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Beta defensins as biomarkers: detectable in LPS-stimulated equine peripheral blood mononuclear cells and normal, aseptic, and probable septic equine synovial fluid

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OBJECTIVES
Diagnosis of equine septic arthritis is not straightforward, and increasing time between onset, diagnosis, and treatment can have serious consequences for quality of life. Defensins are used in diagnosis of human joint infection. The presence of beta defensins (BDs) in equine synovial fluid and their utility as a biomarker of sepsis has not been investigated; therefore, our objectives were to (1) compare in vitro gene expression of lipopolysaccharide (LPS)-stimulated equine neutrophils to unstimulated neutrophils and (2) compare BD protein expression from normal, aseptically inflamed, and septic equine joints.

ANIMALS
5 horses for isolated neutrophil BD expression and 21 synovial fluid samples from 14 horses.

PROCEDURES
RT-qPCR analysis was performed for BD gene expression of stimulated and unstimulated equine peripheral neutrophils. BD protein expression was evaluated from equine joints with no disease, aseptic inflammation, and septic inflammation using a commercial ELISA designed for horses and analyzed with a Kruskal-Wallis test (significant at $P < .05$).

RESULTS
A significant increase was noted in expression of BD-3 in LPS stimulated as compared to unstimulated neutrophils. There were no significant differences in BD expression noted between joints with no disease, aseptic inflammation, and septic inflammation. Low case numbers and different types of cases in the aseptic inflammation group were main limitations. BD expression patterns in samples from stimulated equine peripheral neutrophils and synovial fluid were identified.

CLINICAL RELEVANCE
BDs are detectable in equine synovial fluid and can be stimulated from peripheral neutrophils. Further examination is needed to define their role as biomarkers of joint disease.

Septic arthritis is common in horses of all ages and can result in career ending and life-threatening lameness. Infection occurs when bacteria enter the joint, commonly through hematogenous spread or through a penetrating wound,[1] or less commonly through administration of intra-articular therapies or surgery.[2,3] Bacterial invasion of joints instigates a rapid and robust inflammatory response and results in significant, acute pain for the animal. The presence of inflammatory cells and cytokines within the joint can also lead to the breakdown of normal joint architecture, development of osteoarthritis, and chronic pain.[4] Obtaining a rapid diagnosis in these cases is important to institute timely and appropriate treatment as increasing the time between onset of disease, diagnosis, and treatment increases the risk of permanent joint dysfunction. Diagnosis of septic arthritis is not straightforward and is based on clinical signs coupled with cytologic analysis of synovial fluid. The period from initial insult to presentation of clinical signs can vary with the quantity and virulence of pathogens introduced[5] and with

medications, particularly corticosteroids, administered into the affected joint.[6] Synovial fluid analysis can also be ambiguous, particularly when values for total white blood cell count and total protein fall into an "intermediate range" between normal and abnormal. A positive bacterial culture of synovial fluid is required for definitive diagnosis[2]; however, positive culture results only occur in approximately 50% to 60% of clinical cases.[7,8] Preliminary culture results can take 48 to 72 hours to be acquired, and negative results do not preclude sepsis. Due to the limitations of current diagnostics and poor outcomes with disease if not treated in a timely manner, there is a critical need to identify biomarkers that can provide a more accurate diagnosis of septic arthritis and initiation of appropriate treatment.

Human periprosthetic joint infection presents similarly to septic arthritis in horses. Clinical signs and diagnostic testing can be suggestive but not definitive for infection; however, diagnostic investigation often yields equivocal results.[9,10] Defensins are cysteine-rich proteins of the innate immune system,[11] have antimicrobial properties, and have been found in humans to be highly sensitive and specific for identification of periprosthetic joint infection.[9,12,13] Beta defensin-3 expression has been detected from human bone and synovial tissue with periprosthetic joint infection. Levels within these tissues were found to be different between tissues of periprosthetic joint infection and those with inflammation due to aseptic implant loosening.[14]

Human beta defensins (BDs) and equine BDs are homologous, which allows comparison between species.[11] In horses, BD-1 expression has been examined and recognized in a variety of tissues including the liver, heart, spleen, kidney, gastrointestinal tract, and reproductive tissues.[15–17] The presence of BD has not been examined in equine blood, neutrophils, synovial fluid, or synovial tissue but has been found in both bovine neutrophils[18] and human blood filtrate.[19] Neutrophils are the predominant cell type in septic synovial fluid and have been used as an *in vitro* model of the innate immune response in the horse, by stimulating them with LPS to activate them to model an endotoxic disease process.[20,21] Evaluation of neutrophils in isolation for the production of BDs is a simpler system to assess their possible role in the horse in a cell type of importance in a septic joint.

Our first objective was to identify and compare expression of BDs in nonstimulated and lipopolysaccharide (LPS) stimulated equine neutrophils. Our second objective was to identify and compare expression of BDs in equine synovial fluid from joints with no disease, aseptic inflammation, and septic inflammation. Our first hypothesis was that BD expression would be present in nonstimulated blood neutrophils and that LPS stimulation would increase expression levels. The second hypothesis was that equine BD expression would be present at low levels in normal joints and joints with aseptic inflammation and present at high levels in joints with septic inflammation.

Methods

All experiments were approved by Michigan State University's Institutional Animal Care and Use Committee. Informed owner consent was obtained where appropriate.

Experiment 1

Overview

Equine whole blood was collected from 5 healthy adult research horses. RT-qPCRs were then used to identify gene and protein expression of equine BDs in nonstimulated and LPS-stimulated blood neutrophils.

Horses

Whole blood was collected from the jugular vein of 5 healthy research horses and a complete blood count was performed to ensure adequate neutrophil number (> 2,000 cells/uL).

Sample collection

Prior to blood collection, the area over the jugular vein was rinsed with 70% ethanol. Blood collection was accomplished using a sterile blood collection set and a 17-G/3.75-cm needle. Blood (200 mL) was collected into a sterile bottle containing heparin anticoagulant.

Neutrophil isolation and RT-qPCR

Immediately following collection, blood was used for isolation of nonstimulated neutrophils or was stimulated with *Escherichia coli* LPS (2.5 mg) (Sigma Aldrich), incubated for 90 minutes, and then underwent neutrophil isolation. Neutrophils were isolated on a discontinuous density gradient formed from 2 solutions (Percoll; Sigma Aldrich).[22] Cells isolated were counted using a cell counting chamber (Bright-Line hemocytometer, Fischer Scientific; Waltham, MA) according to the manufacturer's directions. Following isolation, RNA was extracted and equine beta defensin-3 gene expression was identified and quantified via RT-qPCR. Briefly, RNA was isolated using (RNeasy Kit; Qiagen), and its integrity was checked (Agilent Bioanalyzer; Agilent Genomics). cDNA was made (High Capacity cDNA Reverse Transcription Kit; Applied Biosystems) and used to perform RT-qPCR analysis for the presence of beta defensin-1, -2, and -3 (TaqMan BD1, Ec03468234_m1 and 2 custom-made assays for beta defensin-2 and -3). The efficiency of each Taqman assay was performed to ensure the assays were within the proper range (98 to 110%). Reference genes (HPRT1, B2M, and GUSB) were evaluated for their appropriateness with neutrophils and were used as controls to normalize mRNA levels between samples to allow for comparison of relative BD-3 gene expression between stimulated and unstimulated neutrophils. Equine liver (from archived samples) was used as a positive control as it is known to express beta defensin-1.[12] Gene raw cycle threshold (Ct) and mean fold change ($2^{-\Delta\Delta}$Ct) were reported. Fold change results were expressed relative to the nonstimulated blood and statistically analyzed (Biogazelle qBase Plus, Zwijnaarde).

Statistics

Five horses were determined to be a large enough sample size to see differences (alpha 0.05; power 80%; means 0.075 and 0.3; and standard deviation 0.15). Statistical analysis was carried out on a dedicated statistical program (Prism 8.4.3; Graph Pad). Differences between expression of beta defensins between stimulated and nonstimulated neutrophils was evaluated with a Student's *t* test, with significance set at $P < .05$.

Experiment 2

Overview

Synovial fluid was collected from normal, aseptic, and septic joints from equine clinical cases as determined by the combination of clinical signs and diagnostics indicated below. This synovial fluid was analyzed using commercial ELISAs designed for detecting equine BD-1, -2, and -3 expression (Genorise Scientific).

Horses

Fourteen horses that either presented to one large animal referral hospital or were part of other approved research studies were used as subjects in this experiment. Clinical samples were acquired with owner permission. History, including time from when clinical signs were noted by the owner until presentation at the referral hospital, treatment before or at the hospital, and all diagnostic tests and procedures performed were recorded.

Sample collection

Synovial fluid was collected using sterile technique from joints of horses with no known lameness or radiographic signs of joint disease, from horses with aseptic inflammation (lameness and radiographic evidence of osteoarthritis, cellulitis, or known joint trauma), and from horses with lameness (grade 4 of 5 lameness on the American Association of Equine Practitioners lameness grading scale) and evidence of sepsis. Synovial fluid was aliquoted into an EDTA coated vacutainer tube, a serum blood vacutainer tube, an Eppendorf tube, or a blood culture bottle as appropriate for the analysis to be performed. Samples in the Eppendorf tube were placed into a −80 °C freezer. Cells were counted by a commercial machine (Abaxis VetScan HM5 analyzer; Allied Analytic) as well as confirmed by a board-certified pathologist via hemocytometer. Joints were considered to have septic inflammation if they had a positive bacterial culture and/or bacteria seen intracellularly, or based on having a combination of 2 of the 3 following criteria[2,23,24]: marked neutrophilic inflammation (neutrophils > 90%), nucleated cell count > 20 X 10^3 cells/uL, or total protein > 4 g/dL in submitted synovial fluid samples. All samples were collected within 6 hours of the horse presenting to the hospital.

Beta defensin 1, 2, and 3 ELISAs

Equine BD-1, -2, and -3 protein expression was identified using commercially available sandwich ELISAs (Genorise Scientific) specifically made for the horse. The synovial fluid was diluted to between 2- and 4-part assay buffer to 1-part sample to fall within the standard optical density at 450 nm (OD_{450}) curve. The ELISAs were validated for equine use in our laboratory using a spike and recovery assessment and evaluation of dilutions of individual equine samples (neat, 1:2, 1:4).

Statistics

Normality was assessed with a Shapiro-Wilk test and expression of BDs between groups was analyzed via a Kruskal-Wallis test, significant at $P < .05$. Statistical analysis was carried out on a dedicated statistical program (Prism 8.4.3; Graph Pad).

Table 1—Raw Ct means (avg) and standard deviation (SD) of the housekeeping genes HPRT1, B2M, GUSB, and BD-3 gene expression, as well as mean (avg) fold change ($2^{-\Delta\Delta}$Ct) of BD-3 gene expression in control (nonstimulated) and LPS stimulated neutrophils from 5 adult horses.

	Reference genes				
Horse ID and group	HPRT1 Avg Ct ± SD	B2M Avg Ct ± SD	GUSB Avg Ct ± SD	BD-3 gene expression Avg Ct ± SD	BD-3 gene $2^{-\Delta\Delta}$Ct Avg
Nonstimulated					
Horse 1	27.57 ± 0.13	17.84 ± 0.12	27.40 ± 0.22	37.76 ± 0.00	1.02
Horse 2	24.93 ± 0.07	14.70 ± 0.12	24.69 ± 0.04	33.36 ± 0.71	2.98
Horse 3	26.16 ± 0.47	15.56 ± 0.09	26.03 ± 0.11	35.13 ± 0.30	2.00
Horse 4	23.32 ± 0.12	14.32 ± 0.14	23.48 ± 0.18	36.38 ± 0.52	0.18
Horse 5	25.07 ± 0.17	15.04 ± 0.10	25.62 ± 0.06	35.14 ± 0.95	1.22
Mean	25.41	15.5	25.44	35.55	1.48
SD	0.19	0.11	0.12	0.5	1.06
Stimulated					
Horse 1	24.48 ± 0.07	15.60 ± 0.12	25.31 ± 0.08	35.06 ± 0.20	1.31
Horse 2	23.58 ± 0.07	14.53 ± 0.11	23.79 ± 0.04	30.42 ± 0.07	13.34
Horse 3	24.55 ± 0.15	15.05 ± 0.08	24.62 ± 0.06	31.17 ± 0.26	14.13
Horse 4	25.60 ± 0.08	14.42 ± 0.10	23.76 ± 0.04	33.71 ± 0.38	1.54
Horse 5	25.37 ± 0.24	15.13 ± 0.10	26.15 ± 0.16	32.53 ± 0.26	9.17
Mean	24.72	14.95	24.73	32.58	7.90*
SD	0.12	0.1	0.08	0.23	6.20

Ct = Cycle threshold.
*Stimulated significantly increased as compared to unstimulated ($P = .05$).

Table 2—Break down of the joints included in the study, age of horses, cell count and total protein, historical and treatment information, case outcome, form of disease, relevant history and criteria for inclusion as septic if applicable, and beta defensin (BD)-1, -2, and -3 concentrations (pg/mL) rounded to the nearest integer (n = 14 horses).

Joint	Age	Joint cell count (CC) (cells/uμl)	Total protein (TP) (g/dL)	Time until presentation	Treatment	Case outcome	Form (N, A, S)	Relevant history and criteria for septic diagnosis	BD-1, -2, -3 (pg/mL)
Tarsocrural	7 y	720	< 2.5	N/A	N/A	D/C	N	N/A	3, 0, 0
Tarsocrural	9 y	167	4.0	N/A	N/A	D/C	N	N/A	0, 0, 0
Tarsocrural	19 y	111	< 2.5	N/A	N/A	D/C	N	N/A	3, 0, 0
Radiocarpal	25 y	278	< 2.5	N/A	N/A	D/C	N	N/A	3, 0, 3
Middle carpal	25 y	222	< 2.5	N/A	N/A	D/C	N	N/A	3, 0, 3
Radiocarpal	25 y	111	< 2.5	N/A	N/A	D/C	N	N/A	3, 0, 3
Middle carpal	25 y	111	< 2.5	N/A	N/A	D/C	N	N/A	3, 0, 3
Distal interphalangeal	25 y	56	< 2.5	wk	PRE: none POST: steroid injection after fluid obtained	D/C	A	Chronic osteoarthritis	0, 0, 0
Tarsocrural	12 y	3,333	3.8	< 12 h	PRE: none POST: abx, RLP	D/C	A	Cellulitis	1,221, 766, 305
Tarsocrural	12 y	400	< 2.5	< 12 h	PRE: none POST: biologic injection after fluid obtained	D/C	A	Chronic osteoarthritis, acute flare up	125, 446, 862
Tarsocrural	16 y	230	3.7	2 wk	PRE: none POST: IV pain medication	D/C	A	Chronic osteoarthritis, torn short collateral ligament	0, 0, 0
Tarsocrural	2 mo	22,320	2.5	2 days	PRE: plasma POST: IA and systemic abx, needle lavage	D/C	S	Positive culture > 90% neutrophils, CC	0, 0, 0
Tarsocrural	15 y	14,7500	6.3	6 d	PRE: none POST: E	E	S	Intracellular bacteria, CC, TP	0, 0, 0
Coxo-femoral	1 mo	17,500	5.1	4 d	PRE: systemic abx POST: IV, IA, PO abx	D/C	S	Septic systemically CT of her joint abscess seen and osteomyelitis present CC, TP	3, 0, 3
Tarsocrural	19 y	257,125	5.7	< 12 h	PRE: systemic abx POST: arthroscopy, IV and RLP abx, PO abx	D/C	S	Laceration communicating with joint, >90% neutrophils, CC, TP	339, 285, 92
Distal interphalangeal	7 y	42,740	5.9	2.5 wk	PRE: IV abx, POST: E	E	S	History of nail to that foot, laceration communicating with joint, CC, TP, > 95% neutrophils	3, 0, 19
Tarsocrural	8 y	135,900	5	2 d	PRE: none POST: needle lavage, RLP, IV, PO abx, arthroscopy	D/C, returned for arthro-scopy	S	Steroid injections 2 days prior, CC, TP	42, 3, 11
Front metacarpophalangeal	4 d	65,640	3.3	< 48 h	PRE: plasma POST: IV and IA abx, needle lavage,	D/C	S	Septic systemically, positive culture, CC,	0, 0, 0
Hind metacarpophalangeal	4 d	65,040	2.7	< 12 h	PRE: plasma POST: IV and IA abx, needle lavage	D/C	S	Septic systemically, positive culture, CC	0, 0, 0
Tarsocrural	6 d	21,020	2.6	< 12 h	PRE: plasma POST: IV and IA abx, needle lavage, arthrotomy	D/C	S	Septic systemically, positive culture, CC	0, 0, 0
Tarsocrural	2 y	6,278	5.5	11 d	PRE: systemic abx POST: needle lavage, systemic abx	E	S	Positive culture, TP	0, 0, 0

A = Aseptic. abx = Antibiotics. CC = Cell count. D/C = Discharged. E = Euthanized. IA = Intra-articular. N = normal. POST = Treatment in the hospital after synoviocentesis. PRE = Treatment before the synoviocentesis performed by the referring veterinarian. RLP = Regional limb perfusion. S = Septic. TP = Total protein.

Table 3—Mean ± standard deviation (SD) and ranges (pg/ml) of beta defensins (BD)-1, -2, and -3 in normal, aseptic, and septic equine joints (n = 14 horses).

	BD 1			BD 2			BD 3		
	Normal	Aseptic	Septic	Normal	Aseptic	Septic	Normal	Aseptic	Septic
Mean ± SD (pg/ml)	2.65 ± 1.06	520.79 ± 618.97	38.73 ± 14.47	0 ± 1.00	303.19 ± 373.65	28.82 ± 100.69	1.5 ± 1.60	107.85 ± 144.72	12.67 ± 32.19
Range (pg/ml)	0–3	0–1221.13	0-339.72	0–3	0–766.28	0–285.22	0–3	0–306.00	0–92.40

Results

Experiment 1

BD-3 gene expression of neutrophils isolated from whole blood from 5 healthy horses was compared using RT-qPCR. A significant difference (P = .05) in relative BD-3 expression was found when comparing LPS-stimulated to nonstimulated neutrophils after normalization to the reference genes **(Table 1)**. Efficiencies for BD-1 and -2 showed no signal and subsequently were not included in further RT-qPCR analyses.

Experiment 2

Horses and joints sampled

Of the 14 horses in the study, synovial fluid was collected from 21 joints. There were 7 normal joints, 4 aseptic, and 10 septic. Mean total nucleated cell count values for normal, aseptic, and septic joints were 246 ± 202 cells/uL, 1,005 ± 1,349 cells/uL, and 68,453 ± 77,581.31 cells/uL, respectively. Mean total protein values for normal, aseptic, and septic joints were 2.6 ± 0.3 g/dL, 3.3 ± 0.5 g/dL, and 4.4 ± 1.3 g/dL, respectively. The joints sampled included the following: 12 tarsi, 4 carpi, 2 distal interphalangeal (DIPJ), 1 metacarpo- and 1 metatarsophalangeal (fetlock), and 1 coxofemoral. Horse ages ranged from 4 days to 25 years and included 5 mares, 6 geldings, 1 colt, and 2 fillies. Distribution of the joints is shown in **Table 2**. Time until presentation for horses with septic joints ranged from 4 hours to 2.5 weeks. Of the 5 horses which had BD concentrations of > 15 pg/mL, 2 horses with aseptic inflammation, that had the highest concentrations of BDs, had chronic histories of either cellulitis or osteoarthritis (12 years, mare, osteoarthritis; 12 years, gelding, cellulitis), but both presented within 12 hours of an acute worsening of lameness of their chronic issue. The horse with the septic joint that demonstrated the highest BD concentrations (19 years, mare) also presented within 12 hours of injury.

BD-1, -2, and -3 ELISAs

There were no significantly different concentrations of BDs between groups when examining BD-1 (P = .16) or BD-2 (P = .5). For BD-3 the overall P value was .05, with a normal vs. aseptic adjusted P value of .06, a normal versus septic adjusted P value of .842, and an aseptic versus septic adjusted P value of .236. Specific means, standard deviations, and ranges for all BDs are given in **Table 3**.

Discussion

Expression of BD-3 was identified in both non-stimulated and LPS-stimulated equine neutrophils, which is the first report of BD expression by equine neutrophils. We found greater levels of BD-3, but not BD-1 or BD-2, expression in LPS-stimulated neutrophils, which partially supports the first hypothesis that LPS stimulation would increase BD expression from equine neutrophils. Our second hypothesis was not supported as in clinical cases we did not find an increase in BDs in septic joints compared to normal or aseptic joints.

Neutrophil expression of BDs has not been studied extensively in horses, and it was previously unknown if equine neutrophils express BDs. Systemic stimulation with LPS has been used in other equine studies to mimic septicemia and endotoxemia[20,25] and LPS has also been used to stimulate equine neutrophils in-vitro.[21,26] In humans, LPS stimulation has been shown to induce production of BD-1, -2, and -3 from a variety of cell types.[27–29] In this study, we expected that LPS stimulation would increase all BD expression; however, it only increased BD-3 expression from equine neutrophils in a majority of our samples. This was different from a study which examined alpha and beta defensin expression in unstimulated and stimulated whole blood from human patients.[30] In that study, LPS stimulation produced detectable expression of BD-1 in 88.2% of samples, BD-2 in 39.2% of samples, and no expression of BD-3. Differences that could account for these results include species variability, and differential expression of BDs in whole blood versus isolated neutrophils. The sample from our study that did not show a large increase in BDs had a prolonged period between centrifugations steps during the neutrophil isolation procedure due to centrifuge capacity limitations, and this may have impacted accuracy of this sample. Knowledge of BD expression in equine neutrophils is helpful for further understanding of the innate immune response.

In this study BD-1 protein concentration was not significantly different between normal, aseptically inflamed, and septic joints. Current literature suggests that BD-1 is constitutively produced in all healthy synovial membranes[31] and has roles in immune defenses against bacteria, fungi and viruses.[32] BD-1, -2, and -3 have been found in other tissues of the gastrointestinal tract, respiratory tract, eye, and skin.[33] In our study, BD-1 was either not present or not present in high amounts in synovial

fluid of normal horses. This may be an artificially low concentration as synovial membrane samples may have yielded more accurate concentrations; however, it is invasive to obtain clinically from normal horses.

In aseptic forms of inflammation, such as osteoarthritis, a human study found that greater BD concentrations are present due to joint inflammation and the bony remodeling that occurs after cartilage damage.[34] In that study,[34] BD-3 appeared to upregulate the expression of metalloproteinases (MMPs) responsible for cleaving type II cartilage while downregulating MMP endogenous inhibitors. A more recent study[35] found BDs to be involved in catabolic pathways of articular cartilage, thus contributing to ECM degradation. These findings could explain the presence of BD-3 in osteoarthritic joints even without infection present. BDs were traditionally viewed as exclusively antimicrobial molecules, as their induction in response to diverse bacterial, viral, parasitic, and fungal infections was extensively reported[36]; however, it is now clear that the antimicrobial and immunomodulatory functions of beta defensin peptides are not mutually exclusive and that their function is multifactorial and evolving.[36]

Multiple forms of aseptic inflammation (osteoarthritis, cellulitis, acute trauma) were grouped together in this study, which may be oversimplifying different pathologies and immune responses. A human study looking at the expression of BD-2 in normal patients compared to patients with cellulitis found a greater expression of BD-2 in both the infected skin and the distal skin that appeared normal.[37] This could explain why the horse with cellulitis in the aseptic group had such high levels of BD expression (BD-1, 1221.13 pg/ml; BD-2, 766.28 pg/ml; and BD-3, 306.00 pg/ml) as compared to the other aseptic samples. In the future, cases of cellulitis might merit their own category, and BDs may serve as useful biomarkers for disease progression and response to treatment.

The lack of BDs in septic synovial samples was not expected. Both tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) have been shown to be strong inducers of BD-3.[38] While both osteoarthritic and septic joints are subject to inflammatory responses where TNF-α and IL-1 play a factor, we expected septic joints to be undergoing a greater degree of inflammation and therefore predicted higher concentrations of BDs; however, this was not the case. Paulsen et al[31] found BD-3 expression in all of the samples from joints with pyogenic inflammation and osteoarthritis but unfortunately did not state concentrations or relative level of expression. Another study[13] measuring cytokine and peptide expression in human patients undergoing total joint replacement found that BD-3 was increased in cases of periprosthetic joint infection both in serum and synovial fluid. Interestingly, that study[13] found BD-2 to be significantly increased only in serum in cases of periprosthetic joint infection. Studies that evaluated concentrations of BDs over time postinfection and the half-life of BDs are unfortunately not present in the literature. In fact, most of the human studies[12,13,31] have only evaluated BD expression in periprosthetic joint infection at one time point. It is interesting that our highest concentrations were seen in joints from animals with that presented less than 12 hours from the onset or acute worsening of their clinical signs. Future exploration of BD expression should focus on sampling at this earlier stage of disease development. The reasons for the differences seen between our results, and the studies mentioned above are numerous and include variation among species in BD function, inherent variation among clinical cases, different time frame for sample collection, and this study measuring BDs in synovial fluid alone versus synovium,[31,38] bone, or cartilage. Based on the studies mentioned above, it would have been interesting to measure serum levels of BD and compare those to local expression.

Limitations of this study included low case numbers and different types of aseptic cases. Synovial fluid was only collected from clinical cases over the course of a year, and we were limited by the types of cases that presented to this referral hospital. The different pathophysiologies contributing to aseptic inflammation (ie, osteoarthritis versus a cellulitis case) may have recruited different immune proteins. Additionally, concentrations of BDs in synovial fluid were measured in this study versus in synovium, bone, or cartilage as the latter 3 are invasive to obtain in clinical cases and our interest was if beta defensins could serve as biomarkers of sepsis prior to surgery.

Overall, we were able to identify BD expression in samples from stimulated equine peripheral neutrophils and synovial fluid. While BD expression in normal joints was minimal to absent, further studies are warranted to elucidate the role of BDs in aseptic and septic joint inflammation.

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The authors declare that there were no conflicts of interest.

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