Comparative Immunology, Microbiology and Infectious Diseases 68 (2020) 101375

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Acute phase response and clinical manifestation in outbreaks of interdigital phlegmon in dairy herds

Miia Kontturi[a,*], Reijo Junni[a], Minna Kujala-Wirth[a], Erja Malinen[b], Eija Seuna[b], Sinikka Pelkonen[b], Timo Soveri[a], Heli Simojoki[a]

[a] Department of Production Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Paroninkuja 20, 04920, Saarentaus, Finland
[b] Bacteriology and Pathology Unit, Finnish Food Authority, Mustialankatu 3, 00790, Helsinki, Finland

ARTICLE INFO

Keywords:
Interdigital phlegmon
Foot rot
Interdigital necrobacillosis
Foul-in-the-foot
Acute phase response
Serum amyloid A
Haptoglobin
Albumin
Infectious hoof disease

ABSTRACT

Several Finnish dairy herds have suffered from outbreaks of interdigital phlegmon (IP). In these new types of outbreaks, morbidity was high and clinical signs severe, resulting in substantial economic losses for affected farms. In our study, we visited 18 free stall dairy herds experiencing an outbreak of IP and 3 control herds without a similar outbreak. From a total of 203 sampled cows, 60 suffered from acute stage IP. We demonstrated that acute phase response of bovine IP was evident and therefore an appropriate analgesic should be administered in the treatment of affected animals. The response was most apparent in herds with high morbidity in IP and with a bacterial infection comprising *Fusobacterium necrophorum* and *Dichelobacter nodosus*, indicating that combination of these two bacterial species affect the severity of the disease.

1. Introduction

Several Finnish dairy herds have recently suffered from outbreaks of interdigital phlegmon (IP). The affected herds have been naïve – with no previous history of IP. It is also evident that an apparent trauma in the interdigital cleft was not detected prior the cases of IP. In these outbreaks, morbidity of IP has usually been high and clinical signs severe, resulting in sizeable economic losses for affected herds [1]. Prior to these new type outbreaks, infectious hoof diseases were quite rare in Finland.

Most countries with a modern dairy industry have experience with infectious hoof diseases, of which digital dermatitis (DD) is currently considered the most problematic [2,3]. However, cases of IP also appear regularly [4,5] and there are a few earlier reports of outbreaks of IP [6,7]. Typically, the clinical signs of IP are lameness, a symmetric swelling of the interdigital area and the bulbs of the heels, and a fetid odor. A fissure with swollen protruding edges can appear along the interdigital cleft. In severe cases, systemic signs, including fever, recumbency or anorexia appear [8,9]. IP reduces milk yield [10] and can lead to early culling of affected cows [11].

Fusobacterium necrophorum is considered to be a major pathogen of IP [12–14], although in the disease process several other bacteria, including *Dichelobacter nodosus, Porphyromonas levii, Prevotella melaninogenica, Treponema* species and *Trueperella pyogenes* play a probable role [8,13,15–17]. In our recent study, we frequently detected both *F. necrophorum* and *D. nodosus* in IP, and *D. nodosus* was associated with higher morbidity [14].

Several review articles describe an acute phase response (APR) as an early systemic, non-specific response of the host to infection, inflammation or trauma, with the purpose of restoring normal body functions [18–22]. APR includes multiple metabolic, endocrinal and hematological changes, and it is often expressed by clinical signs – fever, anorexia, apathy and pain [18]. APR is induced by cytokines acting as messengers between the local sites of injury and leads to the hepatic synthesis of acute phase proteins (APP) [19–21]. APPs are likely to participate directly in the protection of the host [20]. APPs are a measurable element of APR, and are classified as positive or negative, and according to the magnitude of their increase as minor, moderate or major [19–21].

Reports of APPs in hoof diseases and lameness have been published

Abbreviations: Alb, albumin; APP, acute phase protein; APR, acute phase response; DD, digital dermatitis; HHE, heel horn erosion; ID, interdigital dermatitis; IP, interdigital phlegmon; Hp, haptoglobin; SAA, serum amyloid A

* Corresponding author.
E-mail addresses: miia.kontturi@helsinki.fi (M. Kontturi), reijo.junni@gmail.com (R. Junni), minna.kujala-wirth@helsinki.f (M. Kujala-Wirth), erja.malinen@welho.com (E. Malinen), eijaseuna@gmail.com (E. Seuna), sinikka.pelkonen@foodauthority.fi (S. Pelkonen), timo.soveri@helsinki.fi (T. Soveri), heli.simojoki@helsinki.fi (H. Simojoki).

https://doi.org/10.1016/j.cimid.2019.101375
Received 19 June 2019; Received in revised form 13 October 2019; Accepted 23 October 2019

previously [23–25]. These studies mainly include detection of two major APPs in cattle: serum amyloid A (SAA) and haptoglobin (Hp). Also, some studies of albumin (Alb), a negative APP, exist [26]. However, studies that include cases of infectious hoof diseases and APR are scant [27–29]. Thus, we investigated the association between clinical signs and APR in IP, to evaluate the degree of inflammation in naturally occurring IP. Furthermore, we investigated the characteristics of these new type outbreaks of IP and tested our hypothesis that presence of *D. nodosus* alongside *F. necrophorum* affects the severity of IP.

2. Materials and methods

We carried out a research project on infectious hoof diseases in Finland in 2012–2015. As a part of the project we made a cross-sectional study, visiting several dairy herds affected by outbreaks of IP and control herds.

2.1. Study herds

The study herds, selection of sampled animals, and sampling are described in detail in our previous study [14]. One outbreak herd was excluded from the current study due to missing data and dissimilar barn construction; manure pack flooring in a non-insulated free stall. Only cows with complete blood sample analysis were included in the study.

We collected samples from 21 commercial free stall dairy herds. Eighteen herds suffered from an outbreak of IP (IP herd) and three herds had been free of IP for at least a decade. The criteria for visiting an outbreak of IP herd were; at least three cows affected in one week, and no previous cases of IP in the herd for at least ten years. The outbreak herds were further divided into two categories, herds with high morbidity in the first two months of the outbreak (morbidity ≥50 %) and herds with moderate morbidity (9–33%).

The study herds were located in three large provinces in Finland: western (11 farms), eastern (7) and southern (3). The average herd size was 76 lactating cows (range 37–140, median 62) and the average milk yield of the herds was 9207 kg (8,000–10,914 kg, median 9219). All free stalls were constructed or extended at most seven years before the farm visit. Cows were milked twice a day in a milking parlor in 4 herds, whereas 16 herds were milked using an automatic milking system, and one herd had both. The alleys had slatted concrete floors in 10 barns, slatted floors with rubber in 3, solid with rubber in 7 and in 1 barn solid concrete. During summer, 4 herds had access to pasture, 2 herds access to an outdoors pen and of these 6 herds 2 had access outdoors during winter. A total or partial mixed ration was fed in 13 herds whereas in 8 herds concentrates and silage were fed separately.

During the farm visits we collected background data on barn construction and herd management. An additional checkup was made a year after the visit by phone interview.

2.2. Selection and clinical inspection of the animals

The cows were selected for sampling on the grounds of lameness, prolonged lying-time, or a trouble report from an automatic milking system. All feet were carefully inspected in a trimming chute. Primarily, samples were collected from cows that had IP. If several feet of a cow were affected, the foot with the most severe lesion in the interdigital cleft was selected for sampling. The bacteriological samples were taken from the lesion site. The IP lesions were further classified as acute infection (hereafter termed acute IP) or a healing stage of IP (healing IP). The diagnosis of IP was made according to Gupta et al. [8]: acute IP was diagnosed if the distal foot near the claws was swollen symmetrically with or without a fissure detected in the interdigital cleft, and the healing IP was defined by proliferation tissue or scar formation being apparent in the affected region. Some IP cows had also other hoof diseases, like heel horn erosion (HHE), DD or white line disease. If IP was considered the main reason for lameness the cow was sampled as IP cow.

Some samples were taken from cows that suffered from DD (n = 14). DD diagnosis was made according to Döpfer et al. [30]. All DD lesions represented either stage M1, M2 or M3 and were less than 3 cm in diameter. If observed, we also sampled cows with other hoof diseases, including interdigital dermatitis (ID), sole ulcer, or white line abscess (hereafter termed Other, n = 34).

The study cows were checked for lameness and classified into lame or non-lame categories (0/1) while standing or walking to and from the trimming chute. Cows were scored according to Sprecher et al. [31] so that the category of non-lame cows included cows 1–2 and lame cows 3–5 on this scale. Their body temperature was measured and previous antimicrobial treatments from one month before the herd visit were recorded. A separate form was used to enter all data relating to the cows.

The control cows in both herd types, IP herds and IP-free herds, were selected from non-lame, non-pyretic (T ≤ 39.2 °C), otherwise clinically healthy cows. Their feet were lifted in a trimming chute, and those with no signs of IP, DD, ID, sole ulcer or white line disease were selected as control cows. Occasionally on some farms, it was impossible to find healthy control cows without any hoof diseases. Therefore, a mild HHE i.e. superficial loss of heel horn as categorized 0 on a scale of 0/1 by Manske et al. [32], was accepted on some control cows. In outbreak herds, the control cows are later referred to as control cows (IP herd), and in herds with no outbreak of IP, as control cows (IP-free herd). All the diagnoses were made by one of two experienced veterinarians and were based on clinical signs only. All feet were photographed in a trimming chute and diagnoses were standardized between the two veterinarians by studying the photographs together.

In this study, a total of 203 cows were sampled: 117 (57.6 %) were Ayrshire and 86 (42.4 %) Holstein. Moreover, 95 (46.8 %) were heifers or first parity cows, 46 (22.7 %) second parity and 57 (28.1 %) third or more parity cows. The lactation stage data for sampled cows were: 71 (35.0 %) early lactation (1–120 DIM), 84 (41.4 %) late lactation (121–305 DIM) and 43 (21.2 %) either over 305 DIM, dry cows or heifers. All study heifers (n = 8) were in late pregnancy, 0–2 months prior to calving. The information on parity and lactation stage was missing for 5 (2.5 %) cows.

2.3. Blood samples and analysis of acute phase proteins

We collected the blood samples (9 ml Z Serum Clot Activator, Vacuette, Austria) from jugular or mammary veins for subsequent analysis of APPs. The whole blood samples were centrifuged at 3500 rpm for 15 min, serum was placed in a 2 ml tube (Sarstedt, Germany) and frozen at – 20 °C within 24 h of sampling.

We analyzed SAA, Hp and Alb. Serum SAA concentration was measured with a commercially available solid phase sandwich ELISA kit (Phase TM Range Multispecies SAA ELISA kit, Tridelta Development Ltd., Ireland) according to the manufacturer's instructions for bovines. The lower detection limit was 9.4 μg/ml and all samples with concentrations < 9.4 μg/ml were set to 9.4 μg/ml. Serum Hp concentration was determined using a hemoglobin-haptoglobin binding assay (Phase TM Range Haptoglobin kit, Tridelta Development Ltd., Ireland). Spectrophotometric methods were used for the determination of Alb [33] and were performed with an automatic chemistry analyzer (KONE Pro, Thermo Fisher Scientific, Finland).

2.4. Bacteriological sampling and analysis of hoof samples

The sampling methods, and the results of the bacteriological analyses are described in detail in Kontturi et al. [14]. Briefly, the sampling was performed in a trimming chute and the affected foot was lifted and the two claws were spread with an extensor. The hoof region was cleaned carefully with a hose, rinsed with saline, and dried with gauze. The cytobrush samples were taken from the inflamed region – samples

from IP cows were collected primarily from a lesion in the interdigital cleft.

The primary culture was performed on the farm and further analysis and PCR in a laboratory of the Finnish Food Authority, Helsinki. The species detected in the study were: *D. nodosus, F. necrophorum, P. levii, P. melaninogenica, Treponema* spp and *T. pyogenes*. In most of the acute IP samples (66.7 %), both *F. necrophorum* and *D. nodosus* were detected and a significant association was established between *D. nodosus* in IP lesions and high morbidity outbreak in the herd [14]. Therefore, we tested the effect of *F. necrophorum* and *D. nodosus* together, in comparison with *F. necrophorum* without *D. nodosus* on APPs in samples from acute IP cows.

2.5. Statistical analysis and models

Data recorded during the herd visits and during the phone interviews, the APP values and bacteriological data were collated in Excel spreadsheets. The statistical analyses were carried out using Stata IC version 15 (Stata Corporation, Texas, USA).

The study animals were divided into six disease categories: 1) control cows (IP-free herd), 2) control cows (IP herd), 3) acute IP, 4) healing IP, 5) DD and 6) other. Possible antimicrobial treatment of the cows was divided into three categories; 1) no current or previous antimicrobial treatment during last month, 2) current antimicrobial treatment or treatment within 6 days before sampling and 3) previous treatment with antimicrobials within 7–30 days prior to sampling. The lactation stage was categorized into three groups: 1–120 DIM, 121–305 DIM and > 305 DIM, in which we also included heifers and dry cows. Moreover, the parity of the cows was categorized into three groups: 1) heifers and first parity 2) second parity and 3) third or more parity cows. The breed of the cows was a dichotomous variable, 0) Ayrshire and 1) Holstein.

Each APP was analyzed separately. The values were described by means, standard errors and 95 % confidence intervals. A *T*-test with unequal variances was performed within various animal groups when comparing mean APP values of cows in high and moderate morbidity and control herds, and when comparing mean APP values for acute IP in *F. necrophorum* positive cows with or without *D. nodosus*. A *P*-value < 0.05 was considered statistically significant.

We studied the association of APPs and various disease categories. The values of SAA and Hp did not adhere to a normal distribution and were transformed into a 0 /1 variable. The 90 % values of both groups of control cows (IP-free herd and IP herd) were used as a reference value for a healthy animal. The cut-off values were 80 μg/ml for SAA, and 0.27 g/l for Hp. Logistic mixed models were used to study the association of SAA and Hp and various disease categories. The values for Alb were normally distributed and a regression mixed model was applied. A possible antimicrobial treatment, DIM, parity and breed were considered as confounding variables and were kept in all models, and the herd was included as a random factor. From a total of 203 cows, 198 had a complete dataset and were included in statistical models.

We tested all biologically plausible interactions but detected no significant association. The models were evaluated by sensitivity and specificity test and roc-curve of the model. The assumptions of the model were controlled by normality and scatter plots of the residuals.

2.6. Ethical review

Viikki Campus Research Ethics Committee of Helsinki University reviewed and approved our research protocol. A written informed consent to use the animals in our study was obtained from the herd owners before sampling. After sampling, the farm veterinarian treated IP and DD cows.

3. Results

3.1. Characteristics of the IP outbreak herds and sampled cows

During the first two months of the IP outbreak, the morbidity was high (≥50 %) in 7 herds, moderate (9–33%) in 11 herds, and no herd had an intermediate morbidity. In 6 herds (33.3 %), also cattle less than 2 years old i.e. calves or young heifers had IP cases. In 11/18 herds (61.1 %), 1–3 cows had to be culled because of IP. The highest culling rate was 12.2 % (11/90) of the current herd average. Of all producers, 15 (83.3 %) felt that cows were cured well of IP with parenteral antimicrobial treatment. Approximately one year after the outbreak, 8 herds (44.4 %) still regularly had new sporadic cases of IP.

Several events were reported for the study herds before the IP outbreaks. All 18 outbreak herds included newly purchased cattle during the year before the outbreak; 2 herds (11.1 %) included fewer than five animals, whereas 16 (88.9 %) more. An enlargement of the barn or construction of a new barn had taken place on 12 farms (66.7 %) in the two years before the outbreak. A change in feeding had occurred within one month before the outbreak on 12 farms (66.7 %). Eleven producers (61.1 %) reported that feces of the milking cows had been either too watery or had contained undigested feed particles before the outbreak. Either one or both of these feeding related problems had occurred in 16 farms (88.9 %). In the compartment of milking cows, 2 herds (11.1 %) were overstocked and 6 producers (33.3 %) reported problems with the cow flow. Additionally, 7 producers (38.9 %) reported at least minor problems in ventilation systems. For 13 outbreak herds (72.2 %), producers were unsatisfied with the cleanliness of the alleys, and for 10 herds (44.4 %) with the cleanliness of the cows.

The descriptive statistics for the study cows, their clinical signs and possible antimicrobial treatment is presented in Table 1. At the time of sampling, 148 (72.9 %) of all sampled cows were not treated with antimicrobials, 35 (17.2 %) had current, and 20 (9.9 %) previous antimicrobial treatment.

3.2. Acute phase response

The increase of SAA and Hp and decrease of Alb was evident in acute IP cows. Table 2 contains the means, standard errors and 95 % confidence intervals of APPs in various disease groups.

In the analyses of APPs, the upper limit for reference value for SAA was 80 μg/ml, and 0.27 g/l for Hp. The probability of SAA and Hp exceeding the reference value with acute IP cows was apparent (Table 3). Table 3 presents the results of logistic mixed models of SAA and Hp, and Table 4 the regression mixed model of Alb. The herd affected values for Hp ($P = 0.05$) and Alb ($P = 0.02$).and a trend existed for SAA ($P = 0.06$).

3.3. APP values in high and moderate morbidity herds

We compared the APP values in various disease categories among herds of various morbidity and control herds. The mean values for SAA and Hp of acute IP were elevated in herds of high morbidity in comparison with moderate morbidity herds ($P < 0.01$). Fig. 1 shows the mean values and standard errors of APPs in various disease categories in high morbidity, moderate morbidity and control herds (IP-free herds).

Furthermore, we compared various APPs of acute IP cows with *F. necrophorum* and *D. nodosus* (n = 31) and *F. necrophorum* without *D. nodosus* (n = 13) in high and moderate morbidity herds (n = 44). The mean value and SE for SAA was 450.7 μg/ml ± 59.3 incows with *F. necrophorum* and *D. nodosus*, and 140.2 μg/ml ± 41.1 in cows with *F. necrophorum* without *D. nodosus*. The mean values for Hp were 2.85 g/l ± 0.4 and 1.54 g/l ± 0.4, and Alb 36.4 g/l ± 0.6 and 35.7 g/l ± 0.7, respectively. The values for Alb did not differ between acute IP

Table 1
Descriptive data for the sampled cows (N = 203); affected foot, clinical signs and possible antimicrobial treatment prior to sampling in various disease groups[1]. Number of animals and the percentage in each group is presented.

Variable	Control (IP free herd)	Control (IP herd)	Acute IP	Healing IP	DD	Other
Cow	19 (9.4%)	42 (20.7%)	60 (29.6%)	34 (16.7%)	14 (6.9%)	34(16.7%)
Front feet[2]	0 (0%)	5 (2.5%)	11 (5.4%)	3 (1.5%)	1 (0.5%)	3 (1.5%)
Hind feet[2]	19 (9.3%)	37 (18.2%)	49 (24.1%)	31 (15.3%)	13(6.4%)	31 (15.3%)
Clinical signs						
Lameness	0/19 (0%)	0/42 (0%)	36/53(689%)[3]	16/29 (55.2%)[3]	3/13 (23.1%)[3]	8/32 (25.0%)[3]
Swelling	0/19 (0%)	0/42 (0%)	57/59 (96.5%)[3]	31/34 (91.2%)	4/14 (28.6%)	13/33 (39.4%)[3]
Odor	0/19 (0%)	0/42(0%)	8/43 (18.6%)[3]	0/26 (0%)[3]	4/9 (44.4%)[3]	0/27 (0%)[3]
Antimicrobial treatment						
None	19/19 (100%)	42/42 (100%)	35/60 (58.3%)	6/34 (17.7%)	12/14 (85.8%)	34/34 (100%)
Current	0/19 (0%)	0/42 (0%)	19/60 (31.7%)	15/34 (44.1%)	1/16 (7.1%)	0/34 (0%)
Previous	0/19 (0%)	0/42 (0%)	6/60 (10.0%)	13/34 (38.2%)	1/16 (7.1%)	0/34 (0%)

[1] Control cows with no outbreak of interdigital phlegmon (IP-free herd), control cows in herds with an outbreak of interdigital phlegmon (IP herd, acute IP, healing stage of IP, digital dermatitis (DD) and other hoof diseases (Other). In a group of other hoof diseases were cases of interdigital dermatitis, sole ulcer, and white line abscess. IP = interdigital phlegmon.
[2] Percentage of all sampled feet.
[3] Missing data for some sampled cows.

Table 2
The number of cows, means, standard errors and 95 % confidence intervals for serum amyloid A, haptoglobin and albumin in blood samples from cows in the various disease groups[1].

	n	Mean	SE	95% CI
Serum amyloid A (μg/ml)				
Control (IP-free herd)	19	52.59	16.49	20.08–85.10
Control (IP herd)	42	33.00	6.13	20.83–45.02
Acute IP	60	308.05	38.65	231.83–384.27
Healing IP	34	74.12	18.50	37.65–110.60
DD	14	90.91	40.70	10.66–171.15
Other	34	89.33	19.40	51.07–127.59
Haptoglobin (g/l)				
Control (IP-free herd)	19	0.23	0.07	0.08–0.37
Control (IP herd)	42	0.16	0.03	0.11–0.21
Acute IP	60	2.15	0.25	1.66–2.64
Healing IP	34	0.50	0.13	0.25–0.76
DD	14	0.17	0.29	0.11–0.23
Other	34	0.55	0.15	0.24–0.87
Albumin (g/l)				
Control (IP-free herd)	19	39.54	0.58	38.39–40.69
Control (IP herd)	42	37.79	0.38	37.04–38.55
Acute IP	60	35.91	0.36	35.20–36.62
Healing IP	34	36.76	0.44	35.90–37.63
DD	14	37.70	0.59	36.53–38.86
Other	34	37.50	0.39	36.72–38.28

n = number of cows, SE = standard error; 95 % CI = 95 % confidence interval; IP = interdigital phlegmon.
[1] Control cows with no outbreak of interdigital phlegmon (IP-free herd), control cows in herds with an outbreak of IP (IP herd), IP in acute stage (acute IP) and healing stage (healing IP), digital dermatitis (DD) and other hoof diseases (other).

cows from herds of various morbidities. Fig. 2 shows the results for SAA and Hp in both morbidity categories. Statistical difference existed between acute IP cows with *F. necrophorum* and *D. nodosus* in high (n = 21) and moderate morbidity (n = 10) herds in SAA ($P < 0.01$) and in haptoglobin ($P < 0.05$) values.

4. Discussion

We investigated the association among clinical signs, bacteriological findings and APPs to evaluate the degree of infection in naturally occurring IP. The study cows with clinical signs of acute IP had clearly elevated levels of SAA and Hp, and decreased concentrations of Alb, the negative APP, when compared with clinically healthy control cows. Our findings show that IP causes a strong APR, which was more evident than for DD and other hoof diseases included in this study, and other causes of lameness previously investigated [23–25]. Furthermore, the APR was more distinguishable in herds with high morbidity of IP (> 50 %) in comparison with herds of moderate morbidity (9–33%). This could indicate presence of a more virulent agent in high morbidity herds, a higher infection pressure or a weaker immunity. Moreover, the acute IP cows with both *F. necrophorum* and *D. nodosus* had more elevated concentrations of SAA and Hp than acute IP cows with *F. necrophorum* without *D. nodosus*, and the values were further increased in cows of high morbidity herds. In our earlier study, we demonstrated that in most of the acute IP samples, both *F. necrophorum* and *D. nodosus* were detected and a significant association was established between *D. nodosus* in IP lesions and a high morbidity outbreak in the herd [14]. Thus, our new findings for APR in IP support our hypothesis of *D. nodosus* affecting the severity of IP. Previously, *D. nodosus* was detected in healthy hooves [34] and was associated with ID [34] and DD [34–36]. Rasmussen et al. [37] speculated that *D. nodosus* could break down the epidermal barrier, creating a suitable environment for secondary invaders. *F. necrophorum* is regarded as being the major pathogen in IP [12–14]. However, there remain difficulties in establishing absolute clarity for the role of other bacteria than *F. necrophorum* in the pathogenesis of IP and further studies on IP bacteriology and on virulence of both *F. necrophorum* and *D. nodosus* are merited.

The values for SAA and Hp were considerably increased in the acute stage of IP. This was expected because SAA and Hp are major APPs in cattle [19,21]. The current understanding is that SAA is the first line and Hp the second line APP; first line APPs are primarily induced by IL-1 type cytokines whereas second line APPs are induced by IL-6 type [18,20]. Because we do not know the exact date of the IP onset at the time of sampling, our cases of IP, especially the group of healing IP, can be somewhat divergent. This may be the reason why we observed only a trend ($p = 0.06$) among Hp values in our group of healing IP cows i.e. during the later stage of IP. Horadagoda et al. [38] postulated that an increase in both SAA and Hp in comparison with an increase solely in SAA could indicate that a greater inflammatory reaction has occurred.

Alb is a major plasma protein, featuring the plasma colloidal osmotic pressure and being associated with the balance of body fluids [39,40]. Hypoalbuminemia is detected in several pathologic processes or in physiological conditions [40]. During APR, synthesis of Alb is probably down-regulated and amino acids are used for the synthesis of the positive APPs [18]. In our study cows, the values of Alb were decreased in acute IP, but they were also affected by DIM and parity. Also feeding is known to influence the blood albumin levels in cattle [41,42]. Thus, albumin alone cannot be regarded as a useful marker of infection in a single cow.

Our previous survey detected animal purchase and enlargement of

Table 3
The final logistic mixed models for serum amyloid A to elevate over 80 μg/ml and haptoglobin to elevate over 0.27 g/l with cow-level factors and herd as a random factor[1] (N = 198).

	Serum amyloid A				Haptoglobin			
	OR	*P*-value	95% CI.	Wald	OR	*P*-value	95% CI.	Wald
Disease category				< 0.01				< 0.01
Control (IP herd)	1				1			
Control (IP-free herd)	1.63	0.63	0.23–11.76		0.86	0.89	0.09–7.61	
Acute IP	38.16	< 0.01	9.08–160.37		37.93	< 0.01	8.98–160.29	
Healing IP	2.59	0.31	0.41–16.16		4.96	0.08	0.83–29.51	
DD	6.42	0.05	0.98–42.08		2.83	0.29	0.41–19.54	
Other	3.48	0.09	0.84–14.38		3.58	0.08	0.86–14.82	
Antimicrobial treatment				0.24				0.19
None	1				1			
Current	1.29	0.71	0.34–4.84		2.62	0.18	0.64–10.61	
Previous	0.26	0.16	0.04–1.68		0.71	0.69	0.13–3.87	
DIM				0.17				0.14
1–120	1				1			
121–305	0.41	0.06	0.16–1.05		0.37	0.05	0.14–0.99	
> 305	0.55	0.27	0.19–1.59		0.65	0.43	0.23–1.87	
Parity				0.29				0.37
≤1st	1				1			
2nd	1.06	0.91	0.37–3.04		0.80	0.68	0.27–2.33	
≥3rd	2.12	0.13	0.80–5.64		1.76	0.25	0.68–4.65	
Breed								
Ayrshire	1				1			
Holstein	0.43	0.06	0.17–1.04		0.70	0.44	0.05–1.71	
Constant	0.42	0.31	0.08–2.27		0.27	0.13	0.05–1.47	

IP = interdigital phlegmon; OR = odds ratio; 95 % CI = 95 % confidence interval; Wald = Wald-test was used to test the overall *P*-value of the variable.
[1] The disease categories were control cows with an outbreak of interdigital phlegmon (IP herd), control cows in herds with no outbreak of IP (IP free herd), IP in acute stage (acute IP), and healing stage (healing IP), digital dermatitis (DD) and other hoof diseases (Other). In a group of other hoof diseases were cases of interdigital dermatitis, sole ulcer, and white line abscess.

Table 4
The final regression mixed model for albumin in various disease categories with cow-level factors and herd as a random factor[1] (N = 198).

	Coefficient	*P*-value	95% CI	Wald
Disease category				< 0.01
Control (IP herd)	1			
Control (IP-free herd)	1.23	0.12	−0.30 to 2.77	
Acute IP	−1.85	< 0.01	−2.90 to -0.81	
Healing IP	−0.92	0.20	−2.31 to 0.47	
DD	−0.76	0.34	−2.32 to 0.79	
Other	−0.82	0.14	−1.68 to 0.52	
Antimicrobial treatment				0.53
None	1			
Current	−0.60	0.30	−1.68 to 0.52	
Previous	−0.06	0.94	−1.51 to 1.39	
DIM				< 0.01
1–120	1			
121–305	1.62	< 0.01	0.99 to 2.34	
> 305	1.66	< 0.01	0.76 to 2.55	
Parity				0.04
≤1st	1			
2nd	0.76	0.07	−0.05 to 1.58	
≥3rd	0.90	0.03	0.11 to 1.68	
Breed				
Ayrshire	1			
Holstein	0.51	0.16	−0.20 to 1.21	
Constant	35.88	< 0.01	34.57 to 37.20	

IP = interdigital phlegmon; 95 % CI = 95 % confidence interval; Wald = Wald-test was used to test the overall *P*-value of the variable.
[1] The disease categories were control cows with an outbreak of interdigital phlegmon (IP herd), control cows in herds with no outbreak of IP (IP-free herd), IP in acute stage (acute IP), and healing stage (healing IP), digital dermatitis (DD) and other hoof diseases (Other). In a group of other hoof diseases were cases of interdigital dermatitis, sole ulcer, and white line abscess.

the barn as risk factors for an outbreak of IP to occur [43]. Similarly, every outbreak herd in the current study included newly purchased animals within one year before the outbreak, and 12 (66.7 %) herds had undergone an enlargement or construction of the barn during the two years before the outbreak. Interestingly, in this study, feeding-related problems for several IP herds (88.9 %) were reported just prior to the outbreak. Because of the low number of herds in this study, risk factors could not be investigated, and further research should be performed to confirm this speculation.

Previous research on APR on hoof diseases has typically included various diagnoses in the same study. Kujala et al. [23] reported elevated SAA concentrations in 16 dairy cattle with either sole ulcer, white line disease or both, but did not detect any variation in Hp values between lame and control groups. Tóthová et al. [26] had a group of 35 lame heifers with various causes of lameness and was able to detect a difference between lame and non-lame groups in SAA and Hp, but not in Alb. Tadich et al. [24] studied lame dairy cows and revealed elevated concentrations of Hp for all levels of lameness (locomotion score > 1), when locomotion was scored on a scale of 1– 5. O´Driscoll et al. [25] investigated differences in metabolite status of dairy cows with or without sole ulcer and detected higher Hp levels for lame cows.

Earlier studies of infectious hoof diseases and APR are scant. A study of Smith et al. [27] included 7 IP cases, all with elevated Hp concentrations compared with controls. An Iranian study [28] measured Hp and SAA in a lameness group, a *F. necrophorum*-positive lameness group and a healthy group, and established a significant difference for values of both lameness groups when compared with controls. The cows were diagnosed as ID in the study, but the description of the signs of the affected cows in the article resembles that of IP as described by Gupta et al. [8]. The nomenclature of infectious hoof diseases has varied and therefore, interpretation of older results is occasionally difficult. Tóthová et al. [29] detected significantly lower Alb values in 23 dairy cows with hoof diseases than in controls. In their study hoof diseases included cases of DD, pododermatitis, laminitis and sole ulcer.

Our study design depended on occurrence of IP outbreaks. Due to long distances between farms in Finland, it was occasionally difficult to reach a farm on time and get enough samples from cows during the early acute stage of IP and which had not yet been treated with

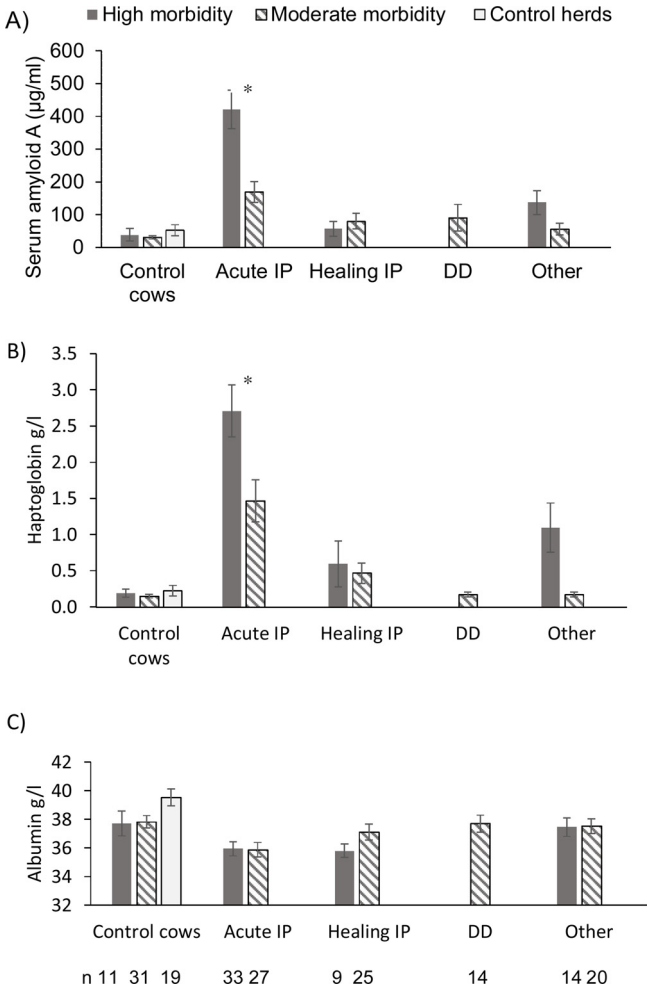

Fig. 1. The mean values and standard errors for A) serum amyloid A, B) haptoglobin and C) albumin in various disease categories in high and moderate morbidity outbreak herds and control herds. The disease categories were control cows, acute interdigital phlegmon (Acute IP), healing stage of IP (healing IP), digital dermatitis (DD) and a group of other hoof diseases (Other) that included cases of ID, sole ulcer and white line abscess. The number of sampled animals (n) in each group is presented under the columns of albumin values. Statistical difference is indicated with * ($P < 0.01$).

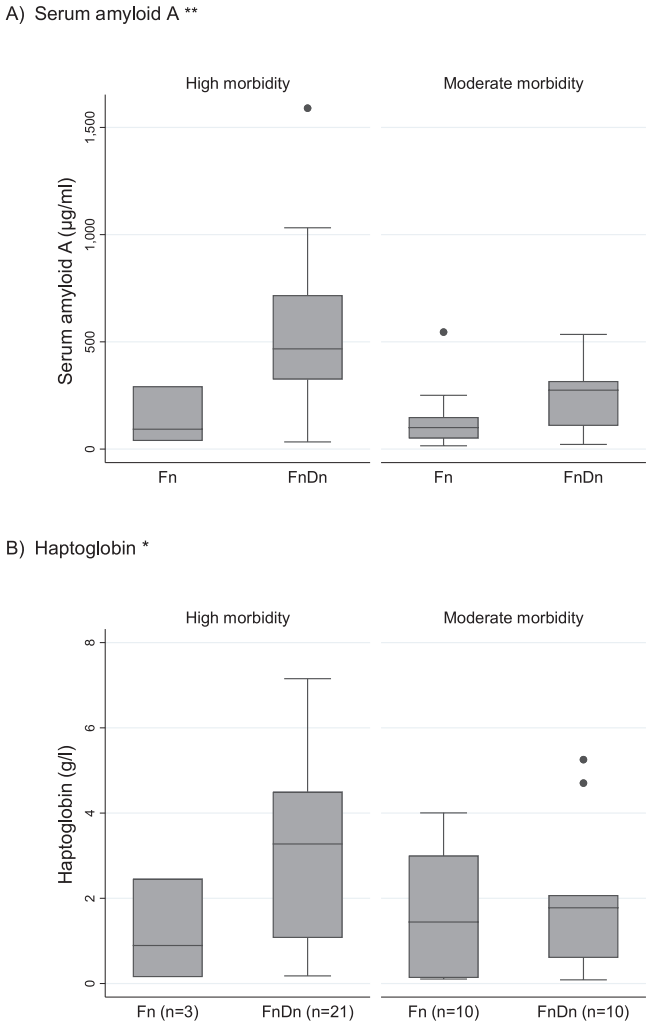

Fig. 2. The concentrations of A) serum amyloid A and B) haptoglobin in high and moderate morbidity herds of cows suffering from acute interdigital phlegmon with *Fusobacterium necrophorum* (Fn) and with *F. necrophorum* and *Dichelobacter nodosus* (FnDn) The number of sampled animals in each group is presented under the columns of haptoglobin values. In box and whiskers plots, the median is represented as a line that divides the box into two parts. The box represents the mid 50 % of the values while the whiskers scores outside the mid 50 %. Statistical difference existed between FnDn cows in high and moderate morbidity herds in SAA ($P < 0.01$) and in haptoglobin ($P < 0.05$) values.

antimicrobials. Considering the severe clinical signs of the cows, the treatment was not delayed for study purposes. However, in both high and moderate morbidity herds we were able to collect almost the same number of samples from acute IP cows. In each herd, we only examined and sampled cows on a single day and thus we did not follow up the healing process of an individual cow. The diagnosing was based on clinical signs only and was occasionally challenging. Even though the cows were categorized as IP or DD cows, in some cases also other hoof diseases may have been present. The control cows were chosen among clinically healthy cows in each herd. However, it is possible some of these cows might have experienced a subclinical disease at the time of sampling.

These results suggest that SAA and Hp could be used in the determination of the severity of IP infection and to a certain extent they can add information on predicting the possible outcome of the disease. Furthermore, these results should be borne in mind when deciding on the treatment of a cow suffering from IP. Considering the strong APR, IP cows should be treated as quickly as possible and an appropriate analgesic should be administered in addition to antimicrobials.

Funding

This study was financially supported by the Ministry of Agriculture and Forestry project (2066/312/2011) for infectious hoof diseases in new free stalls in Finland. Valio Ltd, the Mercedes Zachariassen Foundation, the Walter Ehrström Foundation the Finnish Foundation of Veterinary Research and Helsinki University doctoral programme in Clinical Veterinary Medicine are acknowledged for their financial support in the analysis and interpretation of data and the writing of the manuscript.

Authors' contributions

All authors participated in planning the study. MK, RJ, HS and MKW took the hoof samples. EM and ES performed the PCR of the study samples. MK and HS performed the statistical analyses and MK drafted the manuscript. All authors commented, read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank the study farms, veterinarians, hoof trimmers and others who helped during the farm visits. We also thank Taina Rahkonen and Merja Pöytäkangas at the University of Helsinki for the analysis of APPs.

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