Detection of BRD pathogens in relation to clinical signs of case and case-control veal calves

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Abstract

This paper describes a study aimed at quantification of the prevalence of several known bacterial and viral pathogens, clinical signs and additional conditions leading to Bovine Respiratory Disease (BRD) in veal calves in the Netherlands. For this purpose 168 veal calves with a respiratory rate (RR) higher than 50 rates per minute (cases) and 573 calves with a RR lower than 50 rates per minute (case-controls) were subjected to bronchoalveolair lavages (BAL's) and clinical evaluations, on conventional Dutch veal farms. The most prevalent pathogen was *Pasteurella multocida*, detected in 29% of cases and in 33% of case-controls. Statistically, *Arcanobacterium pyogenes,* Bovine Parainfluenza and Bovine Viral Diarrhea Virus had higher a prevalence in cases than in case-controls.
Clinical signs were significantly more often detected in cases, in both viral and bacterial pathogens. Of the cases 45% had an elevated rectal temperature, 24% was in respiratory distress, but just 6% showed coughing. Analysis showed there was no significance between severity of clinical signs and a specific pathogen related to BRD in cases and case-controls.

Introduction

Clinical diseases of the respiratory tract are very common in veal and dairy youngstock. According to several studies examining the prevalence of diseases almost all animals (80–99%) undergo treatment for a respiratory infection during the first 6 months of life (Kelly et al. 1986, Lerust et al. 2012, Pardon et al. 2011, Pardon et al. 2013, Rérat et al. 2012, Snowder et al. 2006, Van der Fels-Klerx et al. 2000). The respiratory diseases and their clinical signs are most commonly referred to as Bovine Respiratory Disease (BRD) by practicing veterinarians, comprehending for example enzootic pneumonia in dairy calves and shipping fever in feedlot cattle (Confer, A.W. 2009, Pardon et al. 2013). Because it is a blend of several diseases, BRD therefore has a multifactorial etiology. It is a result of interactions between environment, management and multiple pathogens. It can be caused by viral, bacterial or myoplasmal infections or a combination of these pathogens (Apley, M. 2006, Autio et al. 2007, Fulton R.W. 2009). Prevalence of BRD within a herd consists of 17%, according to research of Pardon et al. from 2013 and Snowder et al. from 2006. BRD is mostly treated orally with antimicrobials, treating the entire herd or a large group of the herd (Pardon et al. 2013).

Clinical symptoms accompanying BRD may include an elevated rectal temperature, elevated respiratory rate, discharge from eyes and/or nose, coughing and an overall affected general appearance including anorexia and lethargy (Allen et al. 1991, Fulton et al. 2000, Van der Fels-Klerx et al 2000). Nevertheless, prevalence of clinical symptoms is often reported low. Research by Leruste et al. from 2012 conducted at conventional veal farms reported that only 6.8 % of all animals showed clinical signs of BRD, but at slaughter 50% of the lungs of the clinical evaluated calves had lesions. This study proofed that clinical signs alone are not a reliable predictor of BRD. The absence of clinical signs does not make it of less importance; two studies confirmed that an outbreak of BRD results in growth retardation. Up to 8 kg's in carcass weight is lost due to just one BRD outbreak during a 200-day rearing period (Autio et al. 2007, Snowder et al. 2006).

The Netherlands is one of the biggest veal producers in Europe, providing veal for the world market by rearing of 0.9 million veal calves a year. Because of the financial losses accompanied with BRD and the growing concern around antimicrobial resistance, an extensive study was designed to gain more insight in pathogens and clinical signs of BRD in veal calves in the Netherlands. Six students participated in the study, all of them had to answer their own research question during this trial. All students performed the sampling including taking blood samples on D_0 and D_{84}, performing BAL's on D_0 and D_{84} and during outbreaks within this period. In addition, students collected other information. Protocols for drug use on the farm and health protocols were collected at each farm, the individual

drug registration was digitalized and linked to tested calves, mortality was noted and linked to tested calves, an extensive interview was conducted with the farmer and maps of the concerning stables were drawn.

Given the limited literature on linking clinical symptoms and several of the know pathogens from the same dataset, the decision was made to investigate possible significant differences between these groups. Results from the study could be of help for practicing veterinarians, maybe linking clinical signs to a or several pathogens.
The following hypothesis was tested:
H_0: There is a significant difference between the severity of clinical signs and a specific pathogen related to BRD in cases and case-controls
H_1: There is no significant difference between the severity of clinical signs and a specific pathogen related to BRD in cases and case-controls

Materials and methods

An inventory field study was conducted by six students on 10 farms throughout the Netherlands during the autumn and winter of 2013-2014. The field study was designed by Wageningen University. The development of BRD was observed and sampled in the 10 different herds. During the study the calves were monitored by serology, bronchoalveolair lavages (BAL's) and clinical evaluations. In addition, supplementary information from the herd and individual animals was collected. Veal calves in the study were held at conventional farms, originating from different countries and were usually two to four weeks of age entering the study at D_0. The calves were monitored for 84 days and measurements and samples were taken at least twice, when calves arrived on the farm (D_0) and at day 84(D_{84}), the final day of the study. Between D_0 and D_{84} sampling took place when a BRD outbreak was suspected by the farmer and after inspection the farm veterinarian confirmed that a herd treatment with antimicrobials was needed. Sampling was performed by students before the herd treatment was started.

Selecting the calves
The D_0 samples were taken to obtain the first group of case-control calves, these 20 animals were randomly selected by means of randomization by a biostatistician from Wageningen University. The case-controls needed to have a respiratory rate (RR) lower than 50 rates per minute.
During an outbreak of BRD, sampling took place on 10 affected case calves and 16 case-control calves. The cases were calves having a RR higher than 50 rates per minute (RR>50/min). The case-controls were preferably formed from the animals that were case-controls at D_0. At D_{84} measurements and samples were taken from 20 case-controls.
During the study the case-control and case calves were tagged, so case-control calves could be identified and sampled several times during the study. When calves were sampled as case calves, they could not be used as a case-control later on in the study.

Sampling
The following data was recorded per calf per sampling: animal ID-number, sex, breed, chest girth, rectal temperature, general impression, eye / nose discharge, coughing, and respiratory rate. Processing of the samples was done by Wageningen University.
At D_0 sampling consisted of performing BAL's on selected calves and collecting clinical data of the calves undergoing the BAL's.
At D_x during outbreaks BAL's were performed on case-control and case calves and the clinical data of these calves was recorded.
At D_{84} BAL's were performed on case-control calves.

Scoring clinical signs
Generally, scoring of the clinical signs took place before the BAL was performed. The following clinical signs were monitored and scored: rectal temperature (RT), general appearance (GA), eye and nose discharge (E/N), coughing (C) and respiratory distress (RD). The rectal temperature was noted in 3 decimals in degrees Celsius. The other clinical signs were given a score ranging van 0 to 3, according the severity of the symptom. At score 0 the clinical signs were absent, at score 3 the clinical signs were severe. An overview of the score-system is shown in *Table 6 Scoring system clinical signs* on page 11.

Performing the BAL
Selected animals were restrained by one student, while the other student performed the BAL. Restraining was done by putting the head of the animal through the feeding barrier in a corner of the pen, standing behind the calf, blocking movement of the knee of the calf with one leg whilst hanging over the animal and keeping the head up with both hands. Once the calf was successfully restrained, the other student could perform the BAL from the feed passage. Restraining the animals > D_{40} needed to be done by tailing up and physically blocking one side of the calf, keeping the head of the calf up with one arm whilst holding the boarding with that hand. From that age it was mostly impossible perform a BAL through the feeding barrier. The BAL was performed by holding the nose of the calf, half covering one nostril with one hand and with the other hand putting a sterile silicon tube (1m in length and 4x1 mm in diameter) into the respiratory tract via the nostril. The tube needed to be moved up the airway until it did not continue anymore; this was approximately at ¾ of the length of the tube. When positioned correctly, calves stick out their tongue in a specific way and often start coughing. When wrongly positioned into the rumen, these signs were absent and bubbling could be heard and rumen content could be smelled at the other end of the tube. When placing of the tube into the rumen was suspected, all used materials were discarded and a second BAL was attempted with sterile materials. When calves immediately swallowed the tube and the tube only passed the epiglottis, the tube was withdrawn into the nasal cavity and a new attempt for positioning was made. After correct positioning of the tube into the lower airway, a sterile syringe with 100 mL of sterile PBS was connected to the silicon tube and injected into the lungs of the calf and directly sucked back into the syringe. If less than 15 mL of fluid was retrieved, another 100 mL was administered with a maximum of 300 mL. The collected BAL sample was transferred from the syringe into one or two Falcon tubes with medium. Samples were as soon as possible cooled with provided ice-packs and collected by a courier for transport to the laboratory where further processing took place.

Laboratory testing of pathogens
The collected BAL's were tested for the presence of the following pathogens: *Pasteurella multocida, Arcanobacterium pyogenes, Mannheimia haemolytic, Histophilus somnia,* Corona virus, Bovine Parainfluenza virus type 3 (BPI), Bovine Viral Diarrhea virus (BVDV), Bovine Respiratory Syncytial Virus (BRSV) and Bovine Herpes Virus (BHV). The laboratory viral and bacterial culturing were performed according to laboratory protocol. Bacteriological culturing results were confirmed by the use of a MALDI-TOF ®.

Data processing and analysis
This study does not contain all collected data from the original study. Due to the limited availability of data when first analyses were done, it was decided to use the data obtained from the first 8 herds in this study.
Before analyzing the data the BAL results data of the clinical signs were transformed from the original 0-3 to a 0/1 score, the original score 0 and 1 were noted as score 0 and the original scores of 2 and 3 were noted as score 1. The RT was noted as 0 when it was below the 39.5 °C, it was noted as 1 when it was 39.5 °C and/or higher.
Statistical tests were performed to test whether prevalence of the pathogens differed among the case or case-control calves and between al the measured clinical variables. Multiple groups and

multiple characteristics were tested. Because sample size was small in some tests, a Fisher's T-test was used to perform all tests. Significance level was set on $P<0.05$.
Performed analysis include:
Analysis 1: This analysis tested whether the prevalence of a pathogen, or a bacterium or a virus differs significantly between case and case-control groups.
This test has been performed twice. In the first test the case group was set up according to the original study, using calves with a RR > 50 rates per minute. The second test there was a correction for the case calves, the classification was not only made due to respiratory rate. In this test all animals with a score on any of the clinical signs were noted as case animals.
Analysis 2: This analysis tested whether detection of a specific pathogen differed significantly between case and case-control animals.
This test was performed twice. The first time the 'not detected' group consisted of all results were no pathogen was detected. The second time all results were implemented and the 'not detected' group consisted of all other results that did not detect the specific pathogen considered.
Analysis 3: This analysis tested whether the detection of a specific pathogen, specific per clinical sign, differed significantly among case and case-control groups. To do this, firstly the results of case animals were analyzed before comparing the case and case-controls.
Analysis 4: This analysis tested whether the detection of a pathogen, or a bacterium or a virus, per clinical sign, differs significantly within case and case-control groups.

Results

In total the results of 741 BAL's and the associated clinical signs of calves, derived from 8 different farms, were analyzed. The 741 results consisted of 573 case-controls and 168 cases. In 297 of the 573 (52%) case-control BAL's a pathogen was cultured. In 106 of the 168 (63%) case BAL's a pathogen was cultured. An overview of the collected BAL samples from case and case-control groups is presented in *Figure 1 Overview BAL results* on page 12.
As posted in *Table 1 Prevalence of respiratory pathogens in case and case-control calves*, the results in prevalence sometimes resulted in small sample sizes per pathogen.

Table 1 Prevalence of respiratory pathogens in case and case-control calves

Pathogen detected (pure + mixed cultures)	**Number of case calves** (N_{total} = 168)	**Number of case-control calves** (N_{total} = 573)
Pasteurella multocida	49 (29%)	189 (33%)
Arcanobacterium pyogenes	22 (13%)	30 (5%)
Mannheimia haemolytica	14 (8%)	29 (5%)
Histophilus somni	0 (0%)	3 (1%)
Corona virus	5 (3%)	16 (3%)
BPI	30 (18%)	40 (7%)
BVDV	24 (14%)	33 (6%)
BRSV	7 (4%)	11 (2%)
BHV	1 (1%)	15 (3%)
None	*62 (37%)*	*276 (48%)*

Details on all test results, including the combination of pathogens and clinical signs, are provided in *Table 7 Prevalence in case BAL's per pathogen per clinical sign* and *Table 8 Prevalence in case-control BAL's per pathogen per clinical sign* on page 13.

Table 2 Percentages of positive scores per clinical sign

Clinical sign	**Percentage of case calves** (with a positive score)	**Percentage of case-control calves** (with a positive score)
Rectal temperature	45%	10%
General appearance	15%	1%
Eye -/nose discharge	7%	1%
Coughing	6%	0%
Respiratory distress	24%	0%

As presented in *Table 2 Percentages of positive scores per clinical sign* it can be concluded that 45% of the case calves had an elevated RT and 15% had an overall quiet demeanor. Case-control calves scored fewer clinical signs.

Analysis 1

Results showed that the prevalence of a pathogen, regardless whether it is a virus or bacterium, is significantly higher in case calves. As seen *in Table 3 P-value results of Fisher's T-test of* , the P-value of detecting a bacterium infection in case animals was not significantly higher. When all animals with a score on one of the observed clinical signs were also identified as case animals, it did not affect the results being significant or not.

Table 3 P-value results of Fisher's T-test of Analysis 1

P-value	**Case animals depending on respiratory rate**	**Case animals depending on all clinical signs**
A pathogen	**0.01**	**0.01**
A bacterium	0.29	0.08
A virus	**<0.01**	**<0.01**

Analysis 2

The prevalence prevalence of specific pathogens was compared between case and case-control calves, as show in *Table 4 P-value results of Fisher's T-test of Analysis 2*, prevalence of the pathogens *Arcanobacterium pyogenes,* BPI and BVDV were significantly higher in case animals.

Table 4 P-value results of Fisher's T-test of Analysis 2

P-value	**Not detected = no pathogen**	**All results**
Pasteurella multocida	**<0.01**	0.35
Arcanobacterium pyogenes	**<0.01**	**<0.01**
Mannheimia haemolytica	**<0.01**	0.13
Histophilus somni	1	1
Corona virus	0.56	1
BPI	**<0.01**	**<0.01**
BVDV	**<0.01**	**<0.01**
BRSV	1	0.15
BHV	0.32	0.14

Analysis 3

When all case animals were compared per clinical sign, no results were significant. There was one exception, *Arcanobacterium pyogenes* on GA, with a p-value of 0.025. When case and case-control

animals were compared per clinical sign, we found that all results were significant, with the exception of BHV. None of the results with BHV were significant.

Analysis 4

During this test prevalence of pathogens (bacteria and/or viruses) were tested per the results of the clinical signs. As show in *Table 5 P-value results of Fisher's T-test of Analysis 4*, only the results of viruses and E/N were not significant. This means that having a positive score on clinical signs are indicative for case calves, with the exception of viruses on E/N.

Table 5 P-value results of Fisher's T-test of Analysis 4

P-value	**Pathogen**	**Bacteria**	**Viruses**
Rectal temperature	**0**	**0**	**0**
General appearance	**0**	**0**	**<0.01**
Eye -/nose discharge	**<0.01**	**0.01**	0.22
Coughing	**<0.01**	**<0.01**	**<0.01**
Respiratory distress	**0**	**0**	**<0.01**

Discussion

In this study there were 573 case-controls and 168 cases, resulting in 22% case animals reflecting a situation where a ratio of 22% case animals is normal. However, normally in the Netherlands a herd is treated when 15% of the animals are affected. The ratio of 573/168 may have affected the results even though the study was aimed at obtaining insight in the BRD situation on conventional veal farms and therefore may not be representative.

All calves in a herd could participate in the study, regardless of clinical abnormalities. Selecting for case or case-control was done only by RR, other clinical signs could be aberrant. For example, it was possible that calves were selected as case-control with a RR of 45 rates per minute whilst having a arthritis and an elevated RT. From other studies it is known that common diseases in veal calves, like diarrhea, have an effect on the observed clinical signs (Clark, M.A. 1993, Pardon et al. 2013).

The BAL's were obtained via the nasal cavity, making it possible for nasal commensal bacteria to contaminate the used tube and perhaps influence the test result. The effect of contamination via nasal route was demonstrated in the study of Allen et al. in 1999, therefore a plastic sheath protecting the bronchoscope when entering the nostril was used in their study. The study of Pringle et al. in 1988 also used a bronchoscope with protective sheath. The study of DeRosa et al. from 1999 performed transtracheal swabs, but did not mention contamination or preventative measures. The study of Autio et al. from 2006 performed tracheobronchial lavages and did not mention protective sheaths or other measures. The Animal Health Department in the Netherlands (Gezondheidsdienst Dieren, Deventer) prescribes cleaning the nostril with alcoholic wipes before applying the tube (Protocol Longspoeling bij Kalveren -2015). It is probable that the used procedure to perform the BAL in this study did not provide sufficient methods for the prevention of contamination.

A very important group of pathogens that weren't analyzed in this study, due to time limits, is the group of Mycoplasma's. This group of pathogens is known to cause cranioventral caseousnecrotic bronchopneumonia (Confer, A.W. 2009). Especially *Mycoplasma bovis* is mentioned as being important in BRD, even though it is still unknown whether it is a primary or secondary pathogen (Confer,A.W. 2009, Giovanni et al. 2013, Fulton, R.W. 2009). It was detected in 70.8 % of affected calves in Belgium (Pardon et al. 2011).

The clinical experiment was executed by several persons, leading to some differences. In total 6 students and 10 farm veterinarians performed BAL's. Most BAL's were performed by the students.

Both students as veterinarians were not all consistent in wearing gloves while performing the procedure. Selecting of case and case-control animals should have been done by the students according to RR and as random as possible. Instead, some did not select calves with a RR>50/min that were lame or were suffering from diarrhea. Perhaps this made selection for BRD more specific, but was not according to the study protocol. In addition to that, some farmers were reluctant to let animals be sampled with the highest score on the clinical signs GA, C and RD when there were enough other animals meeting the requirements of a RR>50/min. This may resulted in missing results of representative animals. This may not had an effect on the prevalence of pathogens, but may have had an influence on the data of the clinical symptoms. Perhaps more case animals would have had a score on clinical signs and this would probably affect the results per pathogen (Analysis 3). However, this would only strengthen the current results, it will not change whether results were significant or not-significant.

The results of this study included in total 741 BAL's, but further analysis revealed small sample sizes per pathogen, as seen in in *Table 1 Prevalence of respiratory pathogens in case and case-control calves* on page 6. With regards to prevalence, *Pasteurella multocida* was detected in 29% of case calves, in other studies a similar prevalence varying of 23-40% was recorded (Confer, A.W. 2009, Pardon et al. 2011, Rérat et al. 2012). However, some other pathogens were only found in a few samples even though other studies found higher prevalences. *Histophilus somni* was found in none of the case calves, and in 3 of the case-control calves. Literature is not consistent on the role of *Histophilus* in BRD, overall it is regarded as a pathogen causing multiple diseases (Confer, A.W. 2009, Fulton et al. 2009). Other studies have also found low prevalences of *Histophilus* in calves affected by BRD (Apley, M. 2006, Autio et al. 2007). One of the pathogens being regarded as being of highest importance in BRD is *Mannheimia*, but it was only found in 8% and 5% of respectively case and case-control calves in this study (Confer, A.W. 2009, Pringle et al. 1998). Of the viral pathogens, BVDV is considered to be of great importance to BRD. Not only as primary cause, but also by causing immunosuppression and thus enabling other pathogens to infect the airway of calves (Apley, M. 2006, Pardon et al. 2011, Fulton et al. 2009). Previous studies in Belgium and the United States recorded prevalence around 20% in animals affected by BRD (Fulton et al. 2000, Pardon et al. 2011). The current study demonstrated a prevalence of 14% in affected calves. Perhaps the test method and origin of the calves was of influence. In order to perform the virus isolation, it is of great importance to cool the samples, and keep them cool, until the samples arrived at the laboratory. This was not always successful. The origin of the calves was diverse and there were also animals included from Germany. The prevalence of BVDV is in Germany very low, due to a government guided eradication program.
Results of Analysis 1 showed that the prevalence of a pathogen is significantly higher in case calves. This significant result is largely due to the presence of viruses. It is important to note that the difference between the first and second test is due to a difference of 63 test results, which are mostly due to the number of calves with an elevated RT (n= 55). This particular clinical sign is not noticeable when visually inspecting a herd of veal calves.
In Analysis 2 the prevalence of the pathogens *Arcanobacterium pyogenes,* BPI and BVDV were significantly higher in case animals. *Histophilus somnia* and BVDV were significant in the first test, but proved to be not significant in the test combining all results. It is important to realize that the first test does not represents the clinical situation, but it may be of a predictive value in a group of veal calves having clinical signs matching with BRD. The second test, including all results, is more representative for a clinical situation were affected and not affected animals are present. However, the farmer and farm veterinarian are more likely interested in the pathogens affecting the case calves, giving more information on which pathogens are relevant and giving information about how the animals should be proper medicinally treated.
When comparing the results of case calves per clinical sign per pathogen in Analysis 3, there were no significant except for *Arcanobacterium pyogenes* with regards to GA. This result had a p-value of 0.025, meaning that case animals with *Arcanobacterium pyogenes* were having more E/N compared

with case animals that were tested negative for pathogens. When the results of case and case-control calves was similarly compared, all results seemed to be significant and thus scoring on one of the clinical signs is more likely to appear within the case animals. There only was one pathogen exception: all results of BHV were not significant. However, the results of this tests cannot be directly extrapolated to a farm situation. Having fever or being weak are clinical signs of BRD, but they are not exclusively for BRD (DeRosa et al. 2000, Pardon et al. 2013).
The difference in prevalence of detecting a pathogen, virus or bacterium, and a score on one of the observed clinical signs, is significantly between case and case-control animals as proven in Analysis 4. There is one exception, the score on E/N for viruses. *Table 7 Prevalence in case BAL's per pathogen per clinical sign* and *Table 8 Prevalence in case-control BAL's per pathogen per clinical sign* on page 13 show that very few calves had a score on the clinical sign E/N. Only 6 case calves and 3 case-control calves scored on E/N. Research by Leruste et al. in 2012 in case and case-control animals demonstrated an overall prevalence of 6.3% nasal discharge and 4.7% of the animals showed coughing. A study by Allen et al. in 1991 in case animals demonstrated that 42% had an elevated respiratory rate, 33% showed eye- or nasal discharge and 27% showed signs of coughing. The studies have let to different results and the results of this study is somewhere in between.

Conclusion

In this study a pathogen was detected in 63% of the case calves, and in 52% of the case-control calves. In both groups the pathogen *Pasteurella multocida* was most frequently isolated from the BAL's, being isolated in respectively 29% and 33% of the samples. In case calves *Arcanobacterium pyogenes,* BPI and BVDV were more frequently detected than in the case-control calves. Prevalence was noted as 13% of *Arcanobacterium pyogenes* in case calves versus 5% in case-control calves, in BPI prevalence was 18% versus 7%, in BVDV prevalence was 14% versus 6%. The case animals show generally more clinical symptoms, whereby bacteria seem more likely to achieve a lower P-value, what may suggest it may be observed more frequently in clinical bacterial infections. However, in this study there was no significant difference between the severity of clinical signs and a specific pathogen related to BRD in cases and case-controls.

Appendix

Table 6 Scoring system clinical signs

	Score 0	Score 1	Score 2	Score 3
General appearance	Bright, alert, normal appetite and behavior	Decreased response, decreased appetite	Apathetical, slow, decreased appetite, separates itself from the group	Soporific, barely reacting to stimuli, isn't able to stand without help
Eye -/nose discharge	No eye- or nasal discharge	Eye- or nasal discharge is variably watery - mucous	Eye- or nasal discharge is increased persistent mucous or clear watery with some white/yellow discoloring	Severe eye- or nasal discharge persistent mucous or bloody.
Coughing	No coughing	Occasionally a dry, non-productive cough	Frequent spontaneous dry or productive coughing	Frequent spontaneous productive cough
Respiratory distress	RR < 50 / min	RR 51-70 /min	RR 71-100/min	RR> 100/min

Figure 1 Overview BAL results

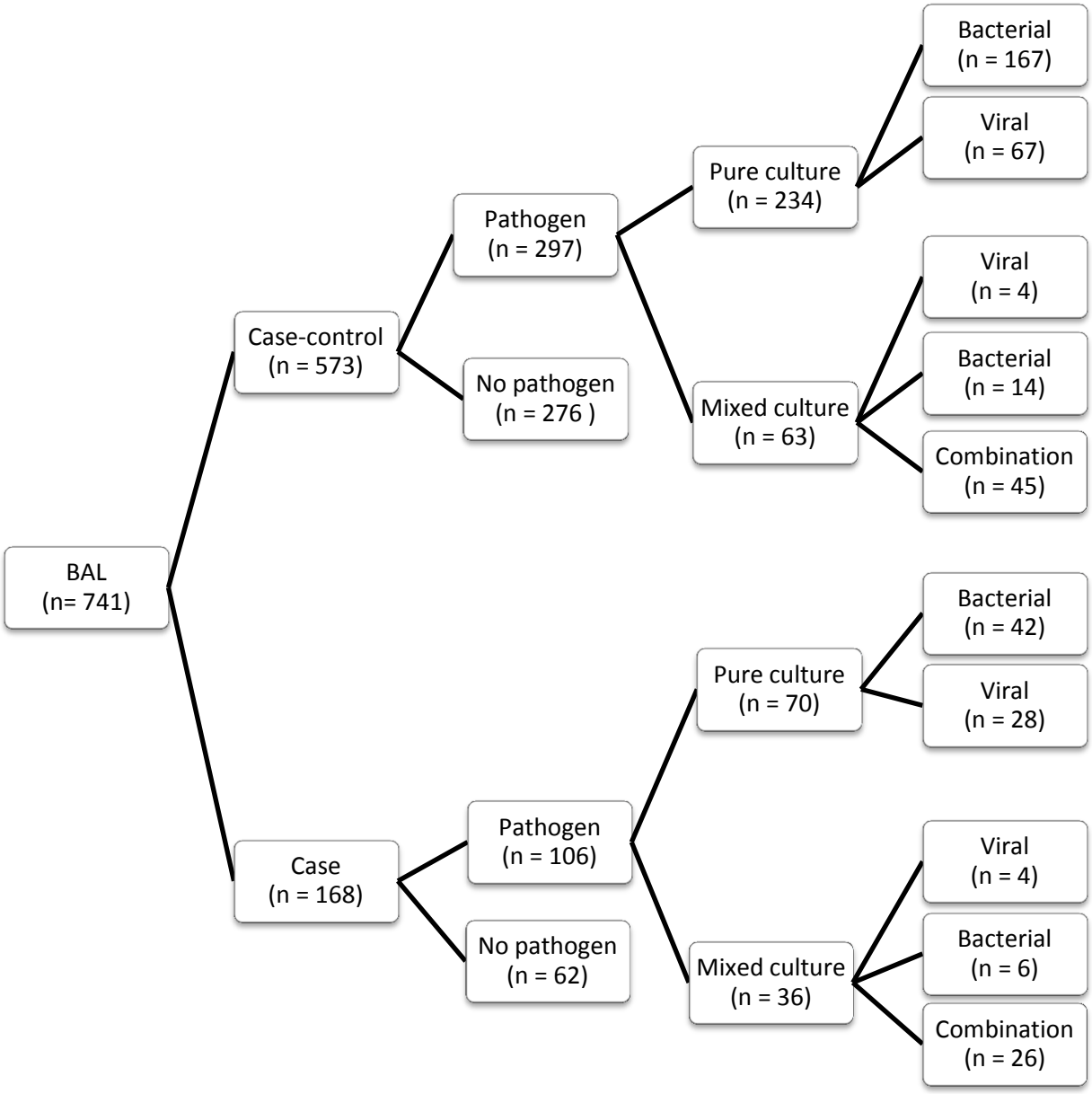

Table 7 Prevalence in case BAL's per pathogen per clinical sign

Number of calves	RT_1	RT_0	GA_1	GA_0	E/N_1	E/N_0	C_1	C_0	RD_1	RD_0
Pasteurella	11	15	0	26	1	25	2	24	6	20
mixed	*12*	*11*	*4*	*19*	*1*	*22*	*3*	*20*	*8*	*15*
Arcanobacterium	8	2	3	7	0	10	1	9	4	6
mixed	*4*	*8*	*4*	*8*	*1*	*11*	*1*	*11*	*2*	*10*
Mannheimia	5	1	2	4	2	4	0	6	2	4
mixed	*4*	*4*	*1*	*7*	*0*	*8*	*0*	*8*	*3*	*5*
Histophilus	0	0	0	0	0	0	0	0	0	0
mixed	0	0	0	0	0	0	0	0	0	0
Corona virus	2	1	0	3	0	3	0	3	0	3
mixed	*1*	*1*	*0*	*2*	*0*	*2*	*0*	*2*	*1*	*1*
BPI	6	5	4	7	1	10	2	9	3	8
mixed	*9*	*10*	*2*	*17*	*1*	*18*	*1*	*18*	*4*	*15*
BVDV	8	6	2	12	2	12	1	13	3	51
mixed	*6*	*4*	*3*	*7*	*1*	*9*	*0*	*0*	*3*	*7*
BRSV	0	0	0	0	0	0	0	0	0	0
mixed	*5*	*2*	*1*	*6*	*1*	*6*	*2*	*5*	*2*	*5*
BHV	0	0	0	0	0	0	0	0	0	0
mixed	*0*	*1*	*0*	*1*	*0*	*1*	*0*	*1*	*0*	*1*
Mixed	16	20	8	28	2	34	3	33	9	27
No pathogen	20	42	6	56	4	58	1	61	14	48
Total pure	40	30	11	59	6	64	6	64	18	52

Table 8 Prevalence in case-control BAL's per pathogen per clinical sign

Number of calves	RT_1	RT_0	GA_1	GA_0	E/N_1	E/N_0	C_1	C_0	RD_1	RD_0
Pasteurella	16	119	0	135	1	134	1	134	0	135
mixed	*5*	*49*	*0*	*54*	*2*	*52*	*0*	*54*	*1*	*53*
Arcanobacterium	1	11	0	12	0	12	0	12	0	12
mixed	*2*	*16*	*0*	*18*	*0*	*18*	*0*	*18*	*0*	*18*
Mannheimia	3	16	1	18	1	18	0	19	0	19
mixed	*0*	*10*	*0*	*10*	*0*	*10*	*0*	*10*	*0*	*10*
Histophilus	1	0	0	1	0	1	0	1	0	1
mixed	0	2	0	2	0	2	0	2	0	2
Corona virus	0	6	0	6	0	6	0	6	0	6
mixed	*0*	*10*	*0*	*10*	*0*	*10*	*0*	*10*	*0*	*10*
BPI	4	25	1	28	1	28	0	29	0	29
mixed	*3*	*18*	*0*	*21*	*1*	*20*	*0*	*21*	*0*	*21*
BVDV	1	16	0	17	0	17	0	17	0	17
mixed	*1*	*15*	*0*	*16*	*1*	*15*	*0*	*16*	*1*	*15*
BRSV	0	7	0	7	0	7	0	7	0	7
mixed	*0*	*4*	*0*	*4*	*0*	*4*	*0*	*4*	*0*	*4*
BHV	0	8	0	8	0	8	0	8	0	8
mixed	*3*	*4*	*0*	*7*	*0*	*7*	*0*	*7*	*0*	*7*
Mixed	4	59	0	63	2	61	0	63	1	62
No pathogen	25	251	1	275	2	274	1	275	1	275
Total pure	26	208	2	232	3	231	1	233	0	234

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