Variation in bacterial presence in the respiratory tract of calves suffering from respiratory disease in relation to clinical signs and treatment

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Abstract

The aim of this study was to analyse variation in the presence of bacteria in the deeper respiratory tract in relation to clinical signs and the effects of treatment for dairy calves suffering from bovine respiratory disease in the Netherlands. For this purpose dairy calves with clear clinical signs were selected and transtracheal aspirated samples were obtained at the day of inclusion in the study and at a later evaluation moment. Coughing, fever, an increased respiratory rate, increased respiratory sounds and nasal discharge were observed clinical signs for diseased animals. The most commonly cultured bacterium was *Pasteurella multocida*. *Mycoplasma spp.*, *M.haemolytica* and *T.pyogenes* were rarely isolated and *H.somni* was not detected at all. Variation in severity of acute infection did not result in considerable differences in cultured bacterial species. Changes in bacterial isolation in the evaluation samples were observed for the majority (78%) of the calves that showed improvement of clinical signs. Improvement of clinical signs was not necessarily associated with a decline in isolated bacteria. It was observed that antibiotic treatment can result in a decline in number of isolated bacteria, but despite an antibiotic treatment also an increase in bacteria still being present or contribution of new bacterial species to an infection already started by other pathogens were found. *Pasteurella multocida* isolates showed susceptibility to 3rd generation cephalosporins, ampi-/amoxicillin, fluoroquinolones, gentamicin, trimethoprim/sulfa, tetracycline/doxycycline, kanamycin, penicillin and spiramycin/tylosin. Resistance against kanamycin, penicillin and spiramycin/tylosin was also detected for some *Pasteurella multocida* isolates. For *M.haemolytica* only resistance against tetracycline/doxycycline was observed.

Keywords
Bovine respiratory disease, calf, pneumonia, economic effects, predisposing factors, virus, bacteria, sampling methods, transtracheal aspiration, *Pasteurella multocida*, *Mannheimia haemolytica*, *Trueperella pyogenes*, *Mycoplasma bovis*, *Histophilus somni*, antimicrobial susceptibility.

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List of abbreviations

Abbreviation	Meaning
BAL	Bronchoalveolar lavage
BCoV	Bovine coronavirus
BHV1	Bovine herpes virus 1
BRD	Bovine respiratory disease
BRSV	Bovine respiratory syncytial virus
BVDV	Bovine virus diarrhoea virus
FPIT	Failure of passive immunity transfer
NPS	Nasopharyngeal swab
NSAIDs	Non-steroidal anti-inflammatory drugs
PI3	Parainfluenza virus 3
STP	Serum total protein
TBL	Tracheobronchial lavage
TTA	Transtracheal aspiration

Introduction

Economic effects of respiratory diseases on beef and dairy industries

Cattle of all ages and different types are susceptible to respiratory diseases and in some of the production settings, bovine respiratory diseases are one of the most important causes of animals' mortality and morbidity. Although respiratory diseases can be of major importance for adult cattle, these diseases are less important with regard to morbidity of these animals in comparison with for example lameness, reproductive disorders, clinical mastitis and several metabolic disorders. Susceptibility to these diseases is also greater in young animals with dairy calf pneumonia traditionally being described to affect animals with an age ranging from 2 to 6 months. In the publication of Virtala *et al.* (1996) it was reported that even calves from 2 weeks of age can get affected by pathogens causing pneumonia (1-4).

Respiratory diseases can still be considered as one of the biggest health concerns in the world's beef industry. In the United States for example, the impact of bovine respiratory diseases on the national beef industry is greater than $500 million on an annual basis (5). For feedlot cattle 40% to 50% and 70% to 80% of the causes of mortality and morbidity respectively can be attributed to respiratory disease. Moreover, these diseases are responsible for 21% of all health problems in pre-weaned beef calves (6,7). Not only costs associated with treatment of diseased animals are important contributors to economic losses, but also cattle's performance can be affected on the long term, which will inevitably impact their final value (8). In the publication of Schneider *et al.* (2009) the relation between negative economic effects and the number of treatments for respiratory diseases was described. Carcass value was reduced with $23.23, $30.15, and $54.01 for animals that have been treated for respiratory diseases once, twice or ≥3 times respectively in comparison to untreated healthy animals. Also in the study described by Cernicchiaro *et al.* (2013) it was reported that net returns of animals which have been treated were decreased compared to untreated animals (8,9).

Moreover, economic losses associated with bovine respiratory diseases are of great importance for the economy of individual farmers in dairy industry. In the NAHMS 2007 Dairy study in the United States it was described that respiratory disease are responsible for 22.5% and 46.5% of the estimated mortalities of unweaned and weaned dairy heifers respectively (10). Also in this sector, the economic losses are caused by treatment and reduction of life-time productivity of diseased cattle. Examples of the latter are a reduction in animal's fertility, an increased mortality, an increase in premature culling, giving birth to the first calf at higher age and a reduction in animal's weight gain (11-14). In the publication of Van der Fels-Klerx *et al.* (2001) economic losses in dairy industry in the Netherlands were analysed. For a seasonal outbreak with heifers up to 15 months old that were showing clinical signs, these average losses were €27.0 per heifer (14,15).

So respiratory diseases are together with diarrhoea the world's most common diseases cattle can suffer from. These diseases are also considered as well-known causes of great economic losses in beef and dairy industry.

Bovine respiratory disease

Cattle can suffer from several respiratory diseases such as calf diphtheria, atypical interstitial pneumonia, lungworms, allergic reaction and emphysema. In literature bovine respiratory disease (BRD) is often described as a multifactorial syndrome referring to a set of several clinical signs caused by different pathogens, e.g. coughing, dyspnoea, an increased respiratory rate, nasal discharge, fever and a decrease in animal's appetite due to depression. For dairy and beef cattle the term is often related to a range of pneumonic illnesses which is associated with inflammation, consolidation and sometimes even with abcessation and fibrosis of the affected animal's lungs. BRD is sometimes subdivided into e.g. enzootic pneumonia for dairy calves and shipping fever in beef calves due to the high incidence of BRD after shipment of calves to feedlots or stocker operations (14,16-20).

In this multifactorial BRD complex several predisposing factors are required to induce disease in susceptible animals. The aetiology of BRD is quite complex with infectious pathogens, disadvantageous environmental factors (e.g. adverse weather conditions and increased concentrations of noxious gasses), but also animal factors (e.g. failure of passive immunity transfer (FPIT), lack of vaccination and age) being involved. Usually a combination of those different predisposing factors is required for the induction of

bronchopneumonia, which is the ultimate result of the BRD complex (3,20-23).

Predisposing factors

Transport of cattle is one of the most accepted stressors responsible for predisposing cattle to BRD. Especially for beef calves, transport is important since these calves will be transported at least once in their lifetime. The result of this particular stressor is immunosuppression and an increase in the host's cellular oxidative challenge. In the publication of Blecha *et al.* (1984) the negative effects of stress, caused by transport, on the animal's cellular immune reactivity were described for feedlot calves. The animals' lymphocyte blastogenic responses were measured at the moment of unloading and these responses turned to be lower for shipped feeder calves (22,24,25).

Commingling of animals from different sources is another stressor which is considered as contributor to the BRD complex. Mixture of animals may increase stress due to interacting with strange animals or can lead to a greater exposure to infectious agents (3,22).

FPIT, which is the result of absorption of inadequate numbers of colostral immunoglobulins during the first hours of a neonatal calf's life, is a predisposing factor concerning the animal itself (26). Due to the cow's syndesmochorial placenta, which prevents immunoglobulins to be received by a bovine fetus, newborn calves depend on their passive immunity. For the latter it is important that calves are fed with adequate amounts of colostrum during the first 24 to 36 hours of their lives (27,28). The publication of Virtala *et al.* (1999) showed that calves being exposed to low levels of IgG had a two times greater odds of developing pneumonia compared to calves with higher measured IgG concentrations (29). In the publication of Windeyer *et al.* (2014) serum total protein (STP) concentrations <5.2 g/dl were considered as FPIT. It was reported that calves with STP concentrations <5.2 g/dl had an increased risk of developing respiratory diseases (30).

Disadvantageous weather conditions and their association with season of birth are also often described in the aetiology of BRD (31). In the publication of Windeyer *et al.* (2014) it was reported that calves born in spring, autumn and winter had 2.1, 1.6 and 2.6 times higher odds of developing BRD respectively in comparison with animals which were born in summer. Calves that were born in winter had also a 1.6 times greater odds of developing respiratory disease in comparison to the ones born in autumn (30). It appears that disadvantageous weather conditions, including low environmental temperatures, contribute to the occurrence of the BRD complex (22,32,33). Rapid changes in ambient temperature are considered as the major cause responsible for elevating BRD incidence, as was described by the publication of Irwin *et al.* (1979) (34). Next to these rapid changes, also influence of the minimum daily temperature was described. In the publication of Cusack *et al.* (2007) it was reported that the lower the minimum daily ambient temperature, the higher the observed daily incidence of BRD (33). Especially young calves have a narrow temperature comfort zone which ranges from about 10 to 25 degrees Celsius (35). Once the ambient temperature reaches values above or below the upper or lower boundaries of this comfort zone, animals will be under stress and become less resistant to respiratory infections. Also other environmental factors such as wind speed, shade and wind protection can contribute to ambient temperatures reaching values above or below the upper or lower boundaries of an animal's comfort zone (32).

Another predisposing factor is a poor air quality. Elevations in concentration of noxious gasses and both the pathogen density and survival time can be the result of an inadequate ventilation within barns together with a high air humidity (3,36). In the publication of Lago *et al.* (2006) it was described that a poor mixing of air within barns can be responsible for turning calf pens into microenvironments which have a worse air hygiene compared to the rest of the barn. Calf pens often have significantly higher airborne bacterial counts than other parts of the barn. Accumulation of airborne bacteria in areas with a poor air quality is associated with a higher prevalence of calves suffering from respiratory diseases (36).

Pathogenic agents

Several viruses are considered to be important in BRD pathogenesis. According to results from several studies, bovine coronavirus (BCoV), bovine respiratory syncytial virus (BRSV), bovine viral diarrhoea virus (BVDV), parainfluenza virus 3 (PI3) and bovine herpes virus 1 (BHV1) are viral agents which are most often isolated from diseased animals (37,38). For BRSV, PI3 and BHV1 it was already known that these viruses are capable of acting as primary respiratory pathogens. Also the role of BVDV as major pathogenic agent in BRD aetiology has been recognized during the last decades (20,23,39,40).

Nevertheless, an infection with one of these viruses may not be enough to cause severe

disease in animals only by themselves. Viral infections are mostly described as antecedent to bacterial infections or as concurrent with the latter. Around 90% of all bacterial pneumonias are preceded by viral infections (22,25,41). The major role of viruses is to assist in establishing a suitable environment in the respiratory tract to contribute to the colonisation and replication of pathogenic bacteria, which can result in pneumonia (23,39). Viral agentia are believed to fulfil this role of predisposing factors in two different ways. First of all, viruses can cause direct damage to both the lung parenchyma and mechanisms which are responsible for the clearance of the respiratory tract. This facilitates the translocation of pathogens from the upper to the lower parts of the respiratory tract and the establishment of a bacterial infection (25). Secondly, it is possible that an infection with a virus is able to interfere with the ability of the host's innate and adaptive immune responses to combat bacterial infections (25,42).

The most common bacterial agents isolated from animals suffering from BRD are *Pasteurella multocida, Mannheimia haemolytica* (formerly *Pasteurella haemolytica*), *Mycoplasma bovis, Trueperella pyogenes* (formerly *Arcanobacterium pyogenes*) and *Histophilus somni* (formerly *Haemophilus somnus*) as described by studies about several production systems and from different parts of the world (16,43-45). In the publication of Klima *et al.* (2014) all previous described bacteria except for *T.pyogenes* were isolated from feedlot cattle that had suffered and died from an acute fibrinous pneumonia in Canada and the USA. *M.haemolytica* was the most common bacterium that was isolated and was present in 91% of the animals studied. This bacterium was followed by *M.bovis* (63%), *H.somni* (57%) and *P.multocida* (13%) (16). Additionally, in the European study described by Tegtmeier *et al.* (1999) *H.somni*, *P.multocida*, *T.pyogenes* and *M.haemolytica* were isolated from the lungs of calves that suffered from calf pneumonia in Denmark in respectively 15%, 14%, 13% and 1% of the cases. In 84% of the cases also several mycoplasmas (e.g. *M.bovis*, *M.dispar*, *M.bovirhinis* and *U.diversum*) were isolated (45).

The fact that bacteria seldom act as primary pathogens in BRD aetiology and also the multifactorial nature of BRD are underlined by observations from different studies. Most of the described bacteria can be considered as commensals being part of the normal resident flora in the nasopharynx of cattle. Several of these bacterial agents can therefore be isolated from the airways of both healthy and diseased animals (3,21,46). The studies described by Angen *et al.* (2009) and Autio *et al.* (2007) reported isolation of potentially pathogenic bacteria from the lower parts of the bovine respiratory tract in clinically healthy calves (38,47). Furthermore, in the study described by Timsit *et al. (*2013) *M.haemolytica* was cultured from inter alia samples obtained from the lower respiratory tract of animals suffering from BRD in France. Subsequently, these *M.haemolytica* isolates were analysed for genetic diversity to determine presence of a single or multiple isolate(s) of this bacterium within a pen during a BRD outbreak. A significant within-pen diversity of this pathogen was reported during BRD episodes with up to three different isolates detected. This significant diversity supports the multifactorial nature of BRD, which is therefore not primarily caused due to the contagious spread of only one virulent isolate among animals. It is more likely the result of combined actions of several predisposing factors which allow the animal's resident flora to overcome the host's immune system and migrate and colonize the lower parts of the bovine respiratory tract (48,49).

Sampling methods

To study presence of bacterial pathogens in both healthy and diseased animals and also differences in bacterial flora between several parts of the respiratory tract, samples have to be obtained. For collection of these samples from different parts of the respiratory tract, various techniques can be used. Among these are less invasive methods such as the nasal and nasopharyngeal swab (NPS). Several studies described different variants of these NPS and nasal swabs (46,50-53). In the publication of Thomas *et al.* (2002) a normal nasal swab was used to obtain samples from the animal's nostril to conclude about the suitability of these swabs for the prediction of Mycoplasma species present in the animal's lungs. For a few seconds, these swabs were deeply introduced into the animal's nostril. However, cultures from nasal swabs were considered as not-suitable to predict which Mycoplasma species are present in the animal's lungs. A disadvantage of nasal swabs mentioned in this study is that rapid desiccation of those swabs negatively influences sample quality and hampers diagnosis of respiratory pathogens (50). The so-called enclosed nasal swab was used to obtain samples in the study described by Magwood *et al.* (1969). This swab, composed of an outer and inner tube, was passed through the animal's nares to a certain point posterior to the maxillary sinus' ostium. Subsequently, the inner tube was inserted 8 to

10 centimetres further to obtain a sample from the inferior meatus. Environmental contamination of the swab was low in this study. A publication of DeRosa *et al.* (2000) describes the use of a guarded nasal swab in order to prevent contamination of the swab with flora resident in the upper parts of the respiratory tract (51,52).

To obtain samples from the nasopharyngeal area one could use a NPS. In the publication of Allen *et al.* (1991) a NPS was used to obtain samples from the more upper parts of the respiratory tract of both healthy calves and animals suffering from respiratory diseases. The isolates from the NPSs were compared to the ones cultured from the animal's lungs. To avoid unwanted contamination, the NPSs were enclosed within a sleeve made of sterile material which can be removed when the swab has reached its desired depth. However, cultures from a NPS turned out not to be predictive for the ones isolated from the lungs at individual calf level (46). Another variant of this NPS is the so-called deep nasopharyngeal swab (deep NPS). The 76 cm long deep NPS described in the publication of Godinho *et al.* (2007) is guarded by a sheath and the tip of the swab is protected by a plug of gelatine. The deep NPS is inserted into the nasal cavity as far as possible. To obtain a sample from the deep nasopharyngeal mucosa, the swab's tip is extruded beyond its protective sheath and firmly rotated against the mucosa. These deep NPSs are a useful method for sampling larger herds in a short timeframe since it is a fast and simple method with minimal stress for the animal being sampled (53).

Next to these techniques, more invasive ones can be used to obtain samples from the deeper parts of the bovine respiratory tract. An example is the guarded transtracheal swab, which was used in the study described by DeRosa *et al*. (2000) to compare isolates from this swab with the ones obtained with a guarded nasal swab in calves with clinical signs of BRD. For 96% of the sampled animals, the same bacterial agents were identified from these guarded swabs. About 70% of the isolates from the guarded nasal swab were also genetically identical to the ones cultured from the guarded transtracheal swab (52). Other examples of more invasive techniques are bronchoalveolar lavage (BAL), tracheobronchial lavage (TBL) and transtracheal aspiration (TTA). One of the main benefits of these sampling methods is the avoidance of contamination with bacteria from the upper respiratory tract. This contamination was avoided by covering the bronchoscope in a clear plastic sheath, which improved the quality of BAL samples in the publication of Allen *et al.* (1991) (46). TTA was used to obtain samples without contamination in the publication of Angen *et al.* (2008). This method of sampling has been recommended as an optimal way of sampling to evaluate the microbiological status of the lower parts of the bovine respiratory tract (38,46,47).

Aim of the present study

In the publication of Magwood *et al.* (1969) the within-day frequency, daily frequency and long-term variation in presence of bacteria in the airways of calves suffering from respiratory diseases were described (51). However, to our knowledge, not in this publication and neither in other studies the relation between variation in bacterial presence, clinical signs of BRD and the effects of treatment has been described. Moreover, in the publication of Magwood *et al.* (1969) only the variation in nasal bacterial flora was reported. The aim of the present longitudinal study was to analyse differences in composition of the bacterial flora in the deeper parts of the respiratory tract of calves suffering from BRD in the Netherlands related to severity of clinical signs and with or without an intermediate treatment. The null hypothesis (H0) of the present study is that the composition of the bacterial flora in the deeper parts of the respiratory tract cannot be linked to the severity of clinical signs and/or the effects of an intermediate treatment. The alternative hypothesis (H1) is that the composition of the bacterial flora in the deeper parts of the respiratory tract can be linked to the severity of clinical signs and/or the effects of an intermediate treatment.

Simultaneously, it was our intention to contribute to the selection of an effective antimicrobial therapy when it was decided to treat patients with antibiotics.

Materials & methods

Animals and housing

Calves that were sampled in this longitudinal study were patients present at the Department of Farm Animal Health of the Faculty of Veterinary Medicine of Utrecht University in the Netherlands. The animals included in the present study were either bought by the Department of Farm Animal Health from different farms in the Netherlands or were born at this location itself to cows which were already present here. A total number of 14 calves has been examined between October 2016 and December 2016. The age of the animals ranged

from 1 to 14 months with an average of 6 months of age. The calves were housed on floors covered with straw in either individual boxes or groups (2 to 3 animals per pen) depending on the animal's age and type and severity of the disease the calf was suffering from. Pens were separated from each other by concrete walls or metal pipes. Calves housed in groups had unlimited access to water and were daily fed with silage, hay and corn

Study design and criteria

All calves were examined at least twice a week and sometimes daily by a veterinarian and/or students and all observations of clinical signs were recorded. For the enrolment of calves in the present study one or a combination of the following clinical signs of BRD had to be present: coughing, nasal discharge and/or abnormal respiratory signs (an abdominal character of breathing, an increased respiratory rate (>45 breaths/minute) and/or increased respiratory sounds). Also calves showing an elevated rectal temperature (>39.5°C) in combination with other clinical signs of BRD were included in the present study.

At the day of inclusion also a first sample from the deeper parts of the respiratory tract was obtained by transtracheal aspiration to analyse the bacterial composition in relation to severity of acute infection. After collection of the first sample, a calf could be treated with different drugs for one or several days, such as antibiotics or non-steroidal anti-inflammatory drugs (NSAIDs). Metacam was the NSAID which was used for treatment of calves in the present study. Antibiotics that were administered to the patients were Nuflor®, Procapen® and ampicillin. In the Netherlands Nuflor® is a registered antibiotic which contains florfenicol and can be given to cattle and sheep that are suffering from respiratory diseases (54). Procapen® is an antibiotic which contains benzylpenicillin and is registered in the Netherlands for cattle, horses and pigs. For cattle it can be administered to treat respiratory diseases, but also urogenital, skin and also joint infections (55).

Subsequently, when clinical signs were improved or deteriorated in comparison to the ones present at the day of inclusion in the study an evaluation sample was obtained to evaluate differences in the composition of the bacterial flora in relation to the first sample. When a calf was treated between the first and evaluation sample also the effects of treatment on the bacterial flora were studied. Some of the animals were only sampled once since it was not possible to obtain an evaluation sample due to a natural death, euthanasia or culling shortly after obtaining the first sample.

When it was not possible to obtain a proper first sample, another attempt was made at another day if clinical signs persisted or occurred again at a later moment. Clinical signs and data were excluded from the present study for animals that were not successfully sampled again. For calves another attempt resulted in a successfully obtained sample, data and clinical signs present at that day were included in the study.

Sampling

Sedation of calves was performed by giving an intravenous injection with xylazine. The dose in mg/kg bodyweight was calculated on the basis of the calf's weighed or estimated weight. This injection was given in either the jugular or coccygeal vein. In one of the cases also a small longitudinal incision in the skin and underlying tissues was made after injection of a local analgesic (lidocaine) to facilitate the perforation of the trachea for this animal.

Then a sterile Centracath – 75 cm catheter was used to obtain transtracheal aspirated samples from the lower part of the bovine respiratory tract. A small area about 7 to 10 centimetres distal to the larynx was cleaned using lukewarm water and burlap. Hibiscrub (chlorhexidine gluconate 40 milligram/ml) was used to further clean the area. Then lukewarm water was used to wash away the remaining dirt. Subsequently, the area was shaved and decontaminated with 70% ethanol before perforation of the trachea was performed. The animal's trachea was fixated firmly with one hand while the other was used for perforation of it with the needle of the catheter. This perforation was performed between two cartilaginous rings. The needle was then further inserted into the trachea and its position was checked for correctness by moving and turning it around very carefully. Then the catheter was gently introduced into the trachea until a certain slight resistance was felt and it was not possible to move it any further. A total of 25 to 90 ml sterile 0.9% NaCl were injected through this catheter and this injection was followed by immediate aspiration. Normally, this resulted in 2 to 9 ml aspiration fluid.

Detection of microbes

Fluid was kept in the fridge overnight at low temperatures or immediately transported to the Veterinary Microbiological Diagnostic Centre of Utrecht University for detection of bacterial pathogens. Firstly, samples were centrifuged

for 10 minutes (3000 rotations per minute). The centrifuged fluid was then drained and the sediment was streaked on different plates using an inoculation loop. For the isolation of bacteria agar plates containing sheep blood were used under aerobic, anaerobic and CO_2 conditions. Also MacConkey agar plates were inoculated with sediment. Culturing of *Mycoplasma spp.* was performed under previously described conditions, but *Mycoplasma spp.* were not further determined. Incubation of the agar plates at a temperature of 37 °C took 18 to 48 hours and the plates were daily judged by microbiologists. Flukes present in some of the samples were also used for the preparation of GRAM stains. When a mixed culture (two or more different bacterial species) was isolated from a sample, the predominant bacterium present in this sample is presented.

Antibiogram

When a predominant bacterial pathogen was cultured from a sample also an antibiogram was made. An antibiogram was not made for *Mycoplasma spp.*. Susceptibility to several antibiotics was tested for the predominant bacterial agent (i.a. penicillin, fluoroquinolones, ampi-/amoxicillin, gentamicin, kanamysin, 3rd generation cephalosporins, spiramysin/tylosin, tetracycline/doxycycline and trimethoprim/sulfa). Susceptibility of a bacterial agent to certain antibiotic drugs was taken into consideration when treatments for diseased animals were set.

Results

Observed clinical signs of respiratory disease

Clinical signs of respiratory disease at the day of inclusion in the study and at the day of obtaining the evaluation sample can be found in Table I. The 14 diseased animals showed coughing, fever, an increased respiratory rate, increased respiratory sounds, an abdominal character of breathing and nasal discharge as clinical signs at the day of inclusion in the study. Coughing was observed for 11 of the sampled animals and fever was reported for 7 calves. An increased respiratory rate, increased respiratory sounds and an abdominal character of breathing were observed for 6, 11 and 4 of the diseased animals respectively. Nasal discharge was reported as clinical sign for 9 of the cases. For 3 animals a first sample was not successfully obtained at first attempt. One of these calves (patient number 3) was successfully sampled at another moment, while for the other 2 another attempt was not performed.

Between the first and evaluation sample 7 patients were treated. An antibiotic treatment was administered to 6 out of 7 patients. The different treatments are illustrated in Table III. All of the 9 patients that were sampled for evaluation showed improvement in clinical signs at the day of obtaining the evaluation sample. Coughing, fever, an increased respiratory rate, increased respiratory sounds and nasal discharge were observed as clinical signs still being present at the day of obtaining the evaluation sample. However, clinical signs were less severe in comparison to the ones present at the day of obtaining the first sample. Coughing as clinical sign was reported for 4 of the calves and fever was only observed for 1 animal. An increased respiratory rate and increased respiratory sounds were observed for 3 and 5 of the calves respectively. Nasal discharge was reported for only 1 of the animals.

Bacterial pathogens cultured from both the first and evaluation samples

Bacterial species cultured from both the first and evaluation samples can be found in Table II. Bacteria were also isolated in different numbers (sporadic (+), few (++), moderate (+++) and many (++++)). No bacterial pathogens were cultured from 3 of the first samples. In the first samples 3 different bacterial species were detected. *P.multocida* was the most commonly cultured bacterium and was detected in 10 out of 14 samples. For the majority of the first samples containing *P.multocida*, it was the only bacterial species present. *T.pyogenes* and *Mycoplasma spp.* were both isolated from 2 out of 14 samples. *T.pyogenes* was only isolated concomitantly with other bacterial species. *Mycoplasma spp.* was either cultured as single pathogen present in a sample or isolated together with other bacterial species. An anaerobic mixed culture was isolated from 1 of the animals (patient number 11), but no predominant anaerobic bacterial species was isolated from this mixed culture.

Table I

Clinical signs observed in calves at the day of inclusion in the study and at the day of obtaining the evaluation sample. For the first samples patients were ranked by severity of clinical signs.

Clinical signs of respiratory disease													
	First samples							Evaluation samples					
	Coughing	Fever (in °C)	Increased respiratory rate (breaths/min)	Increased respiratory sounds	Abdominal character of breathing	Nasal discharge		Coughing	Fever (in °C)	Increased respiratory rate (breaths/min)	Increased respiratory sounds	Abdominal character of breathing	Nasal discharge
Patient							Patient						
1	+	40.5	60/min	+	-	-	-	-	-	-	-	-	-
2	+	39.6	52/min	+	+	+	2	+	-	-	-	-	-
3	+	40.3	-	+	+	+	3	+	-	-	-	-	-
4	+	40.3	-	+	-	+	4	-	39.6	-	+	-	-
5	+	39.6	-	+	-	+	5	+	-	-	+	-	+
6	+	-	60/min	+	+	+	-	-	-	-	-	-	-
7	+	-	88/min	+	-	+	7	-	-	72/min	+	-	-
8	+	40.0	-	-	-	-	8	+	-	-	-	-	-
9	+	40.0	-	-	-	-	9	-	-	48/min	+	-	-
10	+	-	52/min	-	+	-	10	-	-	-	-	-	-
11	-	-	48/min	+	-	+	-	-	-	-	-	-	-
12	+	-	-	+	-	-	-	-	-	-	-	-	-
13	-	-	-	+	-	+	13	-	-	48/min	+	-	-
14	-	-	-	+	-	+	-	-	-	-	-	-	-

Table II

Bacterial pathogens cultured from the transtracheal aspirated fluid at the day of inclusion in the study and at the day of obtaining the evaluation sample.

Bacterial pathogens											
	First samples						Evaluation samples				
	P.multocida	*M.haemolytica*	*T.pyogenes*	*Mycoplasma spp.*	*H.somni*		*P.multocida*	*M.haemolytica*	*T.pyogenes*	*Mycoplasma spp.*	*H.somni*
Patient						Patient					
1	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	2	-	-	-	-	-
3	+	-	-	+	-	3	-	++++	-	-	-
4	+	-	+++	-	-	4	-	-	-	-	-
5	+++	-	-	-	-	5	+	-	-	-	-
6	+++	-	-	-	-	-	-	-	-	-	-
7	++++	-	-	-	-	7	-	-	-	-	-
8	+++	-	-	-	-	8	++++	-	-	-	-
9	+++	-	-	-	-	9	-	-	-	++++	-
10	++	-	-	-	-	10	-	-	-	-	-
11	-	-	-	-	-	-		-	-	-	
12	++++	-	++++	-	-	-	-	-	-	-	-
13	++++	-	-	-	-	13	++++	-	-	-	-
14	-	-	-	++	-	-	-	-	-	-	-

-: not infected, +: sporadic, ++: few
+++: moderate and ++++:many

Table III
Treatments administered to patients between the first sample and the evaluation sample.

	Treatment and treatment duration*			
	Antibiotic			NSAID
	Nuflor®	Procapen®	Ampicillin	Metacam
Patient				
2	1	-	-	1
4	4	-	-	7
5	2	-	-	3
7	4	-	-	8
8	-	-	3	1
9	-	3	-	1
10	-	-	-	1

*: Treatment duration is presented in the number of days a drug was administered to an animal.

No bacterial pathogens were cultured from 4 of the evaluation samples. In the remaining samples 3 different bacterial pathogens were detected. *P.multocida* was also the most frequently isolated bacterium from the evaluation samples and was cultured from 3 out of 9 of these samples. For every sample it was the only bacterial species present. *M.haemolytica* and *Mycoplasma spp.* were both isolated from 1 out of 9 evaluation samples. Both pathogens were also not cultured concomitantly with other bacterial species. *T.pyogenes*, *H.somni* and anaerobic bacterial agents were not cultured from the evaluation samples.

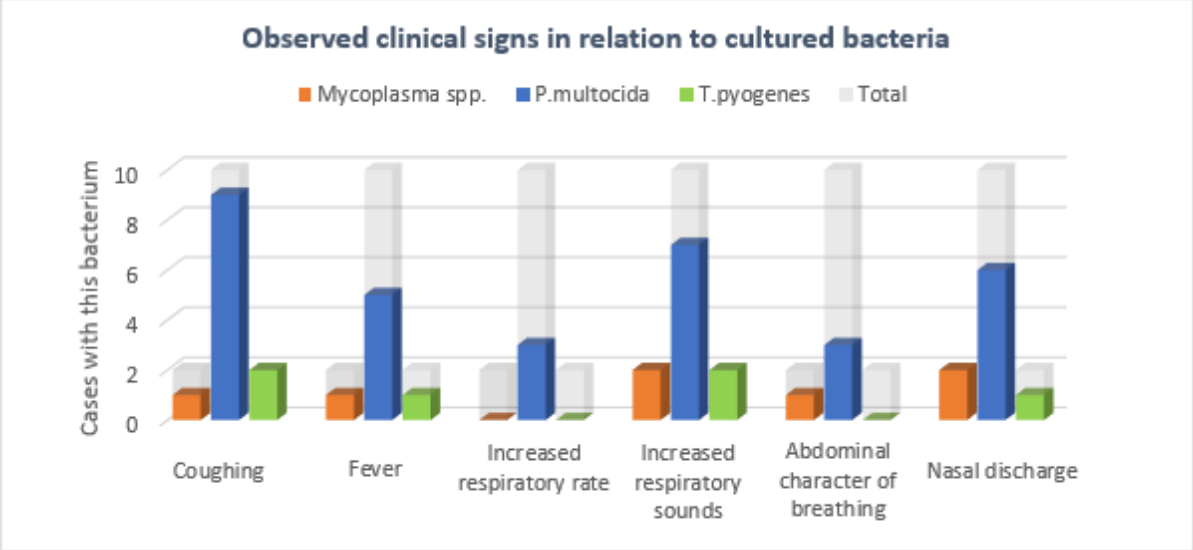
Clinical signs, severity of acute infection, improvement of clinical signs and effects of treatment in relation to cultured pathogens

Observed clinical signs at the day of obtaining the first samples in relation to cultured pathogens are illustrated in Figure I. Coughing was observed as clinical sign in 9 out of 10 cases with *P.multocida*, 1 out of 2 cases with *Mycoplasma spp.* and 2 out of 2 cases with *T.pyogenes*. For 5 out of 10 cases *P.multocida* was isolated from, fever was observed as clinical sign. For both *Mycoplasma spp.* and *T.pyogenes* fever was reported in 1 out of 2 cases. An increased respiratory rate, increased respiratory sounds, an abdominal character of breathing and nasal discharge were observed as clinical signs in 3, 7, 3 and 6 out of 10 cases with *P.multocida* respectively. Increased respiratory sounds, an abdominal character of breathing and nasal discharge were seen in 2, 1 and 2 out of 2 cases with *Mycoplasma spp.* respectively. Increased respiratory sounds and nasal discharge were reported in 2 and 1 out of 2 cases with *T.pyogenes* respectively.

For the first samples *P.multocida*, *Mycoplasma spp.* and *T.pyogenes* were cultured from both calves with more severe clinical signs of respiratory disease and animals with milder clinical signs. Mono and mixed infections with *P.multocida* were seen for both animals with more severe and milder clinical signs. A mono infection with *Mycoplasma spp.* was isolated from a calf with mildest clinical signs. For a patient with more severe clinical signs a mixed infection of *P.multocida* and *Mycoplasma spp.* was cultured. Mixed infections of *P.multocida* and *T.pyogenes* were detected in both calves with more severe and milder clinical signs.

For 7 out of 9 patients (78%) improvement of clinical signs, with or without an intermediate treatment, resulted in changes in cultured bacteria for the evaluation samples in relation to the first samples. For 1 calf (patient number 2) no bacteria were detected in both the first and evaluation sample. For another calf (patient number 13) the same bacterial species was isolated in similar quantity in the evaluation sample. For 3 calves (patient number 4, 5 and 7) antibiotic treatment resulted in culturing less of the same bacterial species, which was also present in the first sample, or detection of no bacteria at all from the evaluation sample. Despite an antibiotic treatment administered to 2 patients (patient number 8 and 9), or more of the same species from the first sample or a new bacterial species was detected in the evaluation sample, while the bacterial species cultured from the first sample was not isolated anymore. For 1 untreated calf (patient number 3), a new bacterial species was also isolated from the evaluation sample, while the bacterial species detected in the first sample were not cultured anymore. Patient number 10 was also not treated with antibiotics, but a decline in isolation of the same bacterial species present in the first sample was observed for the evaluation sample.

Figure I Clinical signs observed at the day of obtaining the first samples in relation to cultured pathogens form these samples.

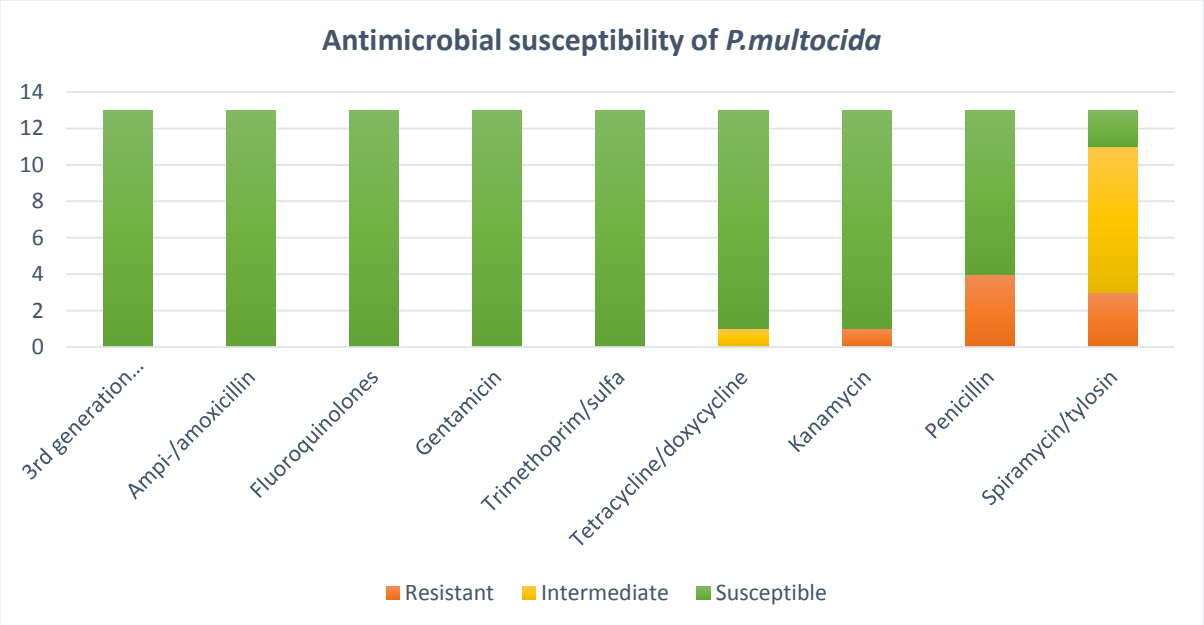


Susceptibility to antimicrobial agents for isolates from both the first and evaluation samples

An antibiogram was made for P.multocida isolates from both the first and evaluation samples. Results of antimicrobial susceptibility of *P.multocida* for both the first and evaluation samples are shown in Figure II. For the 3rd generation cephalosporins, fluoroquinolones, gentamicin, ampi-/amoxicillin, and trimethoprim/sulfa 13 out of 13 *P.multocida* isolates were found to be susceptible to these antibiotic drugs. For tetracycline/doxycycline 12 and 1 isolates showed susceptibility and intermediate susceptibility to this antibiotic drug respectively.

For 12 and 1 of the *P.multocida* isolates susceptibility to and resistance against kanamycin was observed respectively. For penicillin 9 isolates were found to be resistant and 4 isolates showed susceptibility to this drug. For spiramycin/tylosin 2, 8 and 3 of the isolates showed susceptibility, intermediate susceptibility and resistance respectively. The only *M.haemolytica* isolate from the evaluation sample was found to be susceptible to the 3rd generation cephalosporins, fluoroquinolones, ampi-/amoxicillin, gentamicin, kanamycin trimethoprim/sulfa and penicillin. For spiramycin/tylosin and tetracycline/doxycycline intermediate susceptibility and resistance was found respectively.

Figure II Susceptibly to different antibiotics for *P.multocida* isolates from the first and evaluation samples



Discussion

The aim of this longitudinal study was to analyse the variation in bacterial flora in the deeper parts of the bovine respiratory tract in relation to severity of clinical signs and the effects of treatment. Simultaneously, it was our intention to contribute to the selection of an effective antimicrobial treatment by testing antimicrobial susceptibility of cultured bacteria.

In the present study it was assumed that inserting the catheter as far as possible was the best way of obtaining adequate amounts of aspiration fluid. In the publication of Angen *et al.* (2009) a male dog urinary catheter, used for obtaining TTA samples, was introduced into the respiratory tract as far as possible until a certain resistance was felt and no problems were described (47). However, it was experienced in the present study that for some of the patients aspiration fluid was not easily collected. Only after repeated attempts it was possible to collect a little aspiration fluid and in some cases no aspiration fluid at all was obtained from the animal. Possible explanations for this were that coughing by the animal or a collision of the catheter with tracheal cartilage rings had made the catheter going back towards the pharyngeal area of the respiratory tract. In this way the inserted amount of 0.9% NaCl drained very quickly into the animal's airways and was not able to get aspirated again. This improper way of sampling could also contribute to irrelevant cultures since these samples were obtained from areas of the respiratory tract that were situated too cranially. Therefore it was decided that coughing had to be avoided. This was performed by introducing the catheter into the calf's respiratory tract to only the end of the indicated mark (which was at about 20 centimetres). The animal's head was also kept in a more horizontal position in order to prevent a fast draining of the inserted 0.9% NaCl. This resulted in facilitation of collecting aspiration fluid for the remaining samples that had to be obtained.

Cultured bacterial pathogens

In 3 of the first samples no bacterial pathogens were isolated that could have been responsible for causing clinical signs of BRD. One possible reason for this is that these particular samples have been obtained improperly with the catheter present in tissue that is surrounding the animal's trachea. In that case the 0.9% NaCl was injected in these tissues and the aspiration fluid has never been in the trachea and is therefore free of respiratory pathogens. Another explanation is that the patient's clinical signs can be explained by viral infections in the absence of concurrent bacterial pathogens contributing to the disease, because viruses are sometimes also able to cause clinical signs without a bacterial co-infection (22,25,56,57). This was for example demonstrated for BCoV in the publication of Decaro *et al.* (2008) for an outbreak of respiratory disease in cattle herds in Italy (58). The detection of viral presence and their contribution to BRD in dairy calves were not part of the present study. It was therefore not possible to draw conclusions about whether or not observed clinical signs were caused by viral pathogens alone.

P.multocida is often cited as one of the bacterial agents which is most commonly isolated from samples obtained from dairy cattle suffering from BRD (4,47,59-61). In the publication of Härtel *et al.* (2004) *P.multocida* was reported as the most frequently isolated bacterium from TBL samples and also in the study described by Angen *et al. (*2009) *P.multocida* was the most common bacterial agent in samples obtained from diseased animals (47,59). This matches with the results from our study, as *P.multocida* was found to be present in 10 out of 14 of the first samples and also in 3 out of 9 of the evaluation samples.

In samples of the present study *P.multocida* was also often isolated in absence of other bacterial pathogens, which may indicate a possible pathogenic role for this bacterium. However, despite its frequent presence in cases associated with respiratory diseases, *P.multocida* is not considered as a primary pathogen in the aetiology of BRD. Observations of *P.multocida* being a more opportunistic agent were reported by several studies. In the publication of Tegtmeier *et al.* (1999) *P.multocida* was more frequently isolated in coinfections with viral or other bacterial agents rather than alone (45). In the study described by Autio *et al.* (2007) also an association was reported between clinical signs of BRD and cultures containing both *P.multocida* and other bacterial agents. However, such an association was not observed when *P.multocida* was the only bacterium detected in a sample (38). Genetic diversity among *P.multocida* isolates further supports this opportunistic role, as was described in the publication of Taylor *et al.* (2010a). A significant genetic diversity was found for isolates cultured from calves that died from fatal pleuropneumonia. This makes it very likely that *P.multocida* is not horizontally transferred between animals, but is more tended to recrudesce as an opportunistic pathogen from individual animals (62).

Antecedent or concurrent presence of viral pathogens in an infection *P.multocida* was also isolated or analysing genetic diversity between *P.multocida* isolates were both not part of this study. This makes it impossible to conclude about *P.multocida* whether or not being a primary pathogen in the BRD aetiology based on results of the present study.

Mycoplasma spp. are also described as contributors to BRD in cattle with in particular *M.bovis* as one of the main causative bacterial agents. Several studies have reported increasing prevalences of this particular bacterium (59,63-65). Also *M.dispar*, *M.bovirhinis*, *M.bovigenitalum*, *M.alkalenscens*, *M.argini* and *Ureaplasma diversum* can be frequently isolated from animals suffering from respiratory diseases (38,45,59,66). Nevertheless, in the present study *Mycoplasma spp.* were not frequently isolated as these bacteria were only cultured from 2 of the first samples and from 1 evaluation sample. However, only a number of 14 patients was included in the present study due to a limited presence of calves suffering from respiratory diseases at the Department of Farm Animal Health during the period of this research, which makes it sometimes difficult to interpret observed results and compare them to other studies with often many more animals studied. Prevalence of *Mycoplasma spp.* in the present study may also be somewhat underestimated as no special circumstances were used for culturing these bacterial agents. It is known that *Mycoplasma spp.* are pathogens with fastidious requirements for culturing and growth (60). So their actual prevalence in the present study may be some higher. Further determination was also not performed, which makes it not possible to draw conclusions about which Mycoplasma species were present in the samples of calves in this study.

M.haemolytica is traditionally considered as the main pathogen isolated from beef calves suffering from BRD. In the publication of Welsh *et al.* (2004) 46.3% of the isolates obtained from beef cattle suffering from pneumonia consisted of *M.haemolytica* (43). In BRD of dairy calves *M.haemolytica* seems to be of a minor importance with the study described by Autio *et al.* (2007) reporting rare detection of it in their TBL samples obtained from diseased dairy calves (38). In the publication of Nikunen *et al.* (2007) *M.haemolytica* was not cultured at all from TBL samples of affected calves (61). These reports match with the results of the present study, as *M.haemolytica* was only detected in 1 of the evaluation samples.

In the publication of Tegtmeier *et al.* (1999) *T.pyogenes* was most often cultured concomitantly with other bacterial pathogens (45). This is also in accordance with the results of the present study since *T.pyogenes* was only isolated concurrent with *P.multocida*.

H.somni was not detected in any of the samples in this study. In the publication of Härtel *et al.* (2004) *H.somni* was also not detected in any of the lavage samples, but culturing *H.somni* from the lungs after post mortem examination revealed possible failure of detection of this bacterium in lavage samples (59). *H.somni* is also a bacterium with fastidious requirements for growth and culturing, which may contribute to lower isolation rates than the actual prevalence of this bacterium (60).

In the publication of Chirino-Trejo *et al.* (1983) anaerobic bacteria were isolated from cattle's lungs showing acute or chronic bronchopneumonia. The predominant species cultured in this study were *Fusobacterium necrophorum*, *Clostridium perfringens*, *Peptococcus indolicus*, *Bacteroidus asaccharolyticus* and *Bacteroidus fragilis* (67). Only for 1 of the patients in the present study anaerobic bacteria were isolated from a sample. As this was a mixed culture with no predominant anaerobic bacterial agent present, it is not possible to conclude about which anaerobic bacteria possibly had contributed to the occurrence of clinical signs in this animal.

Clinical signs, severity of acute infection, improvement of clinical signs and effects of treatment in relation to cultured pathogens

Clinical signs observed for patients in the present study were in accordance with clinical signs reported for animals suffering from respiratory diseases by other studies. In the publications of Allen *et al. (*1991), Simonen-Jokinen *et al.* (2005) and Nikunen *et al.* (2007) coughing, fever, an increased respiratory rate, increased respiratory sounds and nasal discharge were also reported for affected calves (46,61,68).

The observed clinical signs for diseased animals when *P.multocida* was isolated from the first sample, also match with reports from other studies. Coughing as clinical sign was associated with the presence of *P.multocida* in the study described by de Oliveira *et al.* (2016) (69). An elevated rectal temperature, nasal discharge, an increased respiratory rate and increased respiratory sounds were observed as clinical signs for calves when *P.multocida* was isolated from a TBL sample in the publication of Nikunen *et al.* (2007) (61). In an experimental study described by Dowling *et al.* (2002) fever and nasal discharge were reported after inoculation of calves with *P.multocida* (70).

Since *P.multocida* is considered as a more opportunistic pathogen, one has to keep in mind that its role as cause of respiratory clinical signs can sometimes be questionable. Our results for *Mycoplasma spp.* match with reports from the publication of Stipkovitis *et al.* (2000) who also described coughing, fever, nasal discharge and abnormal respiratory signs for animals suffering from a *M.bovis* infection (71).

Considerable differences in isolation of certain bacterial species in relation to severity of clinical signs of acute infection at the day of inclusion in the study were not observed. For both calves with more severe and milder clinical signs the same bacterial species were isolated. For *P.multocida* the results of being able to cause more severe and also milder clinical signs match with the reports from the study described by Dagleish *et al.* (2016). In this publication differences in severity of clinical signs were reported for calves after experimental inoculation with genetic different *P.multocida* strains. It was suggested that the existence of genetic differences between *P.multocida* strains can be responsible for the production of different with virulence associated proteins. This may contribute to significant variation in severity of BRD infections in calves (72). The role of *Mycoplasma spp.* as contributors to BRD is diverse with studies reporting differences in severity of infection associated with these bacteria. In the present study a *Mycoplasma spp.* infection resulted in milder clinical signs, which is in accordance with reports from other studies (59,73). Experimental infection with *M.dispar* in the publication of Tanskanen (1984) resulted in mild clinical signs, e.g. coughing, nasal discharge and only a slight increase in body temperature (73). Respiratory disease can also be complicated with more severe clinical signs due to mixed infections of *Mycoplasma spp.* and other bacterial agents (66,74). More severe clinical signs were also seen for a patient in our study with a mixed infection of *Mycoplasma spp.* and *P.multocida*. Mixed infections of *T.pyogenes* and *P.multocida* were seen for both calves with more severe and milder clinical signs. In literature *T.pyogenes* is considered as a bacterium with a more secondary character and can be responsible for complicating respiratory disease after damage caused by other bacterial agents (2,45,60).

Improvement of clinical signs for calves treated with antibiotics resulting in a decline in bacterial isolation as was observed for some of the patients in the present study is in accordance with the results from the publication of Allen *et al.* (1992). In this study also a decline in isolation frequency of *Pasteurella spp*. in BAL samples after antibiotic treatment of diseased calves in comparison with previously obtained samples was reported (75). Treatment of patients in the present study, a decline in bacterial isolation was observed for, consisted of repeated administration of Nuflor®. Despite testing susceptibility of *P.multocida* to florfenicol was not part of the present study, florfenicol seemed to be an effective drug for treating BRD infections. This matches with the results of the publication of Thiry *et al.* (2011) who reported improvement of respiratory clinical signs after treatment with the same 300 mg/ml florfenicol formulation as was used in the present study (76). Improvement of clinical signs, with or without an antibiotic treatment, but still the same quantity of the bacterial species also detected in the first sample or presence of a new bacterial species in the evaluation sample was also one of our observations. It may be that due to inability of the animal's immune system and/or lung's clearance mechanisms still significant numbers of bacteria can be present. Also ineffectiveness of antibiotics against certain bacteria, chronic infections and/or abscesses preventing an antibiotic drug to reach place of inflammation in effective concentrations can contribute to this. Opportunistic characteristics of bacteria together with the existence of synergistic bacterial relations being responsible for bacteria starting to contribute to an infection can also be involved. Existence of such synergistic relations between for example *P.multocida* and *Mycoplasma spp.* are described in literature (4,66,68,77).

Antimicrobial susceptibility

In vitro susceptibility to the major part of tested antibiotics was observed for *P.multocida* isolates. *M.haemolytica* showed, except for resistance against tetracycline/doxycycline and intermediate susceptibility to spiramycin/tylosin, susceptibility to all tested antibiotics. Similar results were observed in the publications of El Garch *et al.* (2016) and Härtel *et al.* (2004) who described very high *in vitro* susceptibility percentages for both *M.haemolytica* and *P.multocida* to several licenced antibiotics, such as ampicillin, ceftiofur, fluoroquinolones (cipro-, dano-, enro-, and marbofloxacin), gentamicin and trimethoprim-sulfamethoxazole. *In vitro* resistance for *M.haemolytica* against tetracycline was also observed in the study described by El Garch *et al.* (2016) (59,78). *In vitro* resistance for *P.multocida* against penicillin as was observed in our study was also described by the publication of Jamali *et al.* (2014) who reported 30.5% of *P.multocida* isolates obtained from affected calves being resistant against penicillin G (79). Results from

the present study are in contrast to studies describing decreased susceptibility of *P.multocida* to several antimicrobial agents (78,80). In the publication of El Garch *et al.* (2016) 11.2% of the 134 *P.multocida* isolates showed resistance against tetracycline, which is not in accordance with the great susceptibility of this bacterium to tetracycline observed in our study (78). Nevertheless, in the present study antimicrobial susceptibility was only tested for a limited number of isolates. Moreover, susceptibility to antimicrobial agentia may vary between geographical locations.

Conclusion

In this study *Pasteurella multocida* was most frequently isolated from TTA samples with *Mycoplasma spp., Mannheimia haemolytica*, *Trueperella pyogenes* and *Histophilus somni* rarely cultured or not detected at all. There is no considerable difference in isolation of certain bacterial species from animals with more severe or milder clinical signs of respiratory disease at acute infection. Improvement of clinical signs is often associated with changes in bacterial presence, but does not necessarily result in a decline in number of bacteria still being present in the deeper respiratory tract. Antibiotic treatment can result in culturing less bacteria in comparison samples obtained before treatment, but also increases in bacterial quantity or contribution of a new bacterial species to the infection can occur. In this study *Pasteurella multocida* and *Mannheimia haemolytica* isolates also showed high susceptibility to several licensed antibiotic drugs.

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References

(1) NAHMS. Part I: Reference of 1996 Dairy Management Practices. 1996.

(2) Ames TR. Dairy calf pneumonia: the disease and its impact. Veterinary Clinics of North America: Food Animal Practice 1997;13(3):379-391.

(3) Callan RJ, Garry FB. Biosecurity and bovine respiratory disease. Veterinary Clinics of North America: Food Animal Practice 2002;18(1):57-77.

(4) Virtala AM, Mechor GD, Grohn YT, Erb HN, Dubovi EJ. Epidemiologic and pathologic characteristics of respiratory tract disease in dairy heifers during the first three months of life. J Am Vet Med Assoc 1996 Jun 15;208(12):2035-2042.

(5) NASS Cattle. Cattle And Calves Predator Death Loss in the United States, 2005. 2007;56.

(6) USDA, APHIS, VS. Part II: Reference of 1997 Beef Cow-Calf Health & Health Management Practices. 1997.

(7) Smith RA. Impact of disease on feedlot performance: a review. J Anim Sci 1998;76(1):272-274.

(8) Cernicchiaro N, White BJ, Renter DG, Babcock AH. Evaluation of economic and performance outcomes associated with the number of treatments after an initial diagnosis of bovine respiratory disease in commercial feeder cattle. Am J Vet Res 2013;74(2):300-309.

(9) Schneider M, Tait R, Busby W, Reecy J. An evaluation of bovine respiratory disease complex in feedlot cattle: Impact on performance and carcass traits using treatment records and lung lesion scores. J Anim Sci 2009;87(5):1821-1827.

(10) USDA. USDA (2007). National Animal Health Monitoring Study Dairy Part I. 122. 2007:122.

(11) Waltner-Toews D, Martin SW, Meek AH. The effect of early calfhood health status on survivorship and age at first calving. Can J Vet Res 1986 Jul;50(3):314-317.

(12) Donovan GA, Dohoo IR, Montgomery DM, Bennett FL. Calf and disease factors affecting growth in female Holstein calves in Florida, USA. Prev Vet Med 1998 Jan;33(1-4):1-10.

(13) Sivula N, Ames T, Marsh W, Werdin R. Descriptive epidemiology of morbidity and mortality in Minnesota dairy heifer calves. Prev Vet Med 1996;27(3):155-171.

(14) Van der Fels-Klerx H, Sørensen JT, Jalvingh A, Huirne R. An economic model to calculate farm-specific losses due to bovine respiratory disease in dairy heifers. Prev Vet Med 2001;51(1):75-94.

(15) Patrick RL. A dairy producer's view of respiratory disease. Animal Health Research Reviews 2009;10(02):111-112.

(16) Klima CL, Zaheer R, Cook SR, Booker CW, Hendrick S, Alexander TW, et al. Pathogens of bovine respiratory disease in North American feedlots conferring multidrug resistance via integrative conjugative elements. J Clin Microbiol 2014 Feb;52(2):438-448.

(17) Booker CW, Abutarbush SM, Morley PS, Jim GK, Pittman TJ, Schunicht OC, et al. Microbiological and histopathological findings in cases of fatal bovine respiratory disease of feedlot cattle in western Canada. Canadian Veterinary Journal 2008;49(5):473-482.

(18) Love WJ, Lehenbauer TW, Van Eenennaam AL, Drake CM, Kass PH, Farver TB, et al. Sensitivity and specificity of on-farm scoring systems and nasal culture to detect bovine respiratory disease complex in preweaned dairy calves. J Vet Diagn Invest 2016 Mar;28(2):119-128.

(19) Guterbock WM. The impact of BRD: the current dairy experience. Animal Health Research Reviews 2014;15(02):130-134.

(20) Grissett G, White B, Larson R. Structured literature review of responses of cattle to viral and bacterial pathogens causing bovine respiratory disease complex. Journal of Veterinary Internal Medicine 2015;29(3):770-780.

(21) Smith BP. Large Animal INTERNAL MEDICINE. 2002;1735:551-560.

(22) Cusack P, McMeniman N, Lean I. The medicine and epidemiology of bovine respiratory disease in feedlots. Aust Vet J 2003;81(8):480-487.

(23) Panciera RJ, Confer AW. Pathogenesis and pathology of bovine pneumonia. Veterinary Clinics of North America: Food Animal Practice 2010;26(2):191-214.

(24) Blecha F, Boyles SL, Riley JG. Shipping suppresses lymphocyte blastogenic responses in Angus and Brahman× Angus feeder calves. J Anim Sci 1984;59(3):576-583.

(25) Taylor JD, Fulton RW, Lehenbauer TW, Step DL, Confer AW. The epidemiology of bovine respiratory disease: What is the evidence for predisposing factors? Can Vet J 2010 Oct;51(10):1095-1102.

(26) Raboisson D, Trillat P, Cahuzac C. Failure of Passive Immune Transfer in Calves: A Meta-Analysis on the Consequences and Assessment of the Economic Impact. PloS one 2016;11(3):e0150452.

(27) Weaver DM, Tyler JW, VanMetre DC, Hostetler DE, Barrington GM. Passive transfer of colostral immunoglobulins in calves. Journal of Veterinary Internal Medicine 2000;14(6):569-577.

(28) Stott G, Marx D, Menefee B, Nightengale G. Colostral immunoglobulin transfer in calves I. Period of absorption. J Dairy Sci 1979;62(10):1632-1638.

(29) Virtala A, Gröhn Y, Mechor G, Erb H. The effect of maternally derived immunoglobulin G on the risk of respiratory disease in heifers during the first 3 months of life. Prev Vet Med 1999;39(1):25-37.

(30) Windeyer M, Leslie K, Godden S, Hodgins D, Lissemore K, LeBlanc S. Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. Prev Vet Med 2014;113(2):231-240.

(31) Hay K, Morton J, Mahony T, Clements A, Barnes T. Associations between animal characteristic and environmental risk factors and bovine respiratory disease in Australian feedlot cattle. Prev Vet Med 2016;125:66-74.

(32) Cernicchiaro N, Renter D, White B, Babcock A, Fox J. Associations between weather conditions during the first 45 days after feedlot arrival and daily respiratory disease risks in autumn-placed feeder cattle in the United States. J Anim Sci 2012;90(4):1328-1337.

(33) Cusack P, McMeniman N, Lean I. Feedlot entry characteristics and climate: their relationship with cattle growth rate, bovine respiratory disease and mortality. Aust Vet J 2007;85(8):311-316.

(34) Irwin MR, McConnell S, Coleman JD, Wilcox GE. Bovine respiratory disease complex: a comparison of potential predisposing and etiologic factors in Australia and the United States. J Am Vet Med Assoc 1979 Nov 15;175(10):1095-1099.

(35) Hahn G. Dynamic responses of cattle to thermal heat loads. J Anim Sci 1999;77:10.

(36) Lago A, McGuirk S, Bennett T, Cook N, Nordlund K. Calf respiratory disease and pen microenvironments in naturally ventilated calf barns in winter. J Dairy Sci 2006;89(10):4014-4025.

(37) Gulliksen SM, Jor E, Lie KI, Løken T, Åkerstedt J, Østerås O. Respiratory infections in Norwegian dairy calves. J Dairy Sci 2009;92(10):5139-5146.

(38) Autio T, Pohjanvirta T, Holopainen R, Rikula U, Pentikäinen J, Huovilainen A, et al. Etiology of respiratory disease in non-vaccinated, non-medicated calves in rearing herds. Vet Microbiol 2007;119(2):256-265.

(39) Ellis JA. Update on viral pathogenesis in BRD. Animal Health Research Reviews 2009;10(02):149-153.

(40) Ellis JA, West KH, Cortese VS, Myers SL, Carman S, Martin KM, et al. Lesions and distribution of viral antigen following an experimental infection of young seronegative calves with virulent bovine virus diarrhea virus-type II. Can J Vet Res 1998 Jul;62(3):161-169.

(41) Babiuk LA, Lawman M, Ohmann HB. Viral-bacterial synergistic interaction in respiratory disease. Adv Virus Res 1988;35:219-249.

(42) Czuprynski CJ, Leite F, Sylte M, Kuckleburg C, Schultz R, Inzana T, et al. Complexities of the pathogenesis of Mannheimia haemolytica and Haemophilus somnus infections: challenges and potential opportunities for prevention? Animal Health Research Reviews 2004;5(02):277-282.

(43) Welsh RD, Dye LB, Payton ME, Confer AW. Isolation and antimicrobial susceptibilities of bacterial pathogens from bovine pneumonia: 1994--2002. J Vet Diagn Invest 2004 Sep;16(5):426-431.

(44) Shahriar FM, Clark EG, Janzen E, West K, Wobeser G. Coinfection with bovine viral diarrhea virus and Mycoplasma bovis in feedlot cattle with chronic pneumonia. Can Vet J 2002 Nov;43(11):863-868.

(45) Tegtmeier C, Uttenthal A, Friis N, Jensen N, Jensen H. Pathological and microbiological studies on pneumonic lungs from Danish calves. Journal of Veterinary Medicine, Series B 1999;46(10):693-700.

(46) Allen JW, Viel L, Bateman KG, Rosendal S, Shewen PE, Physick-Sheard P. The microbial flora of the respiratory tract in feedlot calves: associations between nasopharyngeal and bronchoalveolar lavage cultures. Can J Vet Res 1991 Oct;55(4):341-346.

(47) Angen Ø, Thomsen J, Larsen LE, Larsen J, Kokotovic B, Heegaard PM, et al. Respiratory disease in calves: microbiological investigations on trans-tracheally aspirated bronchoalveolar fluid and acute phase protein response. Vet Microbiol 2009;137(1):165-171.

(48) Edwards T. Control methods for bovine respiratory disease for feedlot cattle. Veterinary Clinics of North America: Food Animal Practice 2010;26(2):273-284.

(49) Timsit E, Christensen H, Bareille N, Seegers H, Bisgaard M, Assié S. Transmission dynamics of Mannheimia haemolytica in newly-received beef bulls at fattening operations. Vet Microbiol 2013;161(3):295-304.

(50) Thomas A, Dizier I, Trolin A, Mainil J, Linden A. Comparison of sampling procedures for isolating pulmonary mycoplasmas in cattle. Vet Res Commun 2002;26(5):333-339.

(51) Magwood SE, Barnum DA, Thomson RG. Nasal bacterial flora of calves in healthy and in pneumonia-prone herds. Can J Comp Med 1969 Oct;33(4):237-243.

(52) DeRosa DC, Mechor GD, Staats JJ, Chengappa MM, Shryock TR. Comparison of Pasteurella spp. simultaneously isolated from nasal and transtracheal swabs from cattle with clinical signs of bovine respiratory disease. J Clin Microbiol 2000 Jan;38(1):327-332.

(53) Godinho KS, Sarasola P, Renoult E, Tilt N, Keane S, Windsor GD, et al. Use of deep nasopharyngeal swabs as a predictive diagnostic method for natural respiratory infections in calves. Vet Rec 2007 Jan 6;160(1):22-25.

(54) MSD Animal Health. Nuflor - 300 mg/ml oplossing voor injectie voor runderen en schapen. 2013; Available at: http://www.msd-animal-health.nl/binaries/Nuflor_rund_schaap_IN_145144_2013-11_tcm100-187840.pdf. Accessed 17-12-2016, 2016.

(55) AST Farma B.V. PROCAPEN® 300 mg/ml suspensie voor injectie voor runderen, varkens en paarden. 2015; Available at: http://www.astfarma.nl/wp-content/uploads/2014/03/Procapen_300mg-ml_Bijsluiter_A4_def.pdf. Accessed 17-12-2016, 2016.

(56) Storz J, Purdy CW, Lin X, Burrell M, Truax RE, Briggs RE, et al. Isolation of respiratory bovine coronavirus, other cytocidal viruses, and Pasteurella spp from cattle involved in two natural outbreaks of shipping fever. J Am Vet Med Assoc 2000;216(10):1599-1604.

(57) Lathrop SL, Wittum TE, Brock KV, Loerch SC, Perino LJ, Bingham HR, et al. Association between infection of the respiratory tract attributable to bovine coronavirus and health and growth performance of cattle in feedlots. Am J Vet Res 2000;61(9):1062-1066.

(58) Decaro N, Campolo M, Desario C, Cirone F, D'Abramo M, Lorusso E, et al. Respiratory disease associated with bovine coronavirus infection in cattle herds in Southern Italy. J Vet Diagn Invest 2008 Jan;20(1):28-32.

(59) Härtel H, Nikunen S, Neuvonen E, Tanskanen R, Kivelä S, Aho P, et al. Viral and bacterial pathogens in bovine respiratory disease in Finland. Acta Vet Scand 2004;45(4):1.

(60) Mosier DA. Bacterial pneumonia. Veterinary Clinics of North America: Food Animal Practice 1997;13(3):483-493.

(61) Nikunen S, Härtel H, Orro T, Neuvonen E, Tanskanen R, Kivelä S, et al. Association of bovine respiratory disease with clinical status and acute phase proteins in calves. Comp Immunol Microbiol Infect Dis 2007;30(3):143-151.

(62) Taylor JD, Fulton RW, Dabo SM, Lehenbauer TW, Confer AW. Comparison of genotypic and phenotypic characterization methods for Pasteurella multocida isolates from fatal cases of bovine respiratory disease. J Vet Diagn Invest 2010 May;22(3):366-375.

(63) Nicholas R, Ayling R. Mycoplasma bovis: disease, diagnosis, and control. Res Vet Sci 2003;74(2):105-112.

(64) Brice N, Finlay D, Bryson D, Henderson J, McConnell W, Ball H. Isolation of Mycoplasma bovis from cattle in Northern Ireland, 1993 to 1998. Vet Rec 2000;146(22):643-644.

(65) Kusiluka LJ, Ojeniyi B, Friis NF. Increasing prevalence of Mycoplasma bovis in Danish cattle. Acta Vet Scand 2000;41(2):139-146.

(66) Hirose K, Kobayashi H, Ito N, Kawasaki Y, Zako M, Kotani K, et al. Isolation of mycoplasmas from nasal swabs of calves affected with respiratory diseases and antimicrobial susceptibility of their isolates. Journal of Veterinary Medicine, Series B 2003;50(7):347-351.

(67) Chirino-Trejo JM, Prescott JF. The identification and antimicrobial susceptibility of anaerobic bacteria from pneumonic cattle lungs. Can J Comp Med 1983 Jul;47(3):270-275.

(68) Simonen-Jokinen TL, Eskelinen UM, Härtel HM, Nikunen SK, Saloniemi HS, Maisi PS. Gelatinolytic matrix metalloproteinases-2 and-9 in tracheobronchial lavage fluid obtained from calves with concurrent infections of Pasteurella multocida and Mycoplasma bovirhinis. Am J Vet Res 2005;66(12):2101-2106.

(69) de Oliveira, Bernardo Augusto França Dias, Gaeta NC, Ribeiro BLM, Alemán MAR, Marques LM, Timenetsky J, et al. Determination of bacterial aetiologic factor on tracheobronchial lavage in relation to clinical signs of bovine respiratory disease. J Med Microbiol 2016;65(10):1137-1142.

(70) Dowling A, Hodgson J, Schock A, Donachie W, Eckersall P, McKendrick I. Experimental induction of

pneumonic pasteurellosis in calves by intratracheal infection with Pasteurella multocida biotype A: 3. Res Vet Sci 2002;73(1):37-44.

(71) Stipkovits L, Ripley P, Varga J, Palfi V. Clinical study of the disease of calves associated with Mycoplasma bovis infection. Acta Vet Hung 2000.

(72) Dagleish M, Bayne C, Moon G, Finlayson J, Sales J, Williams J, et al. Differences in Virulence Between Bovine-Derived Clinical Isolates of Pasteurella multocida Serotype A from the UK and the USA in a Model of Bovine Pneumonic Pasteurellosis. J Comp Pathol 2016;155(1):62-71.

(73) Tanskanen R. Colonisation pattern of the respiratory tract of calves by Mycoplasma dispar. Acta Veterinaria Scandinavia (Denmark) 1984.

(74) Stipkovits L, Ripley PH, Varga J, Palfi V. Use of valnemulin in the control of Mycoplasma bovis infection under field conditions. Vet Rec 2001 Mar 31;148(13):399-402.

(75) Allen JW, Viel L, Bateman KG, Rosendal S. Changes in the bacterial flora of the upper and lower respiratory tracts and bronchoalveolar lavage differential cell counts in feedlot calves treated for respiratory diseases. Can J Vet Res 1992 Jul;56(3):177-183.

(76) Thiry J, Rubion S, Sarasola P, Bonnier M, Hartmann M, de Haas V. Efficacy and safety of a new 450 mg/ml florfenicol formulation administered intramuscularly in the treatment of bacterial bovine respiratory disease. Vet Rec 2011;169(20):526-526.

(77) Almeida R, Rosenbusch R. Impaired tracheobronchial clearance of bacteria in calves infected with Mycoplasma dispar. Journal of Veterinary Medicine, Series B 1994;41(1-10):473-482.

(78) El Garch F, de Jong A, Simjee S, Moyaert H, Klein U, Ludwig C, et al. Monitoring of antimicrobial susceptibility of respiratory tract pathogens isolated from diseased cattle and pigs across Europe, 2009–2012: VetPath results. Vet Microbiol 2016.

(79) Jamali H, Rezagholipour M, Fallah S, Dadrasnia A, Chelliah S, Velappan RD, et al. Prevalence, characterization and antibiotic resistance of Pasteurella multocida isolated from bovine respiratory infection. The Veterinary Journal 2014;202(2):381-383.

(80) Catry B, Haesebrouck F, Vliegher SD, Feyen B, Vanrobaeys M, Opsomer G, et al. Variability in acquired resistance of Pasteurella and Mannheimia isolates from the nasopharynx of calves, with particular reference to different herd types. Microbial Drug Resistance 2005;11(4):387-394.

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