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Novel mRNA Biomarkers for Confirming Infection Eradication in Equine Septic Arthritis

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ABSTRACT

Septic arthritis (SA) in horses poses lasting health challenges. Successful recovery depends largely on initiating prompt and intensive therapy that often extends over a long duration. Current diagnostic techniques are inadequate for reliably confirming the full elimination of joint infection. Identifying and characterizing mRNA biomarkers could offer a more accurate alternative. This investigation aimed to pinpoint mRNA biomarkers that indicate the successful clearance of joint infection in equine SA and to compare these findings with previously reported proteomic data. Furthermore, the transcriptomic data were assessed against the human SeptiCyte Lab mRNA panel, which distinguishes sepsis from non-septic shock. A comparative transcriptomic analysis was carried out on synovial fluid from the SA joints of five horses during active infection and after post-treatment clearance, along with five horses exhibiting non-septic synovitis. Eight novel mRNA transcripts were found to be significantly upregulated (>3-fold) in horses with active SA relative to those after infection resolution and those with non-septic inflammation. Two corresponding proteins from the proteomic dataset aligned with these transcripts but were not statistically distinct. The human SeptiCyte transcripts were not differentially expressed in this study. These outcomes suggest that mRNA expression could serve as a promising indicator for infection resolution in equine joints and merits additional study.

Keywords: Horse, Septic arthritis, mRNA, Biomarker, Infection eradication

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Introduction

Septic arthritis is a critical condition in horses with potentially serious outcomes. Early recognition, aggressive therapy, and dependable confirmation of infection clearance are key to positive prognoses. Conventional diagnostic approaches—such as synovial fluid cytology and bacterial culture [1–3]—may not reliably confirm the complete removal of joint infection. Consequently, prolonged antimicrobial treatment is often employed, potentially causing adverse reactions and encouraging antimicrobial resistance [4]. Hence, research has shifted toward discovering improved diagnostic strategies, including the development of biomarkers. The overarching aim of our work is to identify biomarkers capable of pinpointing the exact stage when infection is eliminated in equine SA.

Biomarkers are measurable indicators of disease presence, treatment response, or prognosis [5]. They can consist of proteins, metabolites, mRNA, or other detectable substances in biological materials. Numerous studies have investigated biomarkers associated with joint diseases in horses [6], focusing primarily on protein-based markers [7], while fewer have examined metabolites or other molecular classes [8]. Although many investigations have

identified markers for diagnosing equine SA, none have explored biomarkers for determining when infection has been eradicated following treatment—a central objective of our ongoing research.

An experimental model simulating infection eradication in equine SA demonstrated that traditional synovial fluid parameters—total nucleated cell count, neutrophil percentage, total protein content—and serum amyloid A (SAA) are useful for diagnosing SA but inadequate for confirming eradication after treatment [1, 9]. Consequently, comparative proteomic analysis of synovial fluid from infected and post-treatment joints revealed several proteins potentially linked to infection resolution [10].

The present study expands on that work by analyzing the synovial fluid transcriptome from horses with active and resolved SA and comparing it with previously identified proteomic profiles [10]. This approach was inspired partly by the recently developed [11] and validated [12] human SeptiCyte Lab mRNA biomarker panel, which differentiates septic from non-septic shock. That research demonstrated mRNA's diagnostic value in distinguishing infectious from non-infectious inflammation, prompting us to explore whether synovial fluid mRNA could indicate infection clearance in treated equine SA.

The specific objectives were to:

(1) conduct transcriptomic profiling of synovial fluid from horses with experimental SA before and after infection clearance, and from horses with induced non-septic synovitis;

(2) identify differentially expressed mRNAs between septic, post-eradication, and non-septic conditions;

(3) compare these mRNAs to proteins identified in prior proteomic studies; and

(4) assess overlap with the human SeptiCyte LAB panel used to differentiate septic from non-septic shock.

Materials and Methods

This experiment adhered to the guidelines of the Canadian Council on Animal Care and received approval from the University of Saskatchewan Animal Care and Use Committee and Animal Research Ethics Board (Protocol #20180048). Sample size was determined following the calculations by Hart *et al*. [13], indicating that five horses per group were sufficient to detect a 3-fold change with a 60% coefficient of variation, an average read count of 1000, 80% statistical power, and a significance level of $p < 0.05$.

Ten adult American Quarter Horses (six mares, four geldings) averaging 10 ± 3 years old (range 7–18 years) and weighing 528 ± 29 kg (range 483–580 kg) were used. All were deemed healthy and free from musculoskeletal issues based on comprehensive physical exams, lameness assessments, and blood tests (including CBC, biochemistry, and systemic SAA). Lameness evaluation involved observing the horses walking straight and in circles, and was categorized as sound, mildly lame, or non–weight-bearing lame.

For this controlled trial, ten horses were randomly allocated into two experimental categories: those with induced septic arthritis (n = 5) and those with induced sterile synovitis (n = 5). The induction procedures followed previously validated techniques [1]. In summary, septic arthritis was created by introducing 10^8 colony-forming units of Escherichia coli—originally recovered from an equine joint infection—into the middle carpal joint. Non-septic synovitis was produced by administering 5 ng of lipopolysaccharide (LPS) dissolved in sterile phosphate-buffered saline (PBS, pH 7.4) into the same joint.

At 24 hours following induction (post-induction day 1, PID 1), both experimental groups underwent arthroscopic lavage, followed by a treatment protocol that included local antibiotic perfusion of the limb, intra-articular gentamicin, and systemic antimicrobial therapy, as described earlier [1]. The lavage involved flushing the joint with 20 liters of sterile saline, after which 500 mg of gentamicin was injected intra-articularly. Regional limb perfusion with 1 g gentamicin was carried out on PID 1, 2, and 3. Systemic medications included sodium penicillin (22,000 IU/kg intravenously every six hours), gentamicin sulfate (6.6 mg/kg IV every 24 hours), and phenylbutazone (2.2 mg/kg IV every 12 hours) for six consecutive days, followed by oral trimethoprim–sulfamethoxazole (24 mg/kg every 12 hours) for an additional five days.

In the sterile synovitis group, horses developed temporary and mild lameness that reached its maximum around four hours after induction. Two remained sound, and three showed lameness at the walk. All had returned to soundness by 18 hours post-injection, with no further abnormalities observed afterward. Throughout the trial, physiological parameters stayed within normal limits. In the septic arthritis group, every horse exhibited pronounced effusion in the affected joint and non–weight-bearing lameness that peaked at approximately 12 hours post-bacterial inoculation. Once arthroscopic lavage was completed at 24 hours, all animals regained soundness

at the walk and maintained normal clinical parameters thereafter. All ten horses completed the experiment and displayed no signs of lameness when trotted in a straight line or on a circle.

Samples of blood and synovial fluid were collected from the injected (left mid-carpal) and non-injected (right mid-carpal) joints before induction (PID 0) and at PID 1, 2, 3, 4, 7, and 10. Analyses of synovial fluid and total nucleated cell counts were conducted at Prairie Diagnostic Services (Saskatoon, Canada) using the Advia 2120i system (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Bacteriological assessment of synovial fluid was performed according to previously published procedures [1]. In brief, 100 µL of synovial fluid was added to 100 mL of brain–heart infusion (BHI) broth (BD Bacto Brain Heart Infusion; Becton Dickinson, Franklin Lakes, NJ, USA) and incubated aerobically for three days at 36 °C. Concurrently, aliquots were streaked on Columbia blood agar plates and incubated under identical conditions. When bacterial growth occurred in BHI, colonies were re-cultured on blood agar. Bacterial identification was completed using MALDI-TOF mass spectrometry (Bruker, Billerica, MA, USA) at Prairie Diagnostic Services, following the manufacturer's instructions and the approach detailed previously [14].

RNA isolation and library generation

Synovial samples collected from septic arthritis cases on PID 1 and PID 4, along with samples from sterile synovitis on PID 1, were used for transcriptome sequencing. Total RNA was isolated using Trizol reagent (Thermo Fisher Scientific, Waltham, MA, USA) and purified with the PureLink RNA Mini Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) according to the provided instructions. DNA contamination was eliminated using DNase I (New England Biolabs, Ipswich, MA, USA; Cat. M0303S), followed by an additional cleanup with the Monarch RNA Cleanup Kit (New England Biolabs, Ipswich, MA, USA). RNA quantity and quality were evaluated using the Qubit RNA HS Assay (Thermo Fisher Scientific) and the RNA Screentape System (Agilent Technologies, Santa Clara, CA, USA). Libraries for sequencing were prepared from 50–100 ng of total RNA utilizing the NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) and the NEBNext Ultra II Directional RNA Library Prep Kit (both from New England Biolabs), as instructed by the manufacturer.

RNA sequencing

Prepared cDNA libraries were quantified with the Qubit dsDNA HS Assay (Thermo Fisher Scientific) and checked for fragment size distribution using D1000 Screentape (Agilent Technologies). Barcoded libraries were normalized to equal concentrations, pooled, and sequenced on an Illumina NextSeq 550 platform (San Diego, CA, USA) to produce paired-end reads of 75 base pairs.

Data processing

Raw sequencing outputs were demultiplexed and converted to FASTQ files through the Illumina BaseSpace environment (version 7.2.0; San Diego, CA, USA) using the bcl2fastq utility with standard parameters. To remove unwanted adapters and low-quality sequence fragments, the reads were processed with fastp version 0.20.1 [15]. The cleaned data were aligned against the EquCab3.0 equine genome assembly using STAR (version 2.7.9a) [16]. For inclusion in downstream analyses, genes were required to appear across every sample of at least one treatment category.

Differential gene expression analysis

Quantification of mRNA reads was conducted in R using the Bioconductor tool Rsubread (version 2.105) [17]. Differential gene expression was then determined via DESeq2 (version 1.22.2) [18], applying an adjusted false discovery rate of $p < 0.01$ to define significance. Selection of potential mRNA biomarkers from the significantly altered genes was carried out manually in Excel (version 2404; Microsoft Corp., Redmond, WA, USA) using these specific cutoffs:

1. The lowest transcript count in septic arthritis at PID 1 had to exceed three times the highest value recorded in the same group after infection clearance (PID 4);
2. The lowest transcript count in septic arthritis at PID 1 had to be at least twice the greatest count in the LPS-induced non-septic synovitis group at PID 1;
3. Every septic arthritis horse at PID 1 had to show normalized counts above 1000 **(Figure 1)**.

All visual materials were prepared using GraphPad Prism software (version 8.2.1 for Windows; GraphPad Software, La Jolla, CA, USA).

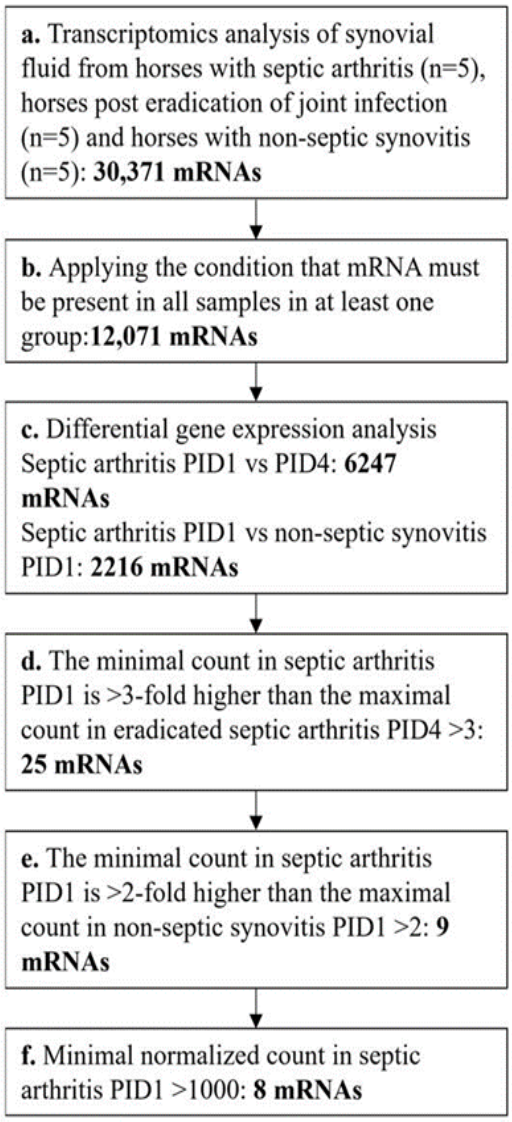


Figure 1. Flow diagram summarizing transcript filtering steps:
(a) Complete set of mRNA transcripts identified; (b) Removal of transcripts absent from any sample within a group; (c) Application of DESeq2 for differential expression; (d) Retention of transcripts with minimal PID 1 values ≥3× the maximal PID 4 values; (e) Retention of transcripts with minimal PID 1 values ≥2× the maximal LPS PID 1 values; (f) Inclusion of transcripts with normalized counts >1000 in septic arthritis PID 1 samples.

Results and Discussion

Experimental model

In horses with septic arthritis, synovial fluid cultures were positive for E. coli in all animals on PID 1 and in two out of five on PID 2. No bacterial growth was observed in any injected joints from PID 3 onward. The LPS-induced non-septic synovitis group remained culture-negative in every sample. The total contamination rate among bacterial cultures was roughly 7% (10 positive out of 140).

At PID 1, the septic arthritis group displayed a marked elevation in nucleated cell count (NCC), reaching a median of 214.8 × 10⁹/L (range 178.2–254.6 × 10⁹/L). The NCC steadily declined through PID 10 **(Figure 2)**.

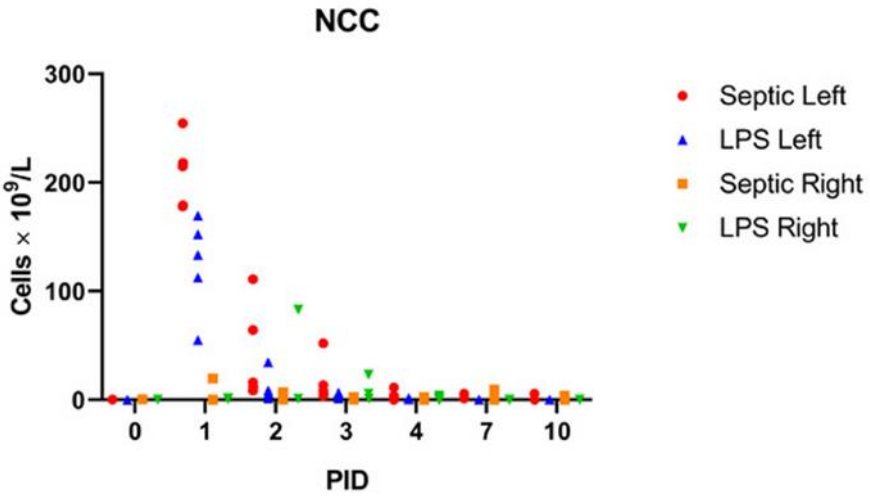


Figure 2. Synovial fluid nucleated cell count (NCC) in the left (E. coli–injected) and right (uninjected) joints of horses affected by septic arthritis (Septic Left ●, Septic Right ■) and LPS-induced non-septic synovitis (LPS Left ▲, LPS Right ▼).

By PID 4, the median NCC of the septic arthritis group had decreased to 3.6 × 10⁹/L (range 0.2–11.4 × 10⁹/L), remaining well below the diagnostic limit for septic arthritis (30 × 10⁹/L [19]), indicating complete clearance of the infection. In the sterile synovitis group, inflammatory reaction was verified by an NCC median of 133.4 × 10⁹/L (range 55.1–133.4 × 10⁹/L). Contralateral control joints in both experimental sets consistently tested negative for bacterial culture.

One horse in the septic arthritis cohort showed a moderate NCC value of 19 × 10⁹/L at PID 1, while one horse in the LPS group had an NCC peak of 83 × 10⁹/L at PID 2, which subsequently fell to 23.4 × 10⁹/L at PID 3 and 3.9 × 10⁹/L at PID 4. These fluctuations were interpreted as transient aseptic responses related to procedural manipulation and sampling [20].

Based on bacterial culture and NCC data, infection elimination was verified in all subjects by PID 4. These samples are referred to as "eradicated septic arthritis at PID 4." Synovial fluid collected at that point was used in transcriptomic evaluations of post-treatment conditions.

Transcriptomics analysis

Across all examined samples, 30,371 genes were detected. Principal component analysis (PCA) clearly separated the three experimental conditions, showing tight clustering of samples within each treatment type **(Figure 3)**.

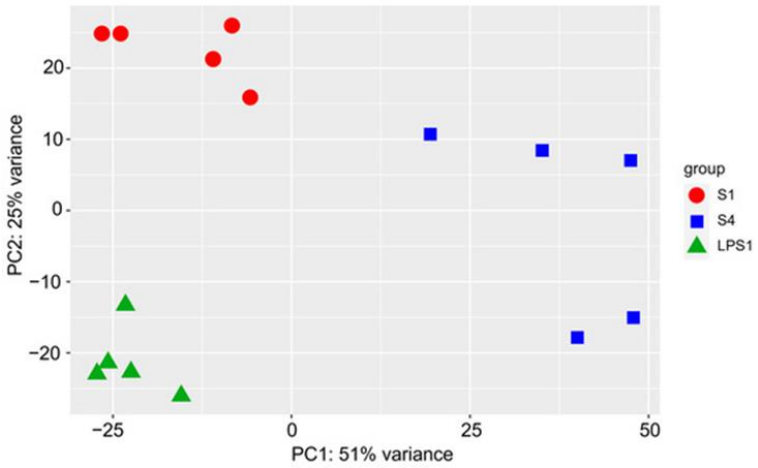


Figure 3. PCA plot showing separation of expression profiles by treatment group, using normalized gene counts.

Symbols: S1 ● = septic arthritis at PID 1; S4 ■ = post-eradicated septic arthritis at PID 4; LPS1 ▲ = LPS-induced non-septic synovitis at PID 1. PID = post-induction day.

Identification of potential mRNA biomarkers

To determine mRNA indicators related to infection clearance, a multi-step filtering strategy was applied **(Figure 1)**. Any gene absent from all samples in at least one treatment category was excluded, leaving 12,071 genes eligible for analysis. From this dataset, 6,247 genes differed significantly between septic arthritis at PID 1 and eradicated septic arthritis at PID 4, while 2,216 genes differed between septic arthritis at PID 1 and LPS-induced non-septic synovitis, based on an FDR-adjusted $p < 0.01$ threshold.

Next, genes were screened for strong expression contrasts. Transcripts were retained only when the lowest expression value recorded in the septic arthritis samples was three times higher than the highest value detected after infection resolution. Twenty-five genes met this strict standard. Of these, nine also showed distinct expression between septic arthritis (PID 1) and LPS-induced non-septic synovitis (PID 1), where the minimum expression in septic arthritis exceeded the maximum in non-septic cases by at least twofold. Finally, only those genes with normalized counts greater than 1,000 in all septic arthritis samples were accepted, reducing the list to eight upregulated transcripts fulfilling every requirement **(Figure 4 and Table 1)**.

Applying the same selection process in reverse to identify downregulated transcripts did not yield any qualifying genes.

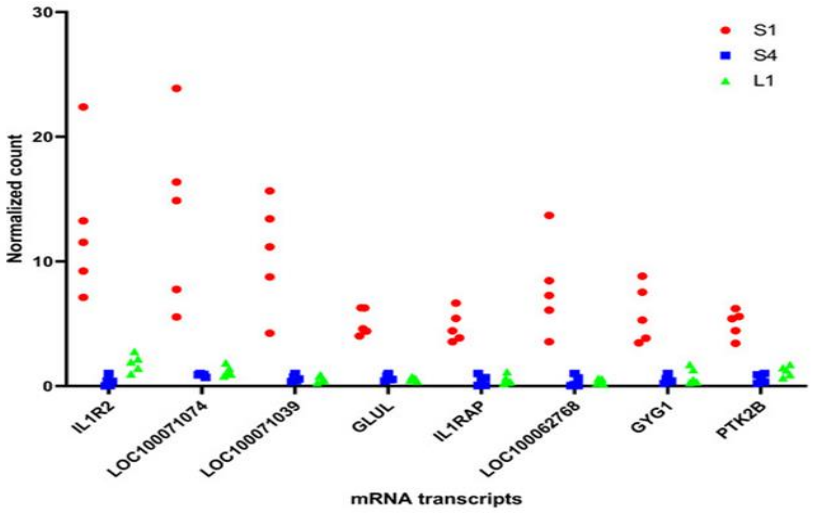


Figure 4. Representation of candidate mRNA transcripts linked to infection clearance in the equine septic arthritis model. Listed genes **(Table 1)** satisfied every selection rule in the study. Counts were scaled relative to the maximum expression found in the post-eradication samples (septic arthritis PID 4).
Symbols: S1 ● = septic arthritis PID 1; S4 ■ = eradicated septic arthritis PID 4; L1 ▲ = non-septic synovitis PID 1. PID = post-induction day.

Table 1. Candidate mRNA markers for infection eradication in the equine septic arthritis model.

Gene Name	Gene ID	UniProt Accession Number	Protein Name
IL1R2	ENSECAG00000000288	F7DK71	Interleukin-1 receptor type 2
LOC100071074	ENSECAG00000039739	F6QB61	Peptidoglycan-recognition protein
LOC100071039	ENSECAG00000032321	F7DQG1	Peptidoglycan-recognition protein
GLUL	ENSECAG00000015865	F6TAZ0	Glutamine synthetase
IL1RAP	ENSECAG00000005083	F7AWQ9	Interleukin 1 receptor accessory protein
LOC100062768	ENSECAG00000006255	F7ADT4	G-protein coupled receptors family 1 profile domain-containing protein
GYG1	ENSECAG00000023192	F6U6H7	Glycogenin 1
PTK2B	ENSECAG00000018924	F7CGD4	Non-specific protein-tyrosine kinase

Differentially expressed genes identified between septic arthritis (PID 1), eradicated septic arthritis (PID 4), and non-septic synovitis (PID 1) met the defined thresholds.

Among the eight transcripts that satisfied all conditions, two—interleukin-1 receptor accessory protein and glycogenin-1—were also present as protein products in our earlier proteomics dataset [10] **(Table 2)**. However, protein abundance for these two did not differ significantly among the three conditions in that previous analysis.

Table 2. Proteins associated with candidate mRNA biomarkers identified in the proteomic study [10].

Gene Name	Statistic	Septic Arthritis PID1	Eradicated Septic Arthritis PID4	Non-Septic Synovitis PID1
IL1RAP	Mean	614,082	623,448	1,358,139
	SD	408,114	241,337	1,041,416
GYG1	Mean	0	0	30,143
	SD	0	0	45,269

Values represent mean ± standard deviation of measured signal intensities.

All 26 proteins previously described as differentially abundant [10] had corresponding mRNA transcripts detected in the current sequencing dataset. Of these, seven transcripts were upregulated, two were downregulated ($p < 0.01$), and seventeen showed no significant difference when comparing septic arthritis (PID 1) to eradicated arthritis (PID 4). In the comparison between septic arthritis (PID 1) and non-septic synovitis (PID 1), seven mRNAs were upregulated, one was downregulated, and eighteen showed no change.

Transcripts encoding neutrophil collagenase, BTB/POZ domain-containing protein KCTD12, ubiquitin-like modifier-activating enzyme 1, and guanine nucleotide-binding protein subunit beta-2 were upregulated in both comparisons involving septic arthritis (PID 1) **(Table 3)**. Although none of these reached the very strict thresholds defined for biomarker selection, mRNAs for neutrophil collagenase (MMP1/8) and interleukin-1 receptor antagonist (IL1RN) consistently showed higher expression in septic joints and may have diagnostic potential.

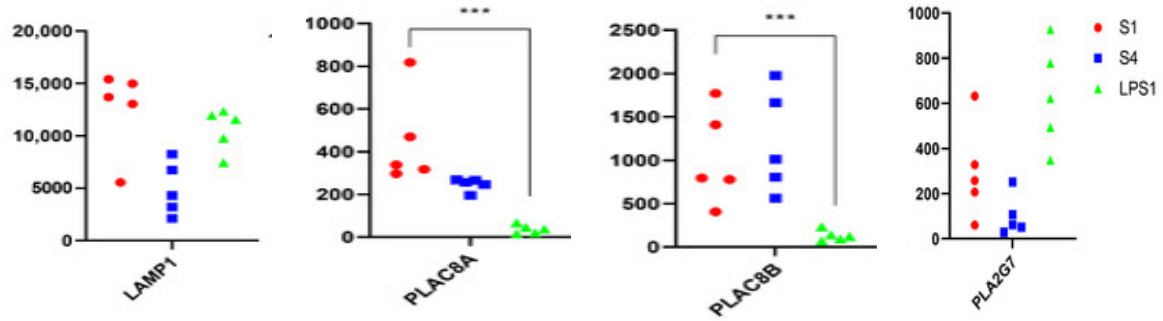
Table 3. Comparison between transcriptomic and previously reported proteomic data [10]. Transcripts detected in this study that correspond to the 26 previously identified proteins are shown with p-values and fold changes obtained from DESeq2 analysis.

Protein Name	Gene ID	Gene Name	p-Value (Septic Arthritis PID1 vs. Eradicated Septic Arthritis PID4)	Fold Change (Septic Arthritis PID1 vs. Eradicated Septic Arthritis PID4)	p-Value (Septic Arthritis PID1 vs. Non-Septic Synovitis PID1)	Fold Change (Septic Arthritis PID1 vs. Non-Septic Synovitis PID1)
E3 ubiquitin-protein ligase TRIM9	ENSECAG00000003278	TRIM9	0.082	−4.1	0.859	1.3
DCC-interacting protein 13-alpha	ENSECAG00000017473	APPL1	0.130	−1.3	0.031	−1.5
Ubiquitin-like-conjugating enzyme ATG3	ENSECAG00000011954	ATG3	0.586	−1.1	0.849	1.1
Aldehyde dehydrogenase family 16 member A1	ENSECAG00000023437	ALDH16A1	0.145	1.3	0.638	−1.1
Serine/threonine-protein kinase 24	ENSECAG00000007682	STK24	0.007	1.5	0.604	1.1
Protein ABHD14B	ENSECAG00000022421	ABHD14B	0.700	−1.1	0.359	−1.2

Transforming protein RhoA	ENSECAG00000033791	RHOA	0.009	1.6	0.032	1.5
Guanine nucleotide-binding protein subunit beta-2	ENSECAG00000017221	GNB2	0.000	2.4	0.000	1.9
Ubiquitin-conjugating enzyme E2 L3	ENSECAG00000032131	UBE2L6	0.170	1.6	0.000	7.1
N-acetyltransferase ESCO1	ENSECAG00000016120	ESCO1	0.000	2.8	0.077	1.6
Twinfilin-2	ENSECAG00000014047	TWF2	0.068	1.3	0.003	1.5
Nuclear transport factor 2	ENSECAG00000021530	NUTF2	0.000	−2.7	0.048	−1.8
Elongation factor 1-gamma	ENSECAG00000014334	EEF1G	0.000	−2.3	0.841	1.1
Septin-7 (Fragment)	ENSECAG00000022693	SEPTIN7	0.216	−1.2	0.473	1.2
GTP-binding nuclear protein Ran	ENSECAG00000020532	RAN	0.071	−1.5	0.283	1.3
Synaptic vesicle membrane protein VAT-1 homolog	ENSECAG00000016700	VAT1	0.790	1.1	0.001	−2.4
Alpha-1-acid glycoprotein 2	ENSECAG00000036760	NA	0.656	−1.3	0.126	−2.2
BTB/POZ domain-containing protein KCTD12	ENSECAG00000002083	KCTD12	0.000	3.6	0.002	2.8
Interleukin-1 receptor antagonist protein	ENSECAG00000027864	IL1RN	0.014	4.2	0.000	9.3
Ubiquitin-like modifier-activating enzyme 1	ENSECAG00000014002	UBA1	0.000	2.6	0.000	2.8
Heterogeneous nuclear ribonucleoprotein D0	ENSECAG00000022692	HNRNPDL	0.051	−1.3	0.678	1.1
Neutrophil collagenase	ENSECAG00000023733	MMP1/8	0.000	22.9	0.000	4.2
Fermitin family homolog 3	ENSECAG00000008107	FERMT3	0.815	1.1	0.332	1.3
Coronin-7	ENSECAG00000024788	CORO1A	0.769	1.1	0.026	1.8

Poly(rC)-binding protein 1	ENSECAG00000024218	PCBP4	0.020	−7.0	0.547	−2.0
Myotrophin	ENSECAG00000024284	MTPN	0.927	−1.0	0.229	1.3

The expression data were also cross-referenced with the SeptiCyte Lab biomarker panel, which includes PLAC8, PLA2G7, LAMP1, and CEACAM4 [11]. In that clinical test, PLAC8 and LAMP1 contribute positively to the diagnostic score (indicating upregulation), while PLA2G7 and CEACAM4 reduce it (indicating downregulation) [11]. In our dataset, PLAC8 (represented by isoforms PLAC8A and PLAC8B), LAMP1, and PLA2G7 were detected, whereas CEACAM4 was not identified **(Figure 5)**.



a) b) c) d)

Figure 5. Expression profiles of SeptiCyte Lab–associated mRNA transcripts detected in this experiment. The figure displays raw count values (y-axis) for the mRNA transcripts included in the SeptiCyte Lab diagnostic panel that were identified in this dataset. Two subunits of PLAC8, designated A and B, were detected, both exhibiting a pronounced increase in expression in horses with septic arthritis when compared with those affected by experimentally induced non-septic synovitis.
Symbol key: S1● = septic arthritis at PID1; S4■ = infection-cleared (eradicated) septic arthritis at PID4; LPS1▲ = experimentally induced non-septic synovitis at PID1.
Asterisks (****) denote $p < 0.001$, indicating statistically significant differences according to DESeq2 analysis.

No statistically significant differences in transcript levels were observed across all experimental groups ($p > 0.01$). Nonetheless, LAMP1, PLAC8A, and PLAC8B tended to show elevated expression in horses with septic arthritis at PID1, while PLA2G7 expression appeared lower in this same group relative to non-septic synovitis **(Figure 5)**. This general pattern is consistent with expression trends reported by the SeptiCyte Lab assay.
This research identified eight mRNA transcripts that may serve as molecular indicators of infection resolution in an equine model of septic arthritis. Each was significantly upregulated in synovial fluid collected from horses with septic arthritis at PID1, in comparison to both the same animals after infection clearance at PID4 and to horses with experimentally induced non-septic synovitis at PID1. A number of these transcripts have previously been linked to inflammatory signaling and sepsis-related immune activity.
For example, glutamine synthetase (GLUL) has been implicated in mitigating the severity of endotoxin-induced sepsis [21] and shows enhanced expression in macrophages exposed to lipopolysaccharide (LPS) [22]. Interleukin-1 receptor type 2 (IL1R2) acts as a decoy receptor, dampening IL-1–mediated signaling when expressed at higher levels [23]. Elevated IL1R2 has been observed in mice challenged with inactive E. coli and Staphylococcus aureus, as well as in human septic patients [24]. Increased expression has likewise been reported in neonates with bacterial infections [25] and in infants experiencing late-onset sepsis [26].
In our prior proteomics analysis [10], interleukin-1 receptor antagonist (IL1RN) was identified as upregulated in the synovial fluid of horses with septic arthritis relative to those with eradicated infection and non-septic inflammation. Although the IL1RN mRNA detected here exhibited slightly higher normalized counts in the septic arthritis group, it did not meet the adjusted statistical threshold for differential expression. Importantly, both IL1R2 and IL1RN inhibit IL-1 signaling, supporting the notion that suppression of this pathway could help distinguish infectious from sterile inflammatory conditions in the joint.

Another transcript of interest, interleukin-1 receptor accessory protein (IL1RAP), is required for effective IL-1 signal transduction [27]. Similarly, glycogenin-1 (GYG1) mRNA has been reported to rise significantly in blood samples from children with pneumococcal meningitis [28], suggesting its association with systemic infection responses.

Among the remaining candidates, PTK2B appears to lack infection specificity, while several other transcripts encode proteins with only predicted existence, inferred from sequence similarity. The biological significance of these genes remains to be established.

Despite these uncertainties, the 3–11× higher abundance of these eight mRNAs in septic arthritis samples (PID1) compared to post-eradication samples (PID4), together with their 2–7× elevation relative to non-septic synovitis (PID1), indicates that they could represent valuable molecular indicators for tracking infection resolution in cases of equine septic arthritis.

Comparison of Transcriptomic and Proteomic Findings

Aligning the transcriptome data from this study with our previously reported discovery proteomics dataset [10] revealed that all mRNA transcripts corresponding to the 26 proteins identified as differentially abundant were detectable. Among these, four transcripts showed higher expression in synovial fluid from horses with septic arthritis.

Neutrophil collagenase (MMP8) exhibited the most pronounced changes, showing a 22.9-fold increase when comparing septic arthritis at PID1 with post-eradication samples at PID4, and a 4.2-fold increase relative to non-septic synovitis at PID1 **(Table 3)**. These expression patterns mirrored the synovial nucleated cell counts observed.

The interleukin-1 receptor antagonist (IL1RN) transcript was also elevated in septic arthritis joints versus non-septic synovitis (fold change 9.3; $p < 0.01$) and showed higher abundance before eradication (PID1) compared to after infection resolution (PID4) (fold change 4.2; $p = 0.014$), which approached our defined significance threshold. These data reinforce the potential utility of the IL-1 signaling pathway as a source of biomarkers for both distinguishing septic from non-septic inflammation and tracking infection clearance.

Despite these observations, neither MMP8 nor IL1RN met the predefined selection criteria for putative biomarkers—specifically, a >3-fold difference between the minimum counts in septic arthritis (PID1) and post-eradication (PID4), and a >2-fold difference compared to non-septic synovitis (PID1)—and were therefore excluded from the final candidate list.

The incomplete overlap between transcriptomic and proteomic outcomes may be explained by multiple factors, including differences in sample preparation (centrifuged synovial fluid for proteomics versus whole synovial fluid for transcriptomics), post-transcriptional regulation, post-translational modifications, proteins originating from extrinsic sources (e.g., liver-derived proteins entering the joint) [9], and biological variability among individual horses.

Comparison with SeptiCyte Lab

The SeptiCyte Lab assay uses four mRNA transcripts to differentiate septic from non-septic shock in humans [12]. Although our model differs in species, tissue type, and pathology, we evaluated the overlap of transcripts. Three of the four SeptiCyte Lab transcripts were detected in equine synovial fluid, but no significant differential expression was observed between septic arthritis and post-eradication samples. Notably, PLAC8 showed higher expression in septic arthritis versus non-septic synovitis, consistent with its expected behavior in the SeptiCyte Lab panel. Divergences between SeptiCyte Lab and our findings likely reflect species-specific immune responses and the differences between whole blood (SeptiCyte Lab) and synovial fluid (current study).

Conclusion

This study performed a comprehensive transcriptomic analysis of equine synovial fluid from horses with septic arthritis, after infection eradication, and experimentally induced non-septic synovitis. From these data, eight mRNA transcripts emerged as the most promising biomarkers of infection clearance.

Interpretation of the results requires consideration of several limitations. The small group sizes contributed to variability, and the experimental model does not fully replicate clinical equine septic arthritis. Biological variation among animals was not fully controlled, and therapeutic interventions may have influenced mRNA levels.

Despite these constraints, the study establishes a foundational transcriptomic dataset for identifying molecular markers of joint infection clearance in horses and demonstrates that mRNA transcripts are a viable target for biomarker development.

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Conflict of Interest: None

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References

1. Koziy RV, Yoshimura S, Dickinson R, Rybicka JM, Moshynskyy I, Ngeleka M, et al. Use of standard diagnostic techniques to determine eradication of infection in experimental equine septic arthritis. Can J Vet Res. 2019;83(1):24–33.
2. Schneider RK, Bramlage LR, Moore RM, Mecklenburg LM, Kohn CW, Gabel AA. A retrospective study of 192 horses affected with septic arthritis/tenosynovitis. Equine Vet J. 1992;24(6):436–42.
3. Taylor AH, Mair TS, Smith LJ, Perkins JD. Bacterial culture of septic synovial structures of horses: does a positive bacterial culture influence prognosis? Equine Vet J. 2010;42(3):213–8.
4. Motta RG, Martins LSA, Motta IG, Guerra ST, de Paula CL, Bolanos CAD, et al. Multidrug resistant bacteria isolated from septic arthritis in horses. Pesqui Vet Bras (Braz J Vet Res). 2017;37(4):325–30.
5. Califf RM. Biomarker definitions and their applications. Exp Biol Med. 2018;243(3):213–21.
6. Fietz SA. Biomarkers in equine synovial fluid and serum for the diagnosis of joint diseases. Ph.D. Dissertation, Freie Universität Berlin, Berlin, Germany; 2009.
7. Rifai N, Gillette MA, Carr SA. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. Nat Biotechnol. 2006;24(8):971–83.
8. Anderson JR, Phelan MM, Foddy L, Clegg PD, Peffers MJ. Ex vivo equine cartilage explant osteoarthritis model: a metabolomics and proteomics study. J Proteome Res. 2020;19(10):3652–67.
9. Yoshimura S, Koziy RV, Dickinson R, Moshynskyy I, McKenzie JA, Simko E, et al. Use of serum amyloid A in serum and synovial fluid to detect eradication of infection in experimental septic arthritis in horses. Can J Vet Res. 2020;84(3):198–204.
10. Koziy RV, Bracamonte JL, Yoshimura S, Chumala P, Simko E, Katselis GS. Discovery proteomics for the detection of putative markers for eradication of infection in an experimental model of equine septic arthritis using LC-MS/MS. J Proteomics. 2022;261:104571.
11. McHugh L, Seldon TA, Brandon RA, Kirk JT, Rapisarda A, Sutherland AJ, et al. A molecular host response assay to discriminate between sepsis and infection-negative systemic inflammation in critically ill patients: discovery and validation in independent cohorts. PLoS Med. 2015;12(12):e1001916.
12. Miller RR, Lopansri BK, Burke JP, Levy M, Opal S, Rothman RE, et al. Validation of a host response assay, SeptiCyte LAB, for discriminating sepsis from systemic inflammatory response syndrome in the ICU. Am J Respir Crit Care Med. 2018;198(7):903–13.
13. Hart SN, Therneau TM, Zhang Y, Poland GA, Kocher JP. Calculating sample size estimates for RNA sequencing data. J Comput Biol. 2013;20(10):970–8.
14. Karunarathna R, Popowich S, Wawryk M, Chow-Lockerbie B, Ahmed KA, Yu C, et al. Increased incidence of enterococcal infection in nonviable broiler chicken embryos in western Canadian hatcheries as detected by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry. Avian Dis. 2017;61(4):472–80.
15. Chen S, Zhou Y, Chen Y, Gu J. Fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics. 2018;34(17):i884–90.
16. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2013;29(1):15–21.
17. Liao Y, Smyth GK, Shi W. The R package Rsubread is easier, faster, cheaper and better for alignment and quantification of RNA sequencing reads. Nucleic Acids Res. 2019;47(8):e47.

18. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550.
19. Steel CM. Equine synovial fluid analysis. Vet Clin North Am Equine Pract. 2008;24(2):437–54.
20. Mahaffey EA. Chapter 10 — Synovial Fluid. In: Cowell RL, Tyler RD, editors. Diagnostic Cytology and Hematology of the Horse. 2nd ed. St. Louis, MO: Mosby; 2002. p. 163–70.
21. Yu J, Zhang J, Shi M, Ding H, Ma L, Zhang H, et al. Maintenance of glutamine synthetase expression alleviates endotoxin-induced sepsis via alpha-ketoglutarate-mediated demethylation. FASEB J. 2022;36(6):e22281.
22. Bianchi MG, Chiu M, Taurino G, Bergamaschi E, Cubadda F, Macaluso GM, et al. The TLR4/NFκB-dependent inflammatory response activated by LPS is inhibited in human macrophages pre-exposed to amorphous silica nanoparticles. Nanomaterials. 2022;12(13):2307.
23. Colotta F, Dower SK, Sims JE, Mantovani A. The type II 'decoy' receptor: a novel regulatory pathway for interleukin 1. Immunol Today. 1994;15(12):562–6.
24. Lang Y, Jiang Y, Gao M, Wang W, Wang N, Wang K, et al. Interleukin-1 receptor 2: a new biomarker for sepsis diagnosis and gram-negative/gram-positive bacterial differentiation. Shock. 2017;47(1):119–27.
25. Smith CL, Dickinson P, Forster T, Craigon M, Ross A, Khondoker MR, et al. Identification of a human neonatal immune-metabolic network associated with bacterial infection. Nat Commun. 2014;5(1):4649.
26. Ng S, Strunk T, Lee AH, Gill EE, Falsafi R, Woodman T, et al. Whole blood transcriptional responses of very preterm infants during late-onset sepsis. PLoS ONE. 2020;15(6):e0233841.
27. Huang J, Gao X, Li S, Cao Z. Recruitment of IRAK to the interleukin 1 receptor complex requires interleukin 1 receptor accessory protein. Proc Natl Acad Sci U S A. 1997;94(24):12829–32.
28. Kulohoma BW, Marriage F, Vasieva O, Mankhambo L, Nguyen K, Molyneux ME, et al. Peripheral blood RNA gene expression in children with pneumococcal meningitis: a prospective case–control study. BMJ Paediatr Open. 2017;1(1):e000092.