

# Equine synovial sepsis laboratory submissions yield a low rate of positive bacterial culture and a high prevalence of antimicrobial resistance

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## OBJECTIVE

To investigate (1) variables associated with the likelihood of obtaining a positive culture, (2) commonly isolated microorganisms, and (3) antimicrobial resistance patterns of isolates from horses with presumptive synovial sepsis.

## SAMPLES

Synovial fluid, synovium, and bone samples from equine cases with presumptive synovial sepsis submitted to the Cornell University Animal Health Diagnostic Center from 2000 to 2020 for microbial culture and antimicrobial sensitivity testing.

## PROCEDURES

Univariable and multivariable analyses were performed to determine the effect of variables on the likelihood of positive culture. Frequency distributions for isolated organisms and antimicrobial resistance were generated. Multidrug resistance patterns and associations were assessed with association rule mining.

## RESULTS

The positive culture rate for all samples was 37.4%, while the positive culture rate among samples confirmed to be septic by a combination of clinical pathological variables and case details was 43%. Blood culture vial submissions were 1.7 times more likely to yield a positive culture compared to samples submitted in a serum tube. Structure sampled, tissue submitted, and horse age were associated with a positive culture. *Staphylococcus* spp (23.7%), *Streptococcus* spp (22.4%), and *Enterococcus* spp (9.67%) were commonly isolated. Multidrug resistance prevalence decreased from 92% (2000 to 2009) to 76% (2010 to 2020) of gram-negative isolates and 60% (2000 to 2009) to 52% (2010 to 2020) of gram-positive isolates.

## CLINICAL RELEVANCE

The positive culture rate from synovial fluid submissions with traditional sampling and culture methods remains low and may be optimized by submitting samples in blood culture vials. Overall, antimicrobial resistance was frequently observed but did not increase from the first to second decade for most genera.

Septic synovitis, which includes septic arthritis, bursitis, and tenosynovitis, is a common emergency in horses evaluated by both primary and tertiary care veterinarians. Survival rates range from 42 to 98%. Factors associated with a poor prognosis include elevated pre- and postoperative synovial fluid total protein concentrations, induction of anesthesia outside of normal working hours, presence of moderate to severe synovial inflammation or pannus at

time of surgery, multiple endoscopic surgeries, bone or tendon involvement, multiple infected synovial structures, treatment with doxycycline, and infections with gram-negative bacteria, particularly those with multidrug resistance (MDR).<sup>1-6</sup> Performance-limiting complications include irreversible cartilage loss, intrathecal adhesions, and rapidly progressing osteoarthritis.<sup>7</sup> Current treatment recommendations include local and systemic antimicrobial therapy;

synovial lavage with or without arthroscopic, bursectoscopic, or tenoscopic treatment; and administration of analgesics.<sup>6-9</sup> Ideally, antimicrobial selection is informed by culture and sensitivity results.

Previously reported positive culture rates from equine synovial sepsis submissions range from 25 to 70%.<sup>3,10,11</sup> Using enrichment broth increases the likelihood of yielding a positive culture in horses, dogs, and humans.<sup>3,10,12-16</sup> In 1 study,<sup>13</sup> enrichment in blood culture from equine synovial fluid yielded a positive culture rate of 78.9% compared to 23.3% from the same samples using traditional direct agar culture. However, epidemiological investigation of case and submission variables associated with the likelihood of positive culture from samples submitted from both primary and referral hospitals to a centralized diagnostic laboratory is limited. These data could help guide clinicians and diagnostic laboratories to enhance the diagnostic value of synovial culture and sensitivity. Empirical antimicrobial selection is often required while awaiting culture and sensitivity results. The use of broad-spectrum systemic antimicrobials, such as penicillin and an aminoglycoside, in addition to local treatment with intrasynovial and/or intravenous regional limb perfusion with an aminoglycoside is common in equine medicine.<sup>13,17</sup>

Historically, *Enterobacteriaceae* spp were the most isolated bacteria from equine septic synovial structures,<sup>11</sup> but reports<sup>10</sup> from 2010 and 2016 isolated *Staphylococcus aureus* most commonly. These more recent reports<sup>10</sup> exclusively studied adult horses, while the older study<sup>11</sup> included both foals and adult horses. All 3 studies<sup>3,10,11</sup> evaluated cases of synovial sepsis treated at referral hospitals. *Staphylococci* and *Streptococci* spp are more commonly isolated from cases of iatrogenic synovial sepsis, while *Enterobacteriaceae* spp are more likely to be associated with sepsis caused by a traumatic wound.<sup>7,11</sup> Literature regarding antimicrobial resistance (AMR) in equine synovial sepsis is scarce. One study<sup>3</sup> reported that the most effective antimicrobials for initial empirical treatment against both gram-positive and gram-negative bacteria are doxycycline, oxytetracycline, gentamicin, and trimethoprim sulphamethoxazole. This study reported a low incidence of MDR isolates, with only 2/114 (1.75%) of samples demonstrating MDR, and both of those were methicillin-resistant *Staphylococcus aureus*. Furthermore, variation in commonly isolated organisms and resistance patterns was linked to both population demographics and geographic regions, emphasizing the importance of regional knowledge about resistance patterns.

Optimization of sample submission techniques for synovial sepsis cases will improve organism identification, aid in accurate treatment, and reduce the development of AMR. This information has the potential to benefit not only veterinary and human medical professionals treating synovial sepsis, but all who are working to reduce the effect of AMR on veterinary, human, and environmental health. The implications of this article in relation to the body of literature regarding synovial sepsis and one health are further

explored in the companion Currents in One Health editorial by Pearson et al, *JAVMA*, August 2023.

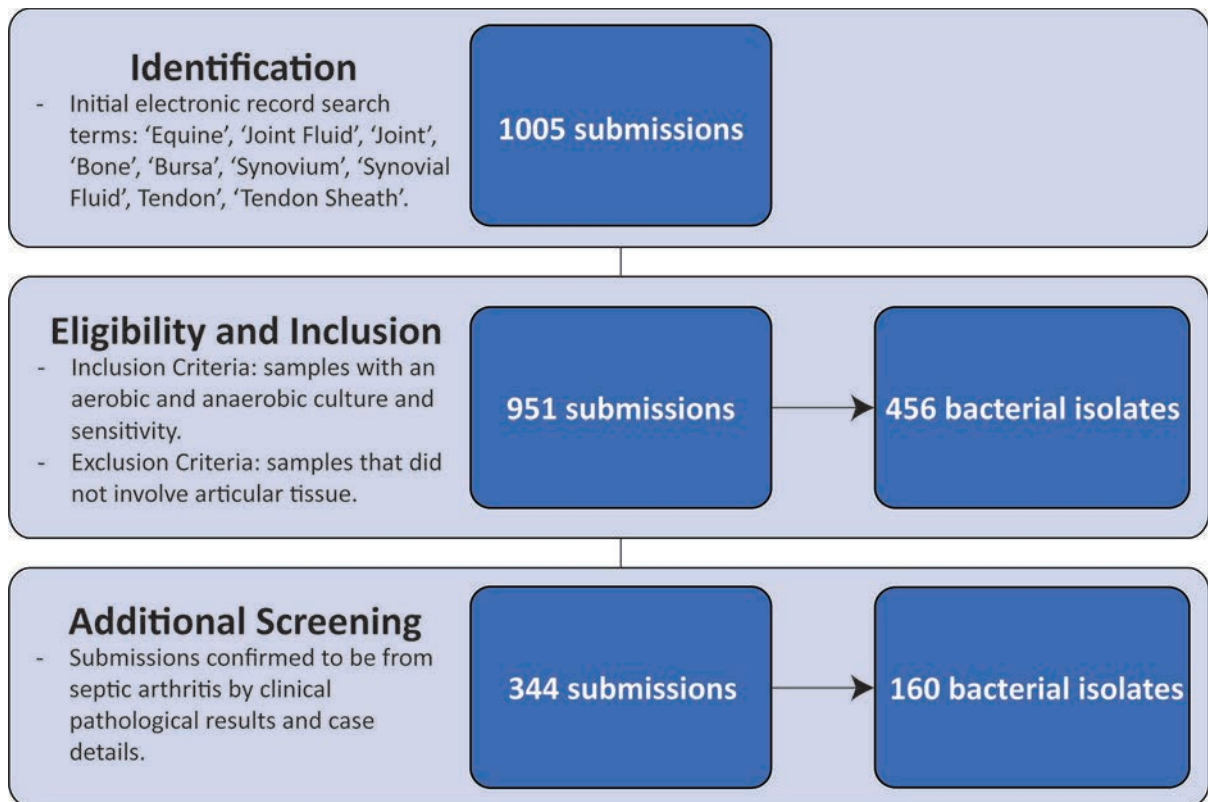
The objectives of this study were to investigate (1) the factors associated with the likelihood of obtaining a positive culture, (2) the most frequently isolated microorganisms, and (3) AMR patterns of isolates from equine cases with presumptive synovial sepsis. This information may help inform future techniques to enhance positive culture rates in equine synovial sepsis and provide insight to guide empirical antimicrobial selection.

## Methods

### Case selection and data retrieval

Submissions from cases of presumptive equine synovial sepsis made to the Cornell University Animal Health Diagnostic Center (AHDC) from January 1, 2000, to December 31, 2020, were identified using the following search terms: "Equine," "Joint Fluid," "Joint," "Bone," "Bursa," "Synovium," "Synovial Fluid," "Tendon," and "Tendon Sheath." Inclusion criteria included samples with an aerobic and anaerobic culture and sensitivity submitted to the AHDC. Submissions were excluded if they did not involve articular tissue or were labeled as non-articular infected tissue (abscess or sequestrum) (**Figure 1**). Data retrieved, when available, included signalment (age, breed, or sex), submitting facility (external veterinary practices or a Cornell-affiliated hospital), year of submission, affected anatomical structure, submitted tissue (synovial fluid, synovium, or bone), suspected cause of infection, submission container, identified organism, and antimicrobial susceptibility report. Age was recorded as a continuous variable. The submitting facility was recorded as either an external veterinary practice or a Cornell-associated hospital (Cornell University Hospital for Animals Equine and Nemo Farm Animal Hospital [ENFAH] and Cornell Ruffian Equine Specialists [CRES]). Submitted tissue was recorded as synovial fluid, synovium, or bone. The suspected cause of infection was determined from accompanying submission paperwork or by the medical record and was classified as hematogenous, iatrogenic, wound, idiopathic, or unknown, meaning the information was not available. The resistant or susceptible interpretation for each antimicrobial tested was extracted from the susceptibility report. All submissions that fit the inclusion and exclusion criteria listed above were included in descriptive statistical analysis.

**Positive culture rate methodology for all samples**—For the positive culture rate analysis, submissions were classified as positive if 1 or more organisms were isolated. Data analysis was performed for all samples that met the inclusion and exclusion criteria above to ensure consistency across submissions. Since medical record information was not available from external veterinary practices, cases could not be definitively classified as synovial sepsis based on historical information, clinicopathological data, clinical progression, or gross pathologic evaluation.



**Figure 1**—Collection and inclusion process for submissions included in this study.

**Positive culture rate methodology for Cornell-affiliated hospitals**—To improve the accuracy of the true positive culture rate, analyses were limited to those submissions from Cornell-affiliated hospitals determined to be septic on the bases of historical information, clinicopathological data, clinical progression, or gross pathologic evaluation from the hospital electronic medical record review. Data retrieved included history, synovial fluid analysis (location sampled, sample type, submission container, synovial fluid color, total nucleated cell count, neutrophil count, total protein concentration, lactate concentration, and serum amyloid A concentration), and treatment. These samples were classified as confirmed cases of synovial sepsis if they met 1 or more of the following criteria: a positive bacterial culture and/or observation of bacteria on a cytological smear; a wound demonstrated to enter a synovial structure; elevation of synovial fluid parameters (nucleated cell count > 10,000 cells/uL and > 80% neutrophils); suppurative or fibrinous inflammation within the synovial structure demonstrated during postmortem exam; or clinical diagnosis of synovial sepsis by the attending clinician.

## Bacteriology

Detailed methods regarding bacterial culture and antimicrobial sensitivity testing are reported (**Supplementary Appendix S1**). Briefly, samples for aerobic culture were directly inoculated onto varying agar and enriched. All were evaluated for bacterial growth at 24 and 48 hours postincubation. Identification of isolated bacteria was performed

either using the Sensititre automated bacterial identification system (GNID/GPID; Thermo Scientific) in combination with 16S rRNA sequencing PCR as needed or by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Susceptibility testing was performed using the Sensititre system according to the recommendations of the manufacturer. Interpretations of MICs were performed in accordance with guidelines and breakpoints established by the Clinical Laboratory Standards Institute at the time of the culture.

## Statistical analysis

Clinical, bacteriological, and susceptibility data were stored in spreadsheet software (Microsoft Office Excel 2023), and statistical analysis was performed using JMP Pro 16. Statistical distributions were evaluated using Q-Q plots and Shapiro-Wilk normality tests. The proportion of positive culture samples and 95% CI by age, submitting facility, decade, affected structure, submitted tissue, suspected cause of infection, and submission container were reported for all submissions and for submissions from confirmed cases of synovial sepsis from Cornell-affiliated hospitals. The effect of the hospital on the proportion of samples being submitted in a blood culture vial was evaluated using a logistic regression model. The association between dichotomized age and cause of infection was evaluated using a logistic regression model.

**Positive culture rate analysis and microorganism descriptive statistical analysis**—Due to CRES submissions only being available from 2014 to 2020

and not the earlier decade, these samples were excluded from the univariate and multivariate model statistical analysis. Univariate analysis, using Pearson chi-squared tests with statistical significance set to  $\alpha < 0.05$ , was performed to evaluate differences in culture positive rate with respect to the following variables: age (continuous), decade, cause of infection, submission container, submitted tissue, and structure affected. When statistical significance was observed, a Bonferroni adjustment was performed for individual contrasts. Multivariate analysis was performed using a backward stepwise approach. Variables that had  $P$  values of  $\leq .1$  from the univariate analysis were considered for inclusion in the logistic regression model. Variables were retained if they significantly reduced the residual deviance of the model (likelihood ratio statistic;  $P < .05$ ). Goodness-of-fit was assessed graphically by plotting the deviance residuals versus the individual observations.

**MDR analysis**—The bacterial isolate data with resistant/susceptible interpretations (407 isolates tested against 30 antimicrobials), and antimicrobial class specifications, were transferred to a data warehouse in Microsoft SQL Server. Isolates without sensitivity test results were excluded.

Trends in the prevalence of resistance to each antimicrobial class, MDR, and coexisting resistance patterns for gram-positive and gram-negative bacteria were analyzed for each study period (2000 to 2009 and 2010 to 2020). MDR was defined as resistance to 3 or more antimicrobial classes, and an isolate was considered resistant to an antimicrobial class if it was resistant to at least 1 antimicrobial within the class. Coexisting resistance patterns were combinations of 2 antimicrobial resistances that occur together in an isolate; the number of isolates containing each resistance pattern was tabulated for each gram classification and study period. For calculating the MDR prevalence and coexisting resistance, all beta-lactams, including carbapenems, penicillins, and cephalosporins, were considered as 1 class. Chi-square tests were conducted to test for changes in antimicrobial class prevalence and MDR prevalence across study periods. Statistical significance was set at  $P = .10$ .

Association rule mining was used to characterize MDR phenotypes. An association rule is represented in the form  $X \rightarrow Y$ , where  $X$  is called the antecedent and  $Y$  is called the consequent. In this case,  $X$  and  $Y$  are 1 or more antimicrobial resistances. Three measures are used to define the relevance of association rules: support (prevalence of  $X$  and  $Y$  occurring together), confidence (the proportion of isolates that contain  $X$  resistances that also contain  $Y$  resistances), and lift (a ratio of the probability of  $X$  and  $Y$  occurring together compared to the condition of  $X$  and  $Y$  being independent; lift  $> 1$  indicates a correlation between  $X$  and  $Y$ ). Association rules containing resistances to antimicrobials commonly used in horses (gentamicin, chloramphenicol, penicillin, trimethoprim-sulfamethoxazole, tetracycline, sulphadimethoxine, enrofloxacin, and amikacin) that had at least 0.05 support, 0.1 robust association rules support, 0.8 confidence, and 1.5 lift were extracted for each gram

classification and decade and additional details are reported (Supplemental Appendix S1). Associations between antimicrobials were visualized using Chord diagrams, with the number of arcs proportional to the summed lift for each antimicrobial pairing.

## Results

### Case selection

The initial electronic record search yielded 1,005 submissions to the AHDC during the study period, of which 951 fit the inclusion criteria and 356/951 (37.4%) yielded a positive culture. The 54 samples that were excluded were comprised of submissions that did not involve a synovial structure. The breed distribution included 283 Thoroughbreds (29.8%), 147 Quarter Horses (15.5%), 136 Standardbreds (14.3%), 128 warmbloods (13.5%), and 41 other breeds. The median horse age was 4 years (range, 0.1 to 33). Sex distribution was 355 mares (37.3%), 355 geldings (37.3%), 196 stallions (20.6), and 45 of unknown sex. Frequency distributions for age, submitting facility, year of submission, affected anatomical structure, submitted tissue, suspected cause of infection, and submission container are represented (**Table 1**). Additional frequency distributions for affected anatomical structures are reported (**Supplementary Table S1**).

Of the cases submitted from Cornell-affiliated hospitals, 344/434 (79.3%) were confirmed cases of synovial sepsis; 148/344 (43.0%) of confirmed cases yielded a positive culture. Blood culture vial submission comprised 14% of all external submissions across both decades and 53% of all submissions from ENFAH samples ( $P < .001$ ).

### Age, synovial structure type, and submitted tissue affect the rate of positive cultures

Univariate and multivariable analysis results are summarized (**Table 2**). The effect of demographic biological variables, including horse age, sex, and breed, on positive culture rate was evaluated. Every 1-year increase in age was associated with a 4% decrease in the odds of a positive culture ( $P = .001$ ). To ensure that this was not due to the proportion of foals included, horse age was dichotomized into immature ( $\leq 1$  year) or mature ( $> 1$  year) animals and was not associated with a positive culture rate. No other demographic biological variable influenced the likelihood of positive culture.

In addition to demographic variables, variables related to etiopathogenesis and sample handling were evaluated. Submissions in a blood culture vial were more likely to yield a positive culture compared to samples submitted in a serum tube (OR, 2.19; 95% CI, 1.21 to 3.95;  $P = .009$ ). Submissions including bone were more likely to result in a positive culture compared to synovial fluid (OR, 6.92; 95% CI, 3.49 to 13.74;  $P < 0.0001$ ). Submissions involving a bursa were more likely to yield a positive culture compared to submissions from a joint (OR, 2.6; 95% CI, 1.43 to 4.74;  $P = .002$ ). There were no significant associations

**Table 1**—Proportion of culture-positive samples by age, submitting facility, year of submission, affected anatomical structure, submitted tissue, suspected cause of infection, and submission container.

	Percent culture positive (n)	95% CI
Overall	37.4 (951)	34.4, 40.6
Age		
< 1	45.1 (204)	38.4, 52
> 1	39.3 (578)	39.4, 43.3
Total	40.8 (782)	
Submitting facility		
ENFAH	37.6 (407)	33.0, 42.4
CRES	77.8 (27)	59.2, 89.3
External	35.2 (517)	31.2, 39.4
Total	36.4 (951)	
Decade		
2000–2009	38.7 (398)	34, 43.6
2010–2020	36.5 (553)	32.6, 40.6
Total	37.4 (951)	
Affected anatomical structure		
Bursa	52.1 (48)	38.3, 65.5
Tendon sheath	40.2 (72)	29.7, 51.8
Joint	29.4 (482)	25.6, 33.7
Total	32.6 (598)	
Submitted tissue		
Synovial fluid	33.3 (859)	30.2, 36.5
Bone	77.6 (49)	64.1, 86.9
Synovium	56 (25)	37.1, 73.3
Total	36.2 (933)	
Suspected cause of infection		
Hematogenous	46.7 (60)	34.6, 59.1
Iatrogenic	39.4 (66)	28.5, 51.5
Idiopathic	33.8 (74)	24.0, 45.1
Wound	40.2 (194)	33.5, 47.2
Unknown	35.7 (557)	31.8, 39.8
Total	37.4 (951)	
Submission container		
Amies transport media	26.3 (80)	17.9, 36.8
Blood culture	40 (95)	30.7, 50.1
Other	34.9 (43)	22.4, 49.8
Serum tube	23.3 (120)	16.6, 31.6
Total	30.2 (338)	

CRES = Cornell Ruffian Equine Specialists. ENFAH = Cornell University Hospital for Animals Equine and Nemo Farm Animal Hospital.

Frequency distribution of affected anatomical structure is reported (Supplementary Table S1).

between the cause of sepsis and positive culture rate. The final multivariable logistic regression model included 3 variables: age, affected structure, and submitted tissue (Table 2). Bursas had 3.24 times ( $P = .002$ ) and tendon sheaths had 1.81 times ( $P = .04$ ) the odds of yielding a positive culture compared to joints. Bone samples had 8.51 times ( $P < .0001$ ) the odds of yielding a positive culture compared to synovial fluid. Every 1-year increase in horse age was associated with a 5% decrease in the odds of obtaining a positive culture result ( $P = .003$ ).

### ***Staphylococcus spp*, *Streptococcus spp*, and *Enterococcus spp* are the most commonly isolated bacteria**

The frequency of isolated bacteria (genus and species) is reported (Supplementary Table S2). Of

**Table 2**—Variables evaluated for effect on positive culture rate.

	OR (95% CI)	P value
Univariable logistic regression		
Categorical variables		
Anatomical structure		.003
Bursa vs joint	2.60 (1.43–4.74)	.002
Submitted tissue		< .0001
Bone vs synovial fluid	6.92 (3.49–13.74)	< .0001
Submission container		.04
Blood culture vs serum tube	2.19 (1.21–3.95)	.009
Cause of infection		.4
Hospital	0.90 (0.68–1.18)	.5
Continuous variable		
Age	0.96 (0.94–0.99)	.001
Multivariable logistic regression		
Age	0.95 (0.92–0.98)	.003
Anatomical structure		
Bursa	3.24 (1.54–6.82)	.002
Tendon sheath	1.81 (1.02–3.21)	.04
Joint	Reference level	Reference level
Submitted tissue		
Bone	8.51 (3.17–22.89)	< .0001
Synovium	1.82 (0.55–5.95)	.3
Synovial fluid	Reference level	Reference level

Univariable logistic regression models and multivariable logistic regression models are shown. Only statistically significant univariate comparisons are reported.

the 43 bacterial genera identified, 22 were gram positive (304/456, 66.7% of total isolates) and 21 were gram negative (148/456, 32.5% of total isolates). The 5 most common bacterial genera were *Staphylococcus spp* (108/456, 23.7%), *Streptococcus spp* (102/456, 22.4%), *Enterococcus spp* (44/456, 9.67%), *Escherichia spp* (43/456, 9.45%), and *Actinobacillus spp* (27/456, 5.93%). *Enterococcus spp* was overrepresented in the population of submissions from external veterinary practices (34/264, 12.9%) when compared to those submitted from the ENFAH (6/160, 3.8%). Cases of synovial sepsis caused by wounds were more likely to culture *Streptococcus spp* (35/90, 38.9%) than *Staphylococcus spp* (17/90, 18.9%,  $P = .04$ ) and other identified spp (20/90, 22.2%,  $P = .03$ ) when compared to iatrogenic cases of synovial sepsis (*Streptococcus spp* 3/29, 10.3%; *Staphylococcus spp* 11/29, 38%; other identified spp 12/29, 41.4%).

### **AMR**

Overall, MDR decreased from 91.53% to 75.95% ( $P = .02$ ) for gram-negative bacteria and trended down from 60.22% to 51.70% ( $P = .18$ ) for gram-positive bacteria after 2010. A similar trend in reduced MDR isolates was observed for submissions from external veterinary facilities: from 50.91% to 48.91% ( $P = .82$ ) for gram-positive and from 93.94% to 79.31% ( $P = .06$ ) for gram-negative bacteria. The prevalence of resistance to each antimicrobial class is reported (Table 3). Gram-negative isolates showed

**Table 3**—Prevalence of resistance by antimicrobial classes.

Antimicrobial class	Gram negative (n = 138)		Gram positive (n = 269)	
	Prevalence (resistant/ tested) 2000–2009 (n = 59)	Prevalence (resistant/ tested) 2010–2020 (n = 79)	Prevalence (resistant/ tested) 2000–2009 (n = 93)	Prevalence (resistant/ tested) 2010–2020 (n = 176)
Aminoglycosides	47.46 (28/59) <sup>a</sup>	19.23 (15/78) <sup>a</sup>	66.67 (62/93)	66.86 (115/172)
Amphenicols	18.64 (11/59)	20.51 (16/78)	13.04 (12/92) <sup>a</sup>	6.90 (12/174) <sup>a</sup>
Ansamycin	68.97 (40/58)	78.26 (54/69)	7.53 (7/93) <sup>b</sup>	15.09 (24/159) <sup>b</sup>
Beta lactam	20.34 (12/59)	15.79 (12/76)	18.28 (17/93)	19.39 (32/165)
Carbapenems	0.00 (0/59)	0.00 (0/77)	8.60 (8/93)	8.00 (14/175)
Cephalosporins	32.20 (19/59) <sup>b</sup>	47.44 (37/78) <sup>b</sup>	33.33 (31/93)	35.43 (62/175)
Fluoroquinolones	8.47 (5/59)	13.04 (9/69)	45.05 (41/91)	49.34 (75/152)
Macrolides	93.88 (46/49)	95.71 (67/70)	34.41 (32/93) <sup>a</sup>	24.28 (42/173) <sup>a</sup>
Penicillins	89.83 (53/59) <sup>b</sup>	97.47 (77/79) <sup>b</sup>	41.94 (39/93)	39.77 (70/176)
Sulfonamides	42.37 (25/59)	50.00 (39/78)	41.94 (39/93) <sup>a</sup>	30.59 (52/170) <sup>a</sup>
Tetracyclines	22.03 (13/59)	28.21 (22/78)	43.48 (40/92)	46.86 (82/175)

Lincosamide resistance was excluded because it was only tested after 2010.

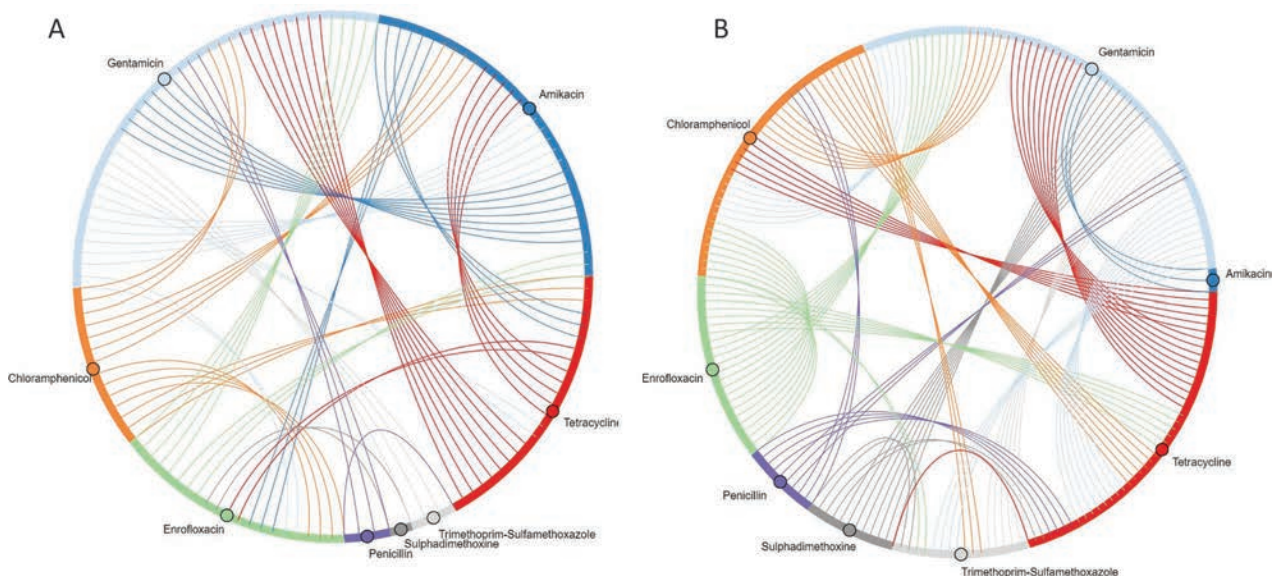
<sup>a</sup>Significant ( $P < .1$ ) decreases in resistance over time. <sup>b</sup>Significant ( $P < .1$ ) increases in resistance.

increased resistance to both cephalosporins and penicillins after 2010 ( $P < .1$ ), while gram-positive isolates showed increased resistance to rifampin only ( $P < .1$ ).

Coexisting resistance patterns among gram-negative bacteria were largely stable over time; 8 of 10 most common patterns before 2010 in gram-negative bacteria are also included among those after 2010 (**Supplementary Table S3**). In gram-positive isolates, the coexistence of erythromycin resistance with other resistances (gentamicin, amikacin, and tetracycline) was more common before 2010 than after 2010 (**Supplementary Table S4**).

To determine which antimicrobial resistances were highly connected, we employed chord diagrams to visualize the MDR association rules. Among gram-positive bacteria (**Figure 2**), aminoglycosides,

chloramphenicol, enrofloxacin, and tetracycline are all highly connected to each other. This suggests that many gram-positive bacteria have resistance to at least 2 of these antimicrobials. Examining individual rules (**Supplementary Table S5**) confirms that approximately one-quarter of isolates have resistance to enrofloxacin, tetracycline, and gentamicin, and the relationship between these resistances is largely unchanged between 2000 to 2009 and 2010 to 2020. Although chloramphenicol resistance is highly connected to other resistances, the specific associations between chloramphenicol and other resistances changed between the 2 decades (i.e., there are different association rules that contain chloramphenicol in 2000 to 2009 and 2010 to 2020). The strongest correlation (i.e., highest total



**Figure 2**—A—Associations of antimicrobial resistance in gram-positive bacteria. Antimicrobials are colored and ordered by class. The arc is colored by the antecedent in the antecedent-consequent pair. The number of arcs is proportional to the total lift of the antecedent-consequent pair. B—Associations of antimicrobial resistance in gram-negative bacteria. Antimicrobials are colored and ordered by class. The arc is colored by the antecedent in the antecedent-consequent pair. The number of arcs is proportional to the total lift of the antecedent-consequent pair.

lift values shown as the greatest number of arcs) occurred between tetracycline and aminoglycoside resistance. Multiple association rules that were the same across decades (**Supplementary Table S6**) also featured aminoglycoside resistance associated with tetracycline resistance.

Gram-negative bacteria had several highly connected resistances: tetracycline, gentamicin, enrofloxacin, chloramphenicol, and sulfonamides (Figure 2). However, only associations between 2 antimicrobials were the same across decades (Supplementary Table S6), suggesting that the relationships between 3 or more resistances are variable. Enrofloxacin resistance had the highest total lift and was strongly associated with chloramphenicol, gentamicin, and tetracycline resistance. The association between trimethoprim-sulfamethoxazole resistance and several other resistances (gentamicin, tetracycline, chloramphenicol) decreased from 2000 to 2009 to 2010 to 2020.

## Discussion

The overall positive culture rate (37.4%) and confirmed septic synovitis positive culture rate (43%) in this study are similar to those reported in 2010<sup>10</sup> and 2016<sup>5</sup> but lower than the 72% rate reported in 1992.<sup>11</sup> In these studies, all submissions were from cases of clinically diagnosed synovial sepsis. In the present study, a complete history or medical record was not available for cases submitted from external veterinary practices to ensure each submission was from a confirmed case of synovial sepsis. Additionally, it is possible that a higher proportion of external submissions received antimicrobials before sample collection, which has been proposed to decrease the positive culture rate in some studies<sup>18,19</sup> but not others.<sup>10</sup> Across all species, positive culture rates for synovial sepsis samples tend to be much lower than other body fluids, such as abdominal, pleural, and cerebral spinal fluid.<sup>20</sup> Challenges with culturing synovial fluid from cases of sepsis have been attributed to multiple factors, including difficulties obtaining sufficient volumes of synovial fluid, presence of a biofilm, and low bacterial load.<sup>18,21,22</sup> The persistence of low positive culture rates despite the use of better submission containers such as blood culture vials warrants continued investigation to optimize sampling and laboratory procedures to more efficiently and accurately diagnose septic synovitis, including consideration of parallel and synergistic detection methods, such as 16S ribosomal RNA sequencing.

Univariate analysis revealed that samples submitted in a blood culture vial were more likely to yield a positive culture compared to samples submitted in a red top tube, consistent with previous research in both horses and other species. A 2020 study<sup>16</sup> in humans reported positive culture rates of up to 76.4% using blood culture vials and 62.3% using blood agar plates in cases of septic arthritis. Studies in horses have also revealed improved culture rates from blood culture vials (78.9%) compared to traditional agar methods (23.3%)<sup>13</sup> and blood culture vials

(77.6%) as compared to agar plate methods (37.8%).<sup>23</sup> The higher positive culture rate from blood agar reported in the human literature may be explained by a greater likelihood of the sample being taken before initiation of antimicrobials or potentially more stringent or regulated sampling practices. In the current study, blood culture vial submissions from ENFAH were 3.7-fold greater than from external veterinary practices. This may reflect the availability of blood culture vials and factors associated with transport and delivery to the diagnostic laboratory, including additional challenges with shipping these samples to diagnostic laboratories. However, the present study and prior work support the use of blood culture vials to maximize the likelihood of a positive synovial fluid culture from cases of suspected synovial sepsis, and the current study revealed that red top vials yielded the lowest likelihood of a positive synovial fluid culture.

Multivariable analysis revealed that 3 variables were associated with a positive culture in the population of submissions evaluated: age, synovial structure type, and submitted tissue. The decrease in odds of a positive culture seen with increasing age may be due to the greater comfort of practitioners attempting to treat adult horses with antimicrobials before collecting a sample or to a more developed adult immune system resulting in a reduced bacterial load. Submissions from joints were less likely to culture positive than submissions from bursae and tendon sheaths. This could be attributed to the difficulty of sampling bursae and tendon sheaths in the absence of severe infection. A prolonged and more severe infection may be more likely to enable bursae or tendon sheath sampling and more likely to culture positive due to a higher density of bacteria. To our knowledge, this association between structure affected and positive culture rate has not been previously reported. Conversely to a previous study<sup>12</sup> suggesting that synovial fluid culture was more likely to yield a positive culture than synovium, submissions including synovial fluid alone were less likely to culture positive than submissions containing synovium or bone in the current study. This is likely, in part, due to the increasing severity of infection that would warrant more invasive sampling but also due to the increased likelihood of trapping bacteria in fibrin and tissue samples. Additionally, this supports the collection of synovial membrane samples during arthroscopic or open lavage to increase the likelihood of a positive culture.

This study is consistent with prior publications<sup>3,10,11</sup> from referral hospitals in both the US and the UK from 1992 to 2016 that have revealed *Enterobacteriaceae* spp, *Staphylococci* spp, and *Streptococci* spp as the most commonly cultured bacterial genera. The majority of equine synovial sepsis literature<sup>24</sup> published before 2010 reported a predominance of gram-negative organisms, which could be due to including a high proportion of septic synovitis from wounds, in addition to including foals. However, the predominance of gram-positive isolates seen in the present study is consistent with what has

been reported since 2010 and suggests that patterns in bacterial isolates from equine synovial sepsis may not differ substantially based on geographic location. The increase in the proportion of *Enterococcus* spp cultured from external hospitals compared to ENFAH may be a result of variation between a clinical setting or a difference in sampling technique.

The prevalence of MDR organisms was high in this study population throughout the study period (60.22% to 51.70% for gram-positive bacteria and 91.53% to 75.95% for gram-negative bacteria). However, information regarding previous antimicrobial therapy before culture is missing in many of these cases and may explain the high prevalence of AMR and MDR observed. Until now, MDR isolates in equine medicine have been thought to be rare.<sup>25,26</sup> Previously reported prevalences of MDR in equine synovial sepsis ranged from 1.8% to 24.4% to 27.2% of isolates.<sup>3,27,28</sup> It is important to note that the reported prevalence of MDR is dependent on the specific antimicrobial drugs and the number of classes tested; if more drugs and classes are tested, the reported prevalence of MDR will likely be higher. In this study, we had susceptibility results from 30 antimicrobials representing 8 classes. One study<sup>3</sup> evaluated 14 antimicrobials from 7 classes and do not state their MDR definition, another study<sup>27</sup> evaluated 9 antimicrobials from 6 classes and used the typical MDR definition of resistance to 3 or more classes, and another study<sup>28</sup> evaluated 17 antimicrobials from 8 classes and used a different MDR definition of resistance to 5 or more classes. The decrease in the prevalence of MDR organisms, both gram positive and negative, is not consistent with the increase in MDR isolates reported in the human literature over the last 10 years.<sup>29,30</sup> It is important to note that all gram-negative bacteria were analyzed together, and some gram-negative bacteria are more likely to be MDR than others. Therefore, the decrease in MDR could be due to changes in the bacterial species or strains cultured and not reflective of MDR decreases within bacterial species. Nonetheless, the prevalence of MDR isolates reported in this study is still very high. AMR has implications not only for veterinary medicine but also for human medicine due to close contact between horses and their owners and the possibility of environmental contamination. One-health considerations relevant to AMR include the implications of antimicrobial prescribing and environmental contamination on antimicrobial resistance, the transfer of resistance mechanisms between bacteria in multiple species, and the interspecies transfer of resistant isolates. These considerations are explored in detail in the companion Currents in One Health by Pearson et al, *JAVMA*, August 2023.

The prevalence of resistance to individual antimicrobial classes, particularly those used frequently in the treatment of septic synovitis, such as penicillins, cephalosporins, sulfonamides, and aminoglycosides, was also quite high (Table 3). Penicillin and cephalosporins are first-line medications frequently used in equine practice, typically combined with an aminoglycoside. Both penicillins and cephalosporins

primarily target gram-positive bacteria with limited gram-negative coverage, making the gram-negative results for these specific antimicrobials of less relevance. An increase in MDR prevalence in gram-positive isolates for ansamycins (rifampin) was seen after 2010. While most of the isolates in this study were not *Rhodococcus equi* cases, this increasing resistance to rifampin is consistent with recent results suggesting increased resistance to antimicrobial classes commonly used for *R equi* prophylaxis in foals.<sup>31-33</sup>

Coresistance patterns remained relatively stable throughout the study period. In the gram-negative population, this consisted of combinations of frequently used antimicrobials including tetracyclines, gentamicin, amikacin, and enrofloxacin. While this is intuitive as these drugs are often used in combination or sequentially, similar patterns of resistance across drug classes presents a specific challenge. Resistance to fluoroquinolones has been reported to be increasing.<sup>34</sup> While the present study did not identify a substantial increase in the prevalence of fluoroquinolone resistance (Table 3), enrofloxacin resistance had the highest total lift of antimicrobials evaluated and strong associations with chloramphenicol, gentamicin, and tetracycline were noted. This may be attributed to the fact that often enrofloxacin is often utilized after treatment with other antimicrobials has already been attempted with response failure.

16S ribosomal gene sequencing by polymerase chain reaction has become more prominent in human and veterinary medicine for more rapid and accurate diagnosis.<sup>35,36</sup> Limitations of 16S PCR include the lack of information regarding the viability of the organisms and the detection of multiple isolates resulting in challenges in identifying relevant bacteria. At this time, 16S PCR should probably always be performed in combination with traditional culture methods.<sup>37</sup>

The main limitations of this study include the inconsistency of available clinical and submission data and the inability to confirm the diagnosis of septic synovitis in cases submitted by external veterinarians. Although the sample size (951 submissions) is greater than previous equine synovial sepsis reports, the substantial diversity in bacterial species represented (Supplementary Table S3) and the small number of submissions per year limit our ability to analyze specific bacterial species or trends over time. Incomplete information for each submission, including submission container and culture and sensitivity results for some samples, resulted in variable numbers of submissions available for analysis. Specifically, submission container was not commonly recorded for cases before 2010. We used antimicrobial susceptibility interpretations from the laboratory susceptibility test reports, rather than reinterpreting the MICs with current breakpoints. Although applying current breakpoints to historical MICs is typically preferred, it also can pose problems for longitudinal studies because historical MICs are sometimes uninterpretable with current breakpoints, leading to a loss of information. The use of historic interpretations can bias the results of our resistance and MDR analysis



because antimicrobial susceptibility breakpoints may have changed throughout the duration of the study. For example, a historic isolate that was classified as susceptible to an antimicrobial with an old breakpoint may be considered resistant with a newer breakpoint. Depending on the changes in breakpoints over time, this can lead to an underestimation or overestimation of historic resistance prevalence.

## Conclusion

The positive culture rate for submissions from presumptive equine synovial sepsis cases remains low with traditional sampling and bacteriology methods. The likelihood of obtaining a positive culture may be optimized by submitting samples in blood culture vials or by including synovium when appropriate. Future studies should consider employing additional methods, such as 16S rRNA sequencing, to help facilitate diagnosis and perhaps even identification of resistance genes and patterns. Similar to past reports of equine synovial sepsis, *Staphylococci* spp, *Streptococci* spp, and *Enterobacteriaceae* spp were the predominant microorganisms, although other organisms not amenable to traditional culture techniques may be underreported given the 37.4% positive culture rate. Commonly used antimicrobials, including penicillins or cephalosporins combined with aminoglycosides, still appear to be appropriate first-line choices for treating septic synovitis, although the prevalence of resistance among gram negatives to aminoglycosides was 20% and among gram positives to cephalosporins and penicillins was 35% to 40% from 2010-2020. Future studies should continue to investigate alternative strategies for the treatment of equine synovial sepsis as AMR is a growing concern in both veterinary and human medicine.

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## Supplementary Materials

Supplementary materials are posted online at the journal website: [avmajournals.avma.org](http://avmajournals.avma.org).