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Association of bovine respiratory disease with clinical status and acute phase proteins in calves

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Abstract

Eighty-four calves with respiratory disease from 18 herds in different parts of Finland were chosen for a study evaluating the capacity of different respiratory pathogens to cause changes in different acute phase protein concentrations, white blood cell (WBC) count and clinical signs. The selected acute phase proteins were fibrinogen, haptoglobin, serum amyloid-A, lipopolysaccharide binding protein and \(\alpha\)-1-acid glycoprotein. From each calf, a paired blood sample was obtained for serological studies of bovine parainfluenza virus-3, bovine respiratory syncytial virus, bovine coronavirus, bovine adenovirus-3 and bovine adenovirus-7. Tracheobronchial lavage was performed to detect bacteria and mycoplasma. Isolation of \textit{Pasteurella multocida} was associated with increased concentrations of all tested acute phase proteins. For
other pathogens, no significant relationships were observed. No association was present between viral or bacterial findings and WBC count.

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1. Introduction

Acute phase response (APR) is a set of reactions in the early protection mechanism of the host against infection, trauma or other tissue damage. One of its main features is hepatic production of acute phase proteins (APPs) [1]. APPs are a large, heterogeneous group of proteins with a wide range of biological activities. Many of their functions in host defence during inflammation are not fully understood. Measuring concentrations of different APPs in plasma can give us valuable information on the APR and the course of disease. The roles and profiles of different APPs differ from each other, and thus, measuring several APPs can provide a more comprehensive picture of APR in individual animals. Many experimental studies of APPs in cattle have been carried out in recent years (e.g. [2–4]). Some of these describe changes in the concentrations of APPs as disease markers in bovine clinical respiratory diseases of feedlot calves [5–8], or in experimental respiratory infection models [9–11]. Bovine respiratory disease (BRD) is a multifactorial disease of cattle that involve many pathogens and APPs may be used to explore impact of different etiological or other factors to this disease complex. To our knowledge, no studies on relationships of different APPs to naturally occurring respiratory pathogens in calves have been conducted.

Fibrinogen, the circulating precursor of fibrin in the blood clotting cascade, was the first APP routinely used in cattle diagnostics [12]. Other proteins, mainly two...
major bovine APPs, haptoglobin (Hp) and serum amyloid-A (SAA), have also been tested to evaluate APR in cattle [11,13]. In addition, some other bovine APPs, such as lipopolysaccharide binding protein (LBP) and moderately responding \( \alpha \)-1-acid glycoprotein (AGP), have been investigated in experimental bacterial respiratory infections in calves [10,14,15].

The objective of our study was to determine concentrations of different APPs in relation to clinical signs and white blood cell (WBC) count in BRD caused by different pathogens in calves in field conditions.

2. Materials and methods

2.1. Herds and animals

Eighteen herds were studied from November 1998 to December 1999. Ten herds were fattening units and 8 were dairy herds. The farms were located in Eastern, Southern and Western Finland. The fattening units had 48–217 young cattle and dairy herds had 30–130 cows. The diseased calves were mostly of the Ayrshire or Holstein–Friesian breed. The mean age of calves was 98 (range 59–137) days and the mean weight 88 (range 61–115) kg.

2.2. Clinical examination

When BRD problems were suspected in herds, the animal owners contacted veterinarians. The farms were then visited by 2 veterinarians who examined all calves with clinical signs. A thorough clinical examination was conducted in which heart rate and respiratory rate were determined, rectal temperature was measured and a description of respiratory sounds and the appearance and amount of nasal discharge were recorded. Any coughing was noted during the clinical examination, and calves were examined for diarrhoea. According to pre-set criteria, abnormal sounds on auscultation of the respiratory tract, 5 calves/ herd, for a total of 90 calves, were chosen for tracheobronchial lavage. In addition all the chosen calves had at least one of the following symptoms: increased respiratory rate (>40/min), rectal temperature >39.5 °C, cough or nasal discharge. For several reasons 6 of the calves had to be left out from the survey, so 84 calves were taken into the research.

2.3. Samples

Tracheobronchial lavage and blood samples were taken from the calves without sedation. The calf was restrained by an assistant while a sterile plastic double catheter [16] was inserted into the nostril through the ventral nose duct into the trachea. When the double catheter was inside the trachea, the inner catheter was pushed through the silicone plug of the outer catheter and into the lungs as far as possible. Thirty to forty millilitres of sterile phosphate-buffered saline (PBS, Dulbecco’s phosphate-buffered saline, Gibco TM, Invitrogen Corporation, Paisley,
Scotland, UK) was injected into the catheter and immediately aspirated. The sample was divided into test tubes with a glucose calf serum broth (GS) for isolation of mycoplasma and transport media (Portagerm multi-transport medium BioMerieux, Lyon, France) for isolation of bacteria. The rest of the sample was frozen at −70 °C for further analyses.

Blood samples for serological tests and determination of WBC counts and APPs (fibrinogen, Hp, SAA, LBP, AGP) were taken from the diseased calves. The second sample for serology was collected from the same calves 3–4 weeks later (paired serum samples). Only those 84 calves for which all samples could be obtained and analysed properly were included in the study. The handling of blood samples has been described elsewhere [17].

2.4. Isolation and identification of bacteria

Tracheobronchial lavage samples were examined for bacterial growth at the National Veterinary and Food Research Institute, Helsinki, according to standard procedures, which have been described in detail elsewhere [17]. The mycoplasma samples were kept frozen at −70 °C until cultured. The media, culturing and identification methods used have been reported elsewhere [17,18].

2.5. Detection of viral antibodies

The serum samples were tested for antibodies to bovine parainfluenza virus-3 (PIV-3), bovine respiratory syncytial virus (BRSV), bovine coronavirus (BCV), bovine adenovirus-3 (BAV-3) and bovine adenovirus-7 (BAV-7). Bovine herpesvirus-1 does not exist in Finland and bovine virus diarrhoea is very rare [19]. These viruses were excluded from the research panel. An ELISA test was used for antibodies to PIV-3, BRSV and BCV. Virus neutralization test was used for BAV-3 and BAV-7. ELISA kits (SVANOVA Biotech, Uppsala, Sweden) were used according to manufacturer’s instructions. If a fourfold increase in antibody titre in the neutralization test was seen or if the ELISA test was seronegative in the first sample and seropositive in the second sample, the calf was considered to have a rise in titre and to be recently infected with the respective virus.

2.6. APPs

Fibrinogen concentration in plasma was determined by the heat precipitation method [20]. Serum Hp was determined using the haemoglobin-binding assay described by Makimura and Suzuki [21], with the modification that tetramethylbenzidine (0.06 mg/ml) was used as a substrate [22]. Serum SAA was measured with a commercially available ELISA kit (Phase SAA kit, Tridelta Ltd., Ireland), according to the manufacturer’s instructions for cattle. Serum AGP was analysed using a commercial radial immunodiffusion kit manufactured by Ecos Institute (Furukawa, Miyagi, Japan). For serum LBP, a commercial ELISA kit was used (LBP ELISA for various species, Hycult Biotechnology, Uden, The Netherlands).
2.7. **WBC count**

WBC count was determined by an automatic cell counter adjusted for animal cell counting (Coulter-Counter Model T850, Coulter Electronics Ltd., Luton, UK).

2.8. **Statistics**

Results where the number of observations was less than five (i.e. for BCV, PIV-3, *Arcanobacterium pyogenes*) were excluded from the statistical analysis. Linear regression analysis was used to study the relationship between results from seroconversion tests (BAV-7, BAV-3), mycoplasmal findings (*Mycoplasma dispar*, *Mycoplasma* sp.) and other bacterial findings (*Pasteurella multocida*, *Fusobacterium necrophorum*) as well as clinical signs, concentrations of APPs and WBC count. If the response variable was dichotomous (cough, diarrhoea, changed respiratory sounds, nasal discharge), logistic regression analysis was applied. Logarithmic transformations were used to achieve normal distributions for all APPs and WBC counts. *P*-values of less than 0.05 were considered significant. Statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS for Windows, version 12.0; SPSS Inc., Chicago, IL, USA).

3. **Results**

3.1. **Viruses and bacteria**

Seroconversion to viruses BAV-7, BAV-3, BCV, PIV-3 and BRSV was observed in 22.6%, 13.1%, 4.8%, 3.6% and 0% of calves, respectively. *M. dispar*, other mycoplasmas, *P. multocida*, *Pasteurella* sp., *F. necrophorum* and *A. pyogenes* could be isolated in 90.5%, 60.7%, 14.3%, 1.2%, 6.0% and 2.4% of calves, respectively. *Mycoplasma bovis* was not found.

3.2. **Clinical signs**

Diarrhoea, increased rectal temperature, increased respiratory rate, crackling respiratory sounds, cough and nasal discharge were present in 14.3%, 15.5%, 44.1%, 41.7%, 78.6% and 45.2% of calves, respectively. Associations between *P. multocida* and elevated respiratory rate, increased rectal temperature, crackling respiratory sounds and nasal discharge were found. A negative association between seroconversion to BAV-7 and crackling respiratory sounds was seen.

3.3. **APPs**

Isolation of *P. multocida* was associated with increased concentrations of all tested APPs (Table 1). For other pathogens, no significant relationships were observed.
3.4. WBC count

No association was present between viral or bacterial findings and WBC count.

4. Discussion

*P. multocida* was the most common bacterium found in the lavage fluid of calves; *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*) was not detected at all. According to other studies, *P. multocida* and *M. haemolytica* are the predominant bacteria associated with BRD [10]. Our most significant result was the relationship between presence of *P. multocida* and both clinical signs and increased concentrations of APPs. This indicates a strong pathogenic role for *P. multocida* in respiratory disease of calves in Finland, at least in situations where other known pathogens, e.g. *M. haemolytica*, *M. bovis* and BRSV, are absent. Our results agree with experimental studies on calves in which *P. multocida* has been reported to cause general symptoms, as well as increased concentrations of the APPs Hp, SAA and AGP [10]. Increases in the concentrations of AGP have been reported to be more gradual and to remain elevated for longer than those observed for SAA or Hp. *M. haemolytica* can cause increased concentrations of Hp and fibrinogen in plasma [6,11] as well as fever [11,23].

We noted no associations between tested viruses and clinical signs or APPs, despite viral seroconversions. This suggests a weak role of these viruses in BRD. Although these viruses seemed not to cause APR directly or clear clinical signs, they may still have some role as predisposing factors for bacterial invasion. The negative association between seroconversion to BAV-7 and crackling respiratory sounds is difficult to explain and may be coincidental. All calves participating in this study were approximately 3 months old. The reliability of antibody determinations based

|                | FIB       | Hp        | SAA       | AGP       | LBP       | WBC       |
|----------------|-----------|-----------|-----------|-----------|-----------|-----------|
| **Absence of P. multocida** |           |           |           |           |           |           |
| Number of calves | 65       | 72        | 72        | 72        | 72        | 69        |
| Mean            | 6.02     | 0.228     | 18.4      | 377.1     | 6.3       | 11.68     |
| ±SE             | 0.157    | 0.030     | 2.13      | 24.64     | 0.72      | 0.382     |
| P-value         | 0.002    | 0.031     | 0.016     | 0.018     | 0.006     | 0.073     |
| **Presence of P. multocida** |           |           |           |           |           |           |
| Number of calves | 11       | 12        | 12        | 12        | 12        | 12        |
| Mean            | 7.73     | 0.465     | 31.5      | 580.0     | 13.5      | 9.58      |
| ±SE             | 0.578    | 0.1246    | 5.97      | 91.17     | 3.29      | 0.758     |
on IgG from serum of calves less than 3 months of age is questionable because of maternal antibodies, which might interfere with seroconversion [24,25]. The results concerning viral seroconversions should thus be interpreted with caution.

Despite *Mycoplasma* sp. and *M. dispar* being isolated in all herds and in over 90% of calves, no association between these mycoplasmas and clinical signs was observed, in agreement with earlier findings [26,27]. Tanskanen [18] did, however, report that *M. dispar* caused some clinical signs, including slight temperature rises, intermittent coughing and nasal discharge, in most calves in an experimental infection model.

In an experimental study in which viral and bacterial responses were compared, fibrinogen, Hp and SAA responded faster to *M. haemolytica* infection than to BVDV infection, and the response lasted longer [11]. When both of these agents were present, concentrations of fibrinogen and Hp increased even more, and the duration of the response was prolonged. Hp has been considered to be a distinguishing marker between viral and bacterial diseases, as it rises significantly in bacterial infections [6]. However, in experimental infection with RSV, authors have also reported high Hp values [9], likewise during the viremic stage of foot-and-mouth disease [28].

Studies on use of different APPs as markers of naturally occurring respiratory disease are somewhat controversial. Hp has been reported to be useful in detecting calves with respiratory disease requiring treatment and in monitoring the efficacy of treatment [5,29,30]. Other researchers have found Hp to have only limited capacity as a clinical tool in the diagnostics of respiratory disease in feedlot cattle [7,8]. Although SAA [14] and LBP [15] have been shown to be more sensitive APPs than Hp, some studies have concluded that SAA is not useful marker of respiratory disease in field conditions [5,29]. Serum SAA concentrations have been reported to be influenced by physical stress [2], which may partially explain its variable behaviour. In our study, establishing the ranking of different APPs as markers of respiratory disease in calves was difficult because associations existed between all APPs and *P. multocida* (Table 1). Nevertheless, the associations between *P. multocida* and LBP and fibrinogen seemed to be slightly stronger than between the other APPs measured. However, this may be due to several confusing reasons such as peak concentrations of different APPs occurring at different stages of the infection and/or other pathogens or inductors causing variable responses of different APPs. Fibrinogen has long been in routine use, and proved to be a useful, easy and inexpensive test for confirming the presence of an infection. WBC count, by contrast, did not correlate well with the type of BRD, and thus, was not useful for clinical purposes in this situation.

Our study confirms the strong pathogenic role of *P. multocida* and the value of APPs in exploring respiratory disease in calves under field conditions.

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