Zuge dessen wurde in dieser retrospektiven Studie untersucht, ob sich der Krankheitsbeginn und -verlauf von Fohlen von Maidenstuten mit bekannter *R. equi* Exposition zu dem von Fohlen von pluriparen Stuten unterscheidet. Dazu wurden Daten von Mutterstuten und Fohlen über drei Jahre gesammelt und ausgewertet. Es wurde ein signifikanter Unterschied zwischen den Fohlen von Maidenstuten und den Fohlen von pluriparen Stuten festgestellt. Dieser zeigt, dass Fohlen von Maidenstuten später im Leben erkranken und einen kürzeren, schwächeren Krankheitsverlauf zeigen. In weiteren Studien sollte der Grund für die unterschiedlichen Krankheitsverläufe von Fohlen von Maidenstuten und pluriparen Stuten untersucht werden.

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## 9. List of figures and tables



Table 1: Temperature [°C] during the study period between January and September.

| | January | February | March | April | May | June | July | August | September |
|---|---|---|---|---|---|---|---|---|---|
| 2018 | 3 | -1 | 2 | 13 | 18 | 19 | 22 | 23 | 17 |
| 2019 | 1 | 4 | 7 | 11 | 13 | 23 | 20 | 21 | 15 |
| 2020 | 4 | 6 | 5 | 11 | 13 | 19 | 19 | 22 | 16 |

Table 2: Precipitation [l/mmm2] during the study period between January and September.

| | January | February | March | April | May | June | July | August | September |
|---|---|---|---|---|---|---|---|---|---|
| 2018 | 66.3 | 5,8 | 36,4 | 41,8 | 3,8 | 27,4 | 75,3 | 9,2 | 24,3 |
| 2019 | 43 | 24,7 | 58,7 | 0 | 15,9 | 1 | 68,6 | 18,3 | 72,5 |
| 2020 | 38,8 | 73,9 | 35,2 | 6 | 30,1 | 64,9 | 39,4 | 39,7 | 70,7 |

Table 3: Humidity [%] during the study period between January and September.

| | January | February | March | April | May | June | July | August | September |
|---|---|---|---|---|---|---|---|---|---|
| 2018 | 87 | 77 | 75 | 68 | 58 | 62 | 56 | 55 | 64 |
| 2019 | 85 | 76 | 78 | 59 | 65 | 56 | 62 | 62 | 71 |
| 2020 | 86 | 79 | 69 | 54 | 64 | 64 | 66 | 62 | 72 |