Pferdeheilkunde – Equine Medicine 39 (2023) 1 (January/February) 37–42 DOI 10.21836/PEM20230104

Microbiological findings in tracheobronchial mucus samples and in the feces of foals with pneumonia at diagnosis and during treatment

Barbara Goebel[1], Fritjof Freise[2] and Monica Venner[3]

[1] University of Veterinary Medicine, Clinic for Horses, Hanover, Germany
[2] University of Veterinary Medicine, Institute for Biometry, Epidemiology, and Information Processing, Hanover, Germany
[3] Equine Clinic, Destedt, Germany

Summary: Pneumonia is one of the most frequent diseases in foals between one and six months of age. Thereby *Rhodococcus equi* (*R. equi*) and *Streptococcus equi subsp. zooepidemicus* (*Strep. zoo.*) are regarded as the most common pathogens causing bacterial respiratory infections. The aim of the current study was to determine in which treatment group a faster reduction of fecal excretion of *R. equi* can be achieved during therapy. The study was designed as a prospective, randomized and blind study. 160 foals older than four weeks of age with moderate (abscess score 10–15 cm) to severe (abscess score >15 cm) pneumonia from a Warmblood stud were examined, randomly assigned to two treatment groups and initially treated for two weeks. Foals of group one (R/T), including 87 foals, were treated with rifampin, in a 10 mg/kg dose orally once daily, in combination with tulathromycin, in a 2.5 mg/kg dose intramuscularly or intravenously once a week. According to the routine treatment of the foals on the study farm, there was a weekly rotation of IM and IV administration during the treatment. In the second group (R/A), 73 foals were treated with rifampin and azithromycin, both in a 10 mg/kg dose orally once daily. All foals were subjected to a weekly weight control using a measuring tape from the start of therapy in order to ensure exact dosages. The success of the treatment was monitored once weekly based on the findings of the clinical examination and the sonographically determined pulmonary abscess score. Twenty-nine randomly chosen foals (R/T n = 14; R/A n = 15) of the two study groups were examined endoscopically on the day of diagnosis and tracheobronchial secretion (TBS) was sampled to identify the bacteria involved by culture. In addition, a native sample of the feces was obtained from each foal and a quantitative, culturally specific isolation and a polymerase chain reaction (PCR) for *R. equi* were performed. Samples from 29 foals were taken from July to September 2019. If *R. equi* was found in the feces, the foals were sampled once weekly with a rectal swab until the expected end of treatment (two weeks after the start of treatment) for *R. equi* PCR. In eight of the 14 foals (57,1 %) from the R/T group and four of the 15 foals (26.6 %) from the R/A group *R. equi* was identified in the tracheobronchial secretions and in the feces at the day of diagnosis. Other pathogens as *Streptococcus equi* subsp. *equi*, *Bordetella bronchiseptica*, *Escherichia coli* and *Staphylococcus aureus* were identified in the airway sample of very few foals of both treatment groups. Season seemed to influence the incidence of pathogens in sick foals. The exact test by Fisher showed that *R. equi* was detected significantly more frequently ($P = 0.0025$) in the very warm and dry early summer months, while *Strep. zoo.* was isolated more frequently in late summer ($P = 0.0007$). In the tracheobronchial secretions of foals with moderate to severe pneumonia, *R. equi* was detected significantly more frequently in high bacterial counts than in the feces ($P < 0.001$). On the other hand, when a high number of *R. equi* was found in the feces, the number of that pathogen in the tracheobronchial secretions was also high. Our hypothesis, that *R. equi* is often isolated concurrently to *Strep. zoo.* detection was rejected with the two-sided test by Fisher ($P = 0,05$). Two weeks after starting antimicrobial treatment, no foal showed positive pathogen detection in the feces anymore. Based on the results of the current study it can be assumed, that both active antimicrobial combinations are equally effective and that *R. equi* was eliminated in the gastrointestinal tract 14 days after starting treatment.

Keywords: pneumonia, foal, macrolide, *Rhodococcus equi, Streptococcus equi subsp. zooepidemicus,* endoscopy, TBS, PCR

Citation: Goebel B., Freise F., Venner M. (2023) Microbiological findings in tracheobronchial mucus samples and in the feces of foals with pneumonia at diagnosis and during treatment. Pferdeheilkunde 39, 37–42; DOI 10.21836/PEM20230104

Correspondence: PD Dr. Monica Venner PhD, ECEIM, Equine Clinic Destedt GmbH, Trift 4, 38162 Destedt, Germany; mvenner@gmx.de

Submitted: November 17, 2022 | **Accepted:** November 30, 2022

Introduction

Pneumonia is one of the most frequent disease in growing foals. *Rhodococcus equi* (*R. equi*) and *Streptococcus equi* subsp. *zooepidemicus* (*Strep. zoo.*) are considered the most common pathogens causing bacterial respiratory infections (*Lavoie* et al. 1994, *Léguillette* et al. 2002). *R. equi* is a gram-positive, facultative intracellular pathogen with high pathogenicity in the alveolar macrophages of foals (*Léguillette* et al. 2002, *Giguere* et al. 2011). The ability to survive and replicate in the host alveolar macrophages is the main key to its pathogenicity. In many foals suffering from bronchopneumonia, an 85 kilo-base-pair (Kb) plasmid was found which codes for several proteins, known as virulence-associated proteins (Vap) (*Léguillette* et al. 2002, *Kuskie* et al. 2011). Only the VapA plasmid in affected foals is necessary, among

other factors, to initiate a disease in the host organism (*Kuskie* et al. 2011). Foals develop pneumonia between one and six months of age, with the majority of foals becoming infected as early as in the first weeks of life (*Giguere* and *Prescott* 1997, *Léguillette* et al. 2002, *Muscatello* 2012a).

Strep. zoo. is a gram-positive, coccoid bacterium that is assigned to Lancefield Group C (*Léguillette* et al. 2002). In several studies *Strep. zoo.* was the most common pathogen isolated in foals (87%) with an infection of the distal respiratory tract and spreads from the tonsils to the deep regions of the lung (*Oikawa* et al. 1994, *Léguillette* et al. 2002). Foals suffering from *R. equi* pneumonia were significantly younger than those in which *Strep. zoo.* was also isolated (*Lavoie* et al. 1994). At the beginning of the disease, foals with abscessing bronchopneumonia usually show unspecific symptoms such as lethargy, fever, loss of appetite, nasal discharge (mucopurulent to purulent) and cough. With increasing severity of bronchopneumonia, tachycardia and tachypnea with marked abdominal effort also occur, and wheezes and crackles can be diagnosed at auscultation (*Giguere* and *Prescott* 1997, *Léguillette* et al. 2002). However, the clinical signs of this disease do not correlate with the severity of the bronchopneumonia (*Giguere* and *Prescott* 1997).

The aims of the current study were, to determine, which pathogens are identified in different sample types (tracheobronchial secretions versus feces) and whether both pathogens (*R. equi* and *Strep. zoo.*) are often found concurrently in sick foals. In addition, the duration of excretion of *R. equi* in the feces in two treatment groups of the foals with pneumonia was described.

Material and methods

Study design and study population

The study was designed as a prospective, randomized and blinded study.

160 foals with moderate (abscess score 10–15 cm) to severe pneumonia (abscess score ≥ 15 cm) from a Warmblood stud with endemic *R. equi* were included in the study (*Fels* et al. 2021, *Arnold-Lehna* et al. 2019). Untreated foals with moderate or severe pneumonia caused by *R. equi* can die. Therefore, we decided not to include an untreated control group for the benefit of the foals. As part of the stud farm routine management of the foals health, all foals underwent a general clinical and ultrasonographic examination of the lung once weekly. In addition, the white blood cell count was determined for all foals once weekly. Ultrasonographic examination of the lung was performed on both sides of the thorax from the 3rd to the 16th intercostal space by screening the lung from dorsal to ventral. All detectable consolidations were added and the addition of the largest diameter of all lesions ≥ 1 cm was defined as the abscess score. To rule out possible spontaneous healing of the foals, an abscess score of ≥ 15 cm (severe pneumonia) in size was used to decide for treatment. Foals older than four weeks of age with moderate (abscess score 10–15 cm) to severe pneumonia (abscess score ≥ 15 cm) were included in the study. The foals were randomly assigned to one of two treatment groups and initially treated for two weeks. Predefined criteria such as severely reduced general health status (apathy, reduced or discontinued drinking, tachycardia, tachypnoea, severe nasal discharge, severe coughing), a rectal body temperature higher than 39.5 °C or the presence of dyspnoea with associated severe pulmonary lesions at sonography excluded foals from the study. Foals of group one (R/T), including 87 foals, were treated with rifampin, in a 10 mg/kg body weight (bw) dose orally once daily, in combination with tulathromycin, in a 2.5 mg/kg bw dose intramuscularly or intravenously once a week. In the second group (R/A), 73 foals were treated with rifampin and azithromycin, both in a 10 mg/kg bw dose orally once daily. All foals were subjected to a weekly weight control using a measuring tape from the start of treatment in order to ensure exact dosages. The success of the treatment was evaluated twice weekly based on the findings of the clinical exam and the abscess score determined by ultrasound. The diseased tested foals were randomly chosen out of 160 above mentioned foals with pneumonia for each group (n = 29; R/T = 14; R/A = 15). On the day of determining pneumonia an aspiration of tracheobronchial mucus was performed for definitively diagnosing *R.*equi pneumonia. In addition, a native sample of the feces was obtained from each of the examined foals and both were sent to a laboratory for microbiological examination (Labor Dr. Böse GmbH, Carl-Zeiss-Straße 6, 31177 Harsum) to do a quantitative, cultural examination and a polymerase chain reaction (PCR) for *R. equi* (Table 1). The native sample of the feces was taken rectally using a sterile swab with Amies medium. Tracheobronchial mucus were sampled with the aid of a sterile plastic catheter which was advanced into the trachea via the working channel of the endoscope. At the same time, the larynx area, the mucous membrane of the airways and the accumulation of mucus were assessed macroscopically. After the endoscopic examination, all patients received their treatment according to the randomized list.

If *R. equi* was found in the feces, the foals were sampled once weekly with a native sample of the feces until the end of therapy, for *R. equi* identification. The repeated microbiological tests were carried out in the same laboratory to follow up the possible pathogen excretion.

Laboratory method

For the microbiological examination of the tracheobronchial samples and the fecal swabs different cultural media were used in order to identify *R. equi*. and further bacterial pathogens: Columbia CNA Agar with 5% Sheep Blood, Water-blue Metachrome-yellow Lactose Agar acc. to GASSNER, Staphylococcal Streptococcal Selective Medium and boiled blood plates. Depending on the culture media, incubation was carried out under aerobic or anaerobic conditions. In addition, the laboratory used nutrient broth, which is required for the subsequent PCR. The cultures appearing on the nutrient media were subjected to a Gram stain and microscopically examined for *R. equi* (*Giguere* and *Prescott* 1997, *Giguere* et al. 2003, *Makrai* et al. 2005). To amplify the VapA gene, which is pathognomonic for *R. equi* infection, a Real-Time-PCR (qPCR) was carried out after the samples have been enriched in a broth.

Data analysis

The assignment of foals to the two treatment groups was done with the randomisation function of Microsoft Excel and prepared with 100 foals for both groups. For the statistical analyses we used the Statistical Analysis System for Windows SAS®.

Results

Evidence of R. equi in the tracheobronchial mucus of foals

One foal from the R/T group (n = 14) had to be withdrawn from the study early during treatment because of developing interstitial pneumonia and this made other treatments necessary for this foal. Therefore, only the microbiological results of 28 (R/T: n = 13; R/A: n = 15) foals were included in the evaluation. The microbiological findings in the tracheal mucus on day of diagnosis identified *R. equi* in 15 foals of all examined foals (n = 28; 53.6%), (Figure 1).

In 9 of the 13 (69.2%) foals of the R/T group and in 6 of the 15 (40%) foals of the R/A group, *R. equi* was isolated in the tracheal mucus samples with different levels of pathogen detection. As shown in Table 2, the detection of *R. equi* in the tracheobronchial mucus was significantly lower in the R/A group than in the R/T group.

Evidence of Strep. zoo. in the tracheobronchial mucus of foals

In 6 foals (46.1%) from the R/T group, *Strep. zoo.* was isolated in the tracheobronchial samples on the day of diagnosis of moderate to severe pneumonia. These included two of the foals (15.4%) that had positive tests for *R. equi* in the tracheobronchial mucus and in the feces (low level: n = 1, high level: n = 1). In 12 foals (80%) from the R/A group, *Strep. zoo.* was isolated in the tracheobronchial samples on the day of diagnosis of pneumonia. These included two of the foals (13.3%) that had positive tests for *R. equi* in the tracheobronchial mucus and in the feces (low level: n = 1, moderate level: n = 1). Figure 2 shows the different pathogen detection levels for *Strep. zoo.* between the two treatment groups at the day of diagnosis.

In both treatment groups, further bacterial pathogens were also detected in addition to *R. equi* and *Strep. zoo.*. *Streptococcus equi subsp. equi* and *Bordetella bronchiseptica* were found in the airway sample of one foal from the R/T group. *Escherichia coli* was isolated in one foal from the R/A group and *Staphylococcus aureus* was identified in one foal in both groups.

Evidence of R. equi in the feces of foals

On the day of diagnosis, *R. equi* was detected in the feces of 12 foals (42,8%) in total. This involved 8 foals from the R/T group (66.7%) and 4 foals from the R/A group (33.3%). In the semiquantitative assessment at microbiological culture of all positively sampled patients, only a low level of *R. equi* was determined in the feces at the day of diagnosis. These 12 foals were sampled weekly with fecal swabs until the end of the two weeks treatment period. There was no further *R. equi* detection at PCR in the fecal swabs of the 12 foals from both treatment groups at T_7 and $T_{14.}$

Evidence of R. equi in the tracheobronchial mucus and in the feces of foals

In 9 of the 13 (69.2%) foals of the R/T group and in 6 of the 15 (40%) foals of the R/A group, *R. equi* was isolated in the tracheobronchial samples and in the fecal swaps at the day of diagnosis pneumonia.

R. equi was detected significantly more frequently in high bacterial counts in the tracheobronchial samples of diseased foals, than in the feces as the sign test showed (p = <0.001).

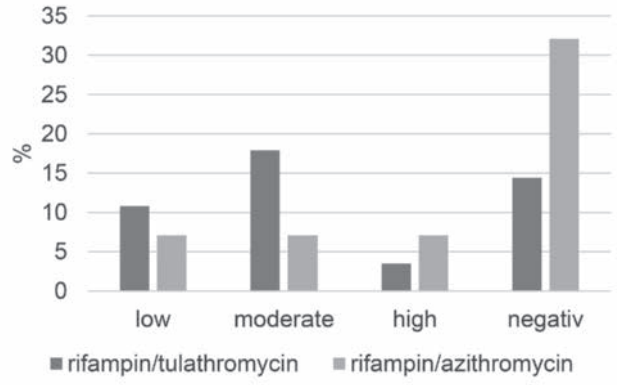

Fig. 1 Identification of *R. equi* in the tracheobronchial mucus in both treatment groups in foals with "moderate to severe pneumonia" at T_0 (T_0 = day of diagnosis). | *Nachweis von R. equi im Tracheobronchialmukus beider Behandlungsgruppen bei Fohlen mit mittel- bis hochgradiger Pneumonie am Tag der Diagnosestellung (T_0 = Tag der Diagnose).*

Table 1 Schedule of the sampling of foals with moderate to severe pneumonia (T_0 = first day of diagnosis, T_7 = seven days after start of treatment, T_{14} = initial end of treatment, PCR = polymerase chain reaction. | *Zeitplan der Probennahme von Fohlen mit mittel- bis hochgradiger Pneumonie (T_0 = Tag der Diagnosestellung, T_7 = 7 Tage nach Behandlungsbeginn, T_{14} = Therapieende).*

Time of sampling	Feces quantitative cultural detection of R. equi	Feces PCR for VapA detection	TBS quantitative cultural detection of pathogens
T_0	X	X	X
T_7	X	X	
T_{14}	X	X	

TBS = tracheobronchial mucus sample | *TBS = Tracheobronchialmukusprobe*

In addition, with a high number of pathogens in the feces, the number of bacteria in the tracheobronchial samples is also high as shown by the Chi-Quadrat test.

Seasonality of pathogens identified in foals with pneumonia

R. equi was detected significantly more often in the hot, dry summer months of July and August. As was shown with the exact test by Fisher ($p = 0.0025$), while *Strep. zoo.* was detected more often in late summer, from late August to September ($p = 0.0007$), (Figure 3). The simultaneous identification of both pathogens did not occur significantly more frequently (two-sided test by Fisher, $p = 0.05$). According to these results, there seems to be a seasonal difference in the incidence of *R. equi* and *Strep. zoo.*.

Discussion

A large number of laboratory diagnostic tools are available for the early detection of the cause of pneumonia in foals (*Lorenz* et al. 2006), which should be preceded by a thorough general clinical examination. Identifying the causative pathogen is crucial for treatment success and here too numerous methods have been investigated. Serological tests show a low sensitivity and in according to the current state of knowledge, they are not useful to diagnose pneumonia caused by *R. equi* (*Giguere* et al. 2003). In sick foals several sample types have been tested such as nasal swabs from foals as a simple method to detect foals with the suspicion of abscessing bronchopneumonia caused by *R. equi*, but with a very low sensitivity (*Meyer-Hamme* 2004). *R. equi* was isolated in only 52 (24%) of the 217 nasal swabs from foals with sonographically detected pneumonia whereby in 118 (54%) of these foals it was detected in the tracheobronchial secretions. Therefore, nasal swabs are not suitable for the detection of *R. equi* and therefore are not recommended (*Meyer-Hamme* 2004, *Lorenz et al*. 2006). The cultural detection of *R. equi* from the tracheobronchial mucus of diseased foals in combination with the detection of the VapA plasmid by using the polymerase chain reaction (PCR) are nowadays the diagnostic method of choice for identification of the pathogen involved in a foal with pneumonia (*Takai* et al.1993, *Giguere* and *Prescott* 1997, *Heyers* 2005, *Kilian* and Feige 2008, *Lämmer* and *Venner* 2010, *Giguere* et al. 2011, *Muscatello* 2012b, *Vitale* et al. 2019). *Oldfield* et al. (2004) examined 35 randomly selected isolates of *R. equi* from various animal and environmental sources and were able to detect the VapA plasmid in 100% of the equine isolates, by using a polymerase chain reaction technique. The molecular biological detection of *R. equi* is much faster and also enables the detection of dead pathogens or their fragments (*Heyers* 2005).

Another simple method for identifying the pathogen in foals with diagnosis "pneumonia" is the detection of *R. equi* in rectal taken swaps. *R. equi* is found quite commonly in the feces of horses (*Takai* et al. 1986). As early as the first week of life, the intestinal tract of the foals can be colonized with *R. equi* (*Takai* et al. 1986). The quantitative, cultural detection of *R.*

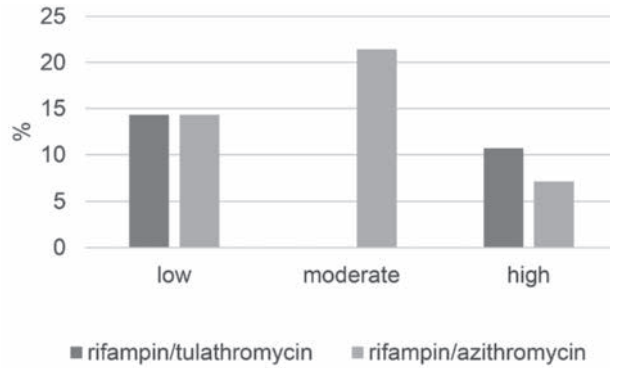

Fig. 2 Amount of pathogen detection of *Strep. zoo.* in the tracheobronchial mucus of foals from both treatment groups with "moderate to severe pneumonia" at T_0 (T_0=day of diagnosis) | *Nachweismenge von Strep. zoo. im Tracheobronchialmukus von Fohlen beider Behandlungsgruppen mit mittel- bis hochgradiger Pneumonie am Tag der Diagnosestellung (n= Anzahl der untersuchten Fohlen, T_0= Tag der Diagnose).*

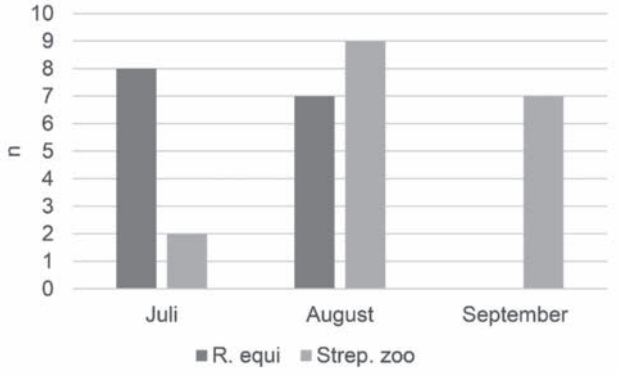

Fig. 3 Number of positive airway and fecal samples to *R. equi* and *Strep. zoo.* in foals with moderate to severe pneumonia at T_0 (T_0= day of diagnosis) between July and September 2019 | *Anzahl des Nachweises von R. equi und Strep. zoo. in den Atemwegen und im Kot von Fohlen mit einer mittel- bis hochgradigen Pneumonie am Tag der Diagnosestellung (T_0= Tag der Diagnose) zwischen Juli und September 2019.*

Table 2 Pathogen detection by microbiological culture. Comparative identification of *R. equi* and *Strep. zooepidemicus*. In the tracheobronchial samples and in the faeces of foals with "moderate to severe pneumonia" at T_0 (n=number of foals, T_0=day of diagnosis). | *Vergleichender Nachweis von R. equi und Strep. zooepidemicus. Im Tracheobronchialmukus und im Kot von Fohlen mit mittel- bis hochgradiger Pneumonie zum Zeitpunkt T_0 (n= Anzahl der Fohlen, T_0= Tag der Diagnose) per mikrobiologischer Kultur.*

	Strep. zoo. TBS	*R. equi* TBS	*R. equi/ Strep. zoo.*	*R. equi* feces	*R. equi* in TBS and feces
rifampin/ tulathromycin n = 13 (46,4%)	3 (23%)	9 (69,2%)	3 (23%)	8 (61,5%)	8 (61,5%)
rifampin/ azithromycin n = 15 (53,6%)	8 (53,3%)	6 (39,9%)	3 (19,9%)	4 (26,6%)	4 (26,6%)

TBS = tracheobronchial mucus sample | *TBS = Tracheobronchialmukusprobe*

equi in the native specimen of the feces is considered as an early diagnostic procedure (*Giguere* et al. 2011). Only a small proportion of foals that show classic symptoms of pneumonia caused by *R. equi* also have positive cultural findings in the feces at the same time. The results of the present study show, that the microbiological detection of *R. equi* in foals with abscessing bronchopneumonia can be provided with the greatest certainty by examining tracheobronchial mucus obtained via endoscopy.

At the day of diagnosis, *R. equi* was identified in 53.6 % of all examined foals in the tracheobronchial samples and in 42.8 % of the foals in the feces. In 9 of the 13 (69.2 %) foals of the R/T group and in 6 of the 15 (40 %) foals of the R/A group, *R. equi* was isolated in both sample types, tracheobronchial samples and in the fecal swaps. The microbiological results in the study of *Lämmer* and *Venner* (2010) showed a 93 % rate agreement of the paired samples from feces and tracheobronchial samples at the day of diagnosis. This statement can be confirmed with the results of the current study as a high germ content of *R. equi* in the feces correlates with a high pathogen content in the tracheobronchial secretions.

Nevertheless, a single positive detection of *R. equi* in fecal sample material does not confirm the diagnosis of pneumonia due to *R. equi*, since a *R. equi* infection can also be present in clinically healthy foals due to the aerogenic uptake of the pathogen (*Venner* et al. 2006). Likewise, a negative fecal sample does not rule out *R. equi* pneumonia (*Giguere* et al. 2011). Therefore, multiple fecal sampling should be performed, when this method is used to detect foals with *R. equi* infections at farms with endemic occurrence of *R. equi* pneumonia.

Based on the current results, we cannot recommend making a diagnosis using fecal swab samples alone. For a reliable diagnosis, this procedure should always be combined with an examination of tracheobronchial mucus samples of the sick foal as the pathogen content of *R. equi* in the tracheobronchial secretions of infected foals is significantly higher than in the feces.

Strep. zoo. as a further opportunistic bacterial pathogen of the lower respiratory tract is diagnosed very frequently in foals with pneumonia (*Lavoie* et al. 1994, *Léguillette* et al. 2002). Clinically, the *Strep. zoo.* pneumonia can hardly be distinguished from a *R. equi* infection. The infection with *Strep. zoo.* in foals often manifests itself as purulent bronchopneumonia. In older suckling foals up to the weaning age it leads to infections of the deep respiratory tract and above all damages the cranial lobes of the lungs (*Lorenz* et al. 2006). The gold standard for differentiating *Strep. zoo.* is the bacteriological examination with subsequent biochemical differentiation of specific DNA via PCR (*Lorenz* et al. 2006). Although the results of the current study did show a seasonal difference in the incidence of *R. equi* and *Strep. zoo.*, the similarity of the clinical signs underlines the importance to determine the causing pathogen in the airway samples of sick foals.

In addition, other possible pathogens can also be identified as the cause of pneumonia and treated accordingly, if these are identified by culture of airway samples. A disadvantage of taking samples via endoscopic examination is certainly the possibility of sample contamination by pathogens from the upper respiratory tract (*Hoffman* et. al 1993, *Kilian* and *Feige* 2008). Therefore, before every endoscopic examination and sample collection, the endoscope should be adequately cleaned and the sample collection should be as sterile as possible (*Hoffman* et. al 1993). Another disadvantage is compared to the transtracheal wash the higher invasiveness of the method, more staff and a sedation are required to restrain the patient and to carry out the endoscopy.

As the current results show, two weeks after starting antimicrobial treatment of pneumonia, no foal showed positive pathogen detection in the feces. These results coincide with those of *Lämmer* and *Venner* (2010), where *R. equi* excretion decreased significantly after two weeks of treatment. Based on the results of the current study, it can therefore be assumed that both active antimicrobial combinations are equally effective in reducing fecal *R. equi* elimination.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Arnold-Lehna D., Venner M., Berghaus L., Berghaus R., Giguere S. (2019) Changing policy to treat foals with Rhodococcus equi pneumonia in the later course of disease decreases antimicrobial usage without increasing mortality rate. Equine Vet. J. 52, 531–537; DOI 10.1111/evj.13219

Fels J., Rohn K., Venner M. (2021) Do the clinical findings correlate with the severity of the pneumonia in foals? Pferdeheilkunde 37, 147–155; DOI 10.21836/PEM20210206

Giguere S., Prescot J. F. (1997) Clinical manifestations, diagnosis, treatment, and prevention of Rhodococcus equi infections in foals. Vet. Microbiol. 56, 313–334

Giguere S., Hernandez J., Gaskin J., Prescott J. F, Takai S., Miller C. (2003) Performance of Five Serological Assays for Diagnosis of Rhodococcus equi Pneumonia in Foals. Clin. Vaccine Immunol. 10, 241–245

Giguere S., Cohen N. D., Chaffin M. K., Slovis N. M., Hondalus M. K., Hines S. A., Prescott J. F. (2011) Diagnosis, treatment, control, and prevention of infections caused by Rhodococcus equi in foals. J. Vet. Intern. Med. 25, 1209–1220; DOI 10.1111/j.1939-1676.2011.00835.x

Giguere S., Hernandes J., Gaskin J., Prescott J. F., Takai S., Miller C. (2003) Performance of five serological assays for diagnosis of Rhodococcus equi penumonia in foals. Clin. Diagn. Lab. Immunol. 10, 241–245; DOI 10.1128/CDLI.10.2.241-245.2003

Giguere S., Prescott J. F. (1997) Clinical manifestations, diagnosis, treatment, and prevention of Rhodococcus equi infections in foals. Vet. Microbiol. 56, 313–334; DOI 10.1016/s0378-1135(97)00099-0

Heyers P. (2005) Vergleich des Nachweises von Rhodococcus equi durch mikrobiologische Kultur mit dem Nachweis durch die Polymerase Chain Reaction in endoskopisch entnommenem Tracheobronchialsekret bei Fohlen. Diss. Med. Vet. Hannover

Hoffman A. M., Viel L., Prescott J. F. (1993) Microbiological changes during antimicrobial treatment and rate of ralapse of distal respiratory tract infections in foal. Am. J. Vet. Res. 54, 1608–1614

Kilian K., Feige K. (2008) Vergleichende Untersuchungen zum Nachweis von Rhodococcus equi in der Atemluft, im Tracheobronchialsekret und im Kot von Fohlen. Diss. Med. Vet. Hannover

Kuskie K. R., Smith J., Wang N., Carter C. N., Chaffin M. K., Slovis N. M., Stepusin R. S., Cattoi A. E., Takai S., Cohen N. D. (2011) Effects of Location for collection of air samples on a farm and time of day of sample collection on airborne concentrations of virulent Rhodococcus equi at two horse breeding farms. Am. J. Vet. Res. 72, 73–79; DOI 10.2460/ajvr.72.1.73

Lämmer M., *Venner M.* (2010) Nachweis von Rhodococcus equi in Kot und Trachealsekret bei Fohlen: Vergleichende Untersuchung zwischen gesunden Fohlen und Fohlen mit Lungenabszessen. Diss. Med. Vet. Hannover

Lavoie J. P., *Fiset L.*, *Laverty S.* (1994) „Review of 40 cases of lung abscesses in foals and adult horses." Equine Vet. J. 26, 348–352; DOI 10.1111/j.2042-3306.1994.tb04401.x

Léguillette R., *Roy M. F.*, *Lavoie J. P.* (2002). „Foal Pneumonia." Equine Respiratory Diseases Ed: P. *Lekeux*, International Veterinary Information Service, Ithaca, New York

Lorenz N., *Hamuth M.*, *Venner M.*, *Strutzberg-Minder K.* (2006) Bedeutende virale und bakterielle Atemwegserreger des Pferdes und deren diagnostischer Nachweis „ Pferdeheilkunde 22, 746–756; DOI 10.21836/PEM20060601

Makrai L., *Fodor L.*, *Vendeg I.*, *Szigeti G.*, *Denes B.*, *Reiczigel J.*, *Varga J.* (2005) Comparison of selective media for the isolation of Rhodococcus equi and description of a new selective plating medium. Acta. Vet. Hung. 53, 275–285

Meyer-Hamme M. B. (2004) Rhodococcus equi und Streptococcus equi subsp. zooepidemicus aus Nasentupfern und Tracheobronchialsekret von lungenkranken Fohlen. Diss. Med. Vet. Hannover

Muscatello G. (2012a) Rhodococcus equi pneumonia in the foal – Part 1: Pathogenesis and epidemiology. Vet. J. 192, 20–26; DOI 10.1016/j.tvjl.2011.08.014

Muscatello G. (2012b) Rhodococcus equi pneumonia in the foal – Part 2: Diagnostics, treatment and disease management. Vet. J. 192, 27–33; DOI 10.1016/j.tvjl.2011.08.009

Oikawa M., *Kamada M.*, *Yoshikawa Y.*, *Yoshikawa T.* (1994) Pathology of equine pneumonia associated with transport and isolation of Streptococcus equi subsp. zooepidemicus. J. Comp. Pathol. 111, 205–212; DOI 10.1016/s0021-9975 (05)80052-0

Oldfield C., *Bonella H.*, *Renwick L.*, *Dodson H. I.*, *Alderson G.*, *Goodfellow M.* (2004) Rapid determination of vapA/vapB genotype in Rhodococcus equi using a differential polymerase chain reaction method. Antonie Van Leeuwenhoek 85, 317–326; DOI 10.1023/B:ANTO.0000020383.66622.4d

Takai S., *Ohkura H.*, *Watanabe Y.*, *Tsubaki S.* (1986) Quantitative aspects of fecal Rhodococcus (Corynebacterium) equi in foals." J. Clin. Microbiol. 23, 794–796; DOI10.1128/jcm.23.4.794–796.1986

Takai S., Watanabe Y., Ikeda T., Ozawa T., Matsukura S., Tamada Y., Tsubaki S., Sekizaki T. (1993) "Virulence-Associated Plasmids in Rhodococcus equi." J. Clin. Microbiol. 1726-1729

Venner M., *Heyers P.*, *Strutzberg-Minder K.*, *Lorenz N.*, *Verspohl J.*, *Klug E.* (2006) Nachweis von Rhodococcus equi durch mikrobiologische Kultur und mittels Polymerasekettenreaktion (PCR) im Tracheobronchialsekret von Fohlen." Berl. Münchn. Tierärztl. Wschr. 120, 126–133

Vitale V., *Sgorbini M.*, *Cuteri V.*, *Preziuso S.*, *Attili A. R.*, *Bonelli F.* (2019) Cytological Findings in Bronchoalveolar Lavage Fluid of Foals With Pneumonia Caused by Rhodococcus equi and Other Bacteria „ J. Equine Vet. Sci. 79, 9–12; DOI 10.1016/j.jevs.2019.05.009