The Association between Results of Broncheoalveolar lavage and Serology in the Detection of BRSV and PI3 in Clinically Healthy Veal Calves

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Contents

Abstract

This paper analyses the relation between brochoalveolaire lavage (BAL) and serology, tested on eighty-one clinically healthy veal calves from six different herds. These herds frequently encounter respiratory outbreaks. In this study, Bovine Respiratory Syncytial Virus (BRSV) and Bovine parainfluenza type 3 virus (PI3) are looked for in the BAL brought in relation with possible seroconversion in the blood.
BRSV was present in de BAL of 10 different calves and seroconversion has occurred twice, both of those instances tested negative in the BAL.
PI3 has been found 26 times in the BAL and seroconversions has occurred 33 times. Of these 33 occurrences of seroconversion there were 12 cases in which the results of the BAL were also positive.
No significant relation between BAL and serology has been found with both of these viruses and in none of the herds. This research can conclude that there is no relation between results from the BAL and serology.

Introduction

The veal calf industry is focused mostly on calves that originate from the dairy cattle branch. Bull calves are redundant in the dairy cattle branch, these types of calves are an important part of the veal sector. While these calves have been bred as part of the dairy industry they eventually contribute to the meat industry.

In the Netherlands there are about 1665 veal calf farms in 2014 which hold about 92000 calves. The average size of a Dutch veal calf farms is about 550 calves. Whit these numbers the Netherlands is the biggest exporter of veal of in the world. Most of the veal (>80%) exporter is exported to Italy, Germany and France.

BRD and diarrhoea are the most prevalent afflictions in the veal sector (Autio, Pohjanvirta et al. 2007). Bovine respiratory disease (BRD) is a collection of diseases in which different pathogens play a role in the respiratory tracts (Snowder 2006). Bovine respiratory syncytial virus (BRSV), Parainfluenza type 3 virus (PI3), Bovine Viral Diarrhoea (BVD), Bovine Coronavirus (BCV), *Mycoplama* spp, *Pasteurella* spp en *Haemopilus somnus* can appear together or individually in BRD (Autio, Pohjanvirta et al. 2007, Van Der Fels-Klerx, Martin et al. 2002).
Predisposing factors in the prevention are age, stress levels, the time of the year, the immune defence system, and shipping conditions. BRD results in a decrease in growth, which has a negative effect on the financial results (Snowder 2006, Van Der Fels-Klerx, Martin et al. 2002). The clinical manifestations of BRD are various and include: fever, nasal spillage, coughing, elevated respiratory rate, and decreased appetite (Van Der Fels-Klerx, Martin et al. 2002, EOtto M. Radostits, et all. 2006).

A field study in collaboration with a.o. WUR and CVI was set up to gain insight into the pathogens that play an important role in the pathogenesis of BRD. Ten different Dutch veal farms participated in the research. The aim of the research is to find a way to preventively anticipate BRD and by doing so reduce antibiotic resistance and control the disease. Six veterinary students have contributed to this study, all with a different research question. Within the research different additional diagnostics were used, namely, serology and broncheoalveolare lavage (BAL). The choice was made to study the association between the results of the BAL and serology concerning BRSV and PI3 to determine the value of these forms of diagnostics. Both BRSV and PI3 are viruses that play an important role in the pathogenesis of BRD. Within this research this was tested on clinically healthy animals. The following hypothesis was drawn up for this research.

H0: There is a significant association between results of the BAL and serology in the detection of BRSV and PI3 in clinically healthy veal calves
H1: There is no significant association between results of the BAL and serology in the detection of BRSV and PI3 in clinically healthy veal calves

Materials & Methods

A total of eighty-four calves from six different herds/farms were used in this research. The number of calves differed per herd, variating from eleven to seventeen. This variation was due to control calves that became sick during the research period, farmers that moved control calves to another barn and ear tag administration errors. The farms that participated in the research had to meet several criteria: no other bovine animals should be kept at the farm, they had to use an all in/all out system, the company had to have at least one barn which offered room to a minimal of 300 calves, and finally, the calves had to arrive on the farm anywhere between week 41 and 48 of 2013.

The control/healthy calves who participated in this research were randomly selected by a biostatistician from Wageningen University. The control calves need to be healthy during the initial activities. To review the healthy calves, a special schedule is used. Different aspects such as, respiratory rate, temperature, ocular discharge, nasal discharge, degree of coughing, respiratory distress and overall impression are rated. The same schedule also applies to the rating of sick calves. Different colors ear tag are used to distinguish between the control and sick calves.

Research procedures

The practical experimental phase of this research lasts 84 days. Day 0 (D0) is defined as the day that the herd arrived at the farm. The measurements are performed until day 84 (D84). The practical procedures started in October and November of 2013 and were performed by a veterinarian or veterinary student.

The BAL's were taken from 20 control calves and during a respiratory disease outbreakA respiratory outbreak was declared when an estimated 10% of the herd showed abnormal respiratory symptoms and the veterinary had decided to treat the herd. All BAL's were taken before treatment was started.
Only at D0 and D84 blood samples are were taken from the corresponding control calves, at which time the BAL was also taken. Once a control calve became sick during the complete research period, that particular calve was excluded from the research.
The BAL fluid and blood samples were shipped under cooled circumstances and exanimated at the Central Veterinary Institute (CVI), Lelystad and both procedures were performed according the laboratory protocol.
All the results are analysed with Microsoft Excel and statistically calculated with IBM SPSS Predictive Analytics-software.

Blood sample

All blood samples were taken from the Jugular vein with a Vacuette serum collection system. During this procedure the calf was fixated. The collected blood was cooled and transported to the Central Veterinary Institute (CVI), Lelystad. The samples are examined with a serological multiplex trivalent ELISA kit which measures the specific IgG1 in the blood (Bio-X Diagnostics). Apart from BRSV and PI3 the antibodies of the Mycoplasma were also measured. However, they were not used for this particular research.

To interpret the test result a serological scale is manually applied during the test. The quantity of antibodies is translated into six groups (-, +, ++, +++, ++++ and +++++). The groups with the corresponding numbers of antibodies are shown in the following table 1.

Table 1: Interpretation of serological groups

Degree of positivity	-		+		++		+++		++++		+++++
BRSV	0	-	26	-	52	-	78	-	104	-	130	>
PI3V	0	-	48	-	95	-	142	-	189	-	236	>

Seroconversion was defined as described by Roshtkhari et al., using the classification of the titer of the manufacturer (BioX Diagnostics). Seroconversion was present when the titer showed an increase of two orders of magnitude (for example, +>+++, ++>++++ or ->++) (Roshtkhari, Mohammadi et al. 2012).

Bronchial alveolar lavage

During the practical procedure of the BAL the calves are fixated as much as possible.
A flexible tube is inserted into the trachea throughout the nasal cavity. The tubes are rounded on one end to minimize damage of the nasal, laryngeal and tracheal mucosa. There were two kinds of tubes available with different diameters. For each calf a tube is chosen, depending on the age and nasal passage. Once the tube was well positioned, 100 ml sterile Phosphate buffered saline (PBS) was inserted in to the lungs, immediately followed by an attempt to aspirate the fluid as much as possible. The position of the tongue during the insertion of the tube acted as an indicator for the position of the tube. If the tongue was curled out this was a sign that the tube was inserted into the trachea. If too little fluid aspirated, another 100ml PBS was administrated. From 100ml, 15ml was the minimal need for examination. When there were difficulties extracting the BAL fluid no more than 300 ml of PBS was administered. Because the number of outbreaks differs per herd there is a table with herd number and the corresponding number of outbreaks. In this table (table 2) the number of calves from each herd is also included.

Results

In table 2 the herd number are put in with the corresponding amount of calves, the number of outbreaks, the time between the first outbreak and D84, and the time between the last outbreak and D84. The table shows that there is some variety in the number of outbreak and the time of the outbreak between D0 and D84.

Table 2 Herd with number of calves, number of outbreaks and time between first outbreak and D84 and between last outbreak and D84.

Herd	Number of calves	Number of outbreaks	Time between first outbreak and D84 (days)	Time between last outbreak and D84 (days)
1	13	2	23	2
2	13	3	77	44
3	13	3	50	1
4	14	1	74	-
5	11	2	74	61
6	17	2	66	48

The results are elaborated on per virus. To establish whether there are variations, the Pearson chi square test has been conducted per herd.
Raw data of all the participating calves is present in table 7a and 7b of the appendix.

Bovine respiratory syncytial virus

BRSV was isolated in the BAL of two calves, while 10 other calves seroconverted.

Table 3 amount of BRSV isolated from BAL and seroconversion

		Seroconversion BRSV		
		Negative	**Positive**	**Total**
BAL BRSV	**Negative**	69	2	71 (87,7%)
	Positive	10	0	10 (12,3%)
Total		79 (97,6%)	2 (2,5%)	81

The following table specifies the serology results per herd. Here the results of the BAL and the serology are combined and where possible the pearson chi square is calculated. Here it becomes clear that there is little discrepancy between the herds.

Table 4 Chi square BRSV per herd

HERD	Fraction BAL negative (BAL negative/serology negative)	Fraction BAL positive (BAL positive/serology positive)	P value Pearson Chi Square
1	100 % (13/13)	0% (0/0)	-
2	83 % (10/12)	0% (0/1)	.657
3	77% (10/13)	0% (3/0)	-
4	86% (12/14)	0% (2/0)	-
5	73% (8/11)	0% (3/0)	-
6	100% (17/17)	0% (0/0)	-
Total	87% (69/79)	0% (0/2)	.591

Seroconverted relative to BAL positive is zero in every herd because of the low numbers of positive serology tests. The chi square test showed that there was no significant association between the results of the BAL and serology.

Parainfluenza virus type 3

In this research PI3 is more often isolated from the BAL in comparison with BRSV Similarly, seroconversion also occurred more often with PI3 than with BRSV.
An overview of the results of the BAL and seroconversion is shown in the following table 5.
This table shows that PI3 was isolated 26 times from BAL and that seroconversion occurred 33 times. Seroconversion can be linked to the BAL in 21 of the 33 occurrences.

Table 5: amount of PI3 isolated from BAL and seroconversion

		Seroconversion PI3		
		Negative	**Positive**	**Total**
BAL PI3	**Negative**	34	21	55 (68%)
	Positive	14	12	26 (32%)
Total		48 (59%)	33 (41%)	81

It can be concluded from table 5 that 32% is positive in in the BAL for PI3 and 40,7% seroconversion. Of all the calves, 26% showed seroconversion with positive BAL. For PI3 the results are also specified per herd, this can be seen in table 6. This table shows that no P value could be calculated for herd 1. Furthermore, it becomes evident that the seroconversion in combination with BAL positive is often lower than seroconversion and BAL negative.

Table 6: Chi square PI3 per herd

HERD	Fraction BAL negative (BAL negative/serology negative)	Fraction BAL positive (BAL positive/serology positive)	P value Pearson Chi Square
1	100% (9/9)	0% (0/4)	-
2	80% (8/10)	33% (1/3)	.631
3	89% (8/9)	25% (1/4)	.522
4	100% (4/4)	20% (2/10)	.334
5	50% (3/6)	40% (2/5)	.740
6	20% (2/10)	86% (6/7)	.761
Total	71% (34/48)	36% (12/33)	.495

Also for PI3 the chi square test showed that there was no significant association between the results of the BAL and serology. With this result H0 is rejected.

Discussion

Broncheoalveolar lavage

A large number of calves were tested negative in the BAL for BRSV as well as PI3. Which means that the virus was discovered in clinically healthy animals. There could be several underlying causes like; poor storage, transport condition, wrong way of taking the BAL, contingencies during the process of isolation of the virus or mistakes in the registration of the results.
Research has shown that Paramyxoviruses have a hard time surviving during storage and transport (Ellis 2010, Brodersen 2010). This can possibly be a reason for the low number of positive BAL's.

During the practical procedure of the BAL there are several factors that can influence the results, for instance, fixation of the calf, sterility of the tube, ability to suck back enough fluid, contamination of the nasal passage and ability to get the tube far enough in to the trachea.

The number of outbreaks is a limiting factor to isolate a pathogen from the BAL. The more often an outbreak has occurred in a herd, the more opportunity for that herd to isolate a virus. It is reported in which herds and how often an outbreak occurred. Consequently, it is unfair to compare all the herds to each other because the chances of being able to isolate the virus from the BAL differs between the different herds. Apart from the amount of outbreaks, it is also important to look at the time between the outbreak and D84. D84 is important because it determines whether or not seroconversion occurred.

Remarkable of herd one is that all the BAL`s that were taken tested negative for both viruses. Because of this the results from the BAL of this herd are questioned.

Serology

Similarly to the BAL procedure there are also mistakes that can be made with the serology. Things to consider are transport, storage, performances in the lab and processing the results. Because the practical procedure of taking blood samples is quicker it is also easier to know whether or not the procedure was successful, as opposed to the BAL procedure which takes longer. Because of this it is less likely to make mistakes during the serology procedure.

Seroconversion appeared In calves that were tested negative in the BAL. This can have different causes. The BAL could be a false negative, the serology could be a false positive. Or at the start of the research the antibody titer was already at a maximum.

After the infection with BRSV, the antibody titer of IGg1 rises somewhere between 6 to 10 days after (Uttenthal, Larsen et al. 2000). With PI3 this takes approximately 6 days after the infection (Ellis 2010). Thus, there was enough time between the outbreak and D84 in all the herds to cultivate antibodies for both BRSV and PI3.

If the half-life of the antibodies is very short it could be that seroconversion did occur but was not observed. The half-life of BRSV is 35,9 days and the half-life of PI3 is 30,3 days (Fulton, Briggs et al. 2004). Because relatively little information about the calves is known, it was assumed that they were not vaccinated. This would mean that seroconversion might have occurred for both viruses in herd 2,4,5, and 6 but was not observed.

Remaining factors

Herds which had to deal with barn conditions that were not up to standard, for instance, bad or outdated ventilation techniques or holes and cracks in the barn, had an increased chance of an BRD outbreak (Guterbock 2014). This was the case for herd 3, where the barn was outdated and it had a poor ventilation system.

Conclusion

In this research 12,3% of the calves tested positive for BRSV in the BAL and in 2,4% of the cases seroconversion occurred. There was no link to a positive BAL in any of these seroconversions. Because of the poor results from the BAL no significant association with the serology could be detected.
This can also be said for PI3. This virus was isolated from the BAL (32%) more often than BRSV and there were more occurrences of seroconversion (40,7%). Of all the calves 26% showed seroconversion for PI3 with a positive BAL. Moreover, after specialisation per herd and virus no statistical significance between BAL and seroconversion was noted. This may suggest that the diagnostic value of the BAL is not high in comparison with serology.

Appendix

Table 7a Overview with results of herd 1,2 and 3.

Herd 1 Calf	BAL	Seroconversion
1	-	-
2	-	-
3	-	PI3
4	-	-
5	-	PI3+BRSV
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	PI3
12	-	PI3
13	-	-

Herd 2 Calf	BAL	Seroconversion
1	BRSV (1)	-
2	PI3 (3)	-
3	-	-
4	-	BRSV + PI3
5	-	-
6	PI3 (1)	PI3
7	PI3 (1)	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	PI3
13	-	-

Herd 3 Calf	BAL	Seroconversion
1	-	-
2	BRSV (2)	-
3	-	-
4	-	-
5	-	-
6	PI3 (1)	-
7	BRSV (1)	-
8	-	PI3
9	-	-
10	-	PI3
11	BRSV (1)	PI3
12	PI3 (1+2)	PI3
13	-	-

- *No positive BAL or no seroconversion*
- *(..) number of outbreak or day of which was positive*

Table 7b Overview with results of herd 4,5 and 6.

Herd 4 Calf	BAL	Seroconversion
1	PI3 (1)	PI3
2	-	-
3	-	PI3
4	-	PI3
5	-	PI3
6	-	-
7	-	PI3
8	BRSV (1)	PI3
9	-	-
10	BRSV (1)	-
11	-	PI3
12	-	PI3
13	-	PI3
14	PI3 (1)	PI3

Herd 5 Calf	BAL	Seroconversion
1	BRSV (2)	PI3
2	BRSV (2)	-
3	PI3 (2, D84)	-
4	-	PI3
5	-	PI3
6	-	-
7	BRSV (2), PI3 (1)	-
8	-	-
9	PI3 (2)	PI3
10	PI3 (2)	-
11	PI3 (2)	PI3

Herd 6 Calf	BAL	Seroconversion
1	PI3 (1)	PI3
2	PI3 (2)	-
3	-	-
4	PI3 (1)	-
5	PI3 (1+2)	-
6	PI3 (1)	-
7	PI3 (1)	-
8	PI3 (2)	-
9	PI3 (1)	PI3
10	PI3 (2)	-
11	PI3 (2)	PI3
12	-	-
13	PI3 (1)	-
14	PI3 (1+2)	PI3
15	PI3 (D84)	PI3
16	PI3 (1+2)	PI3
17	-	PI3

- *No positive BAL or no seroconversion*
- *(..) number of outbreak or day of which was positive*

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