International Journal of Agriculture and Environmental Research

ISSN: 2455-6939

Volume: 10, Issue: 03 "May-June 2024"

RECOVERY OF NOVEL PRESUMPTIVE HORSE THRUSH-CAUSING BACTERIA, *VAGOCOCCUS* SP. AND *CORYNEBACTERIUM* SP., IN RODEO HORSES

[1,2*]Joshua Ellison, [3]Yuhua Pang, [4*]Jing Chen, [1*]Hung King Tiong

[1]Department of Biological and Environmental Sciences, University of West Alabama, USA.

[2]Ellison Farrier Services, Calera, Alabama, USA.

[3]Department of Marketing and Finance, University of West Alabama, USA.

[4]Department of Mathematics, University of West Alabama, USA.

*Corresponding author

DOI: https://doi.org/10.51193/IJAER.2024.10301

Received: 19 Apr. 2024 / Accepted: 02 May. 2024 / Published: 22 Jun. 2024

ABSTRACT

Bacteria-attributed thrush infections of horse hoofs could be detrimental to animal performance. *Fusobacterium necrophorum* (leukotoxin-positive, *lkt*A$^+$) and *Dichelobacter nodosus* (fimbrial-protease-exporter-positive, *fim*A$^+$) bacteria were documented to associate with thrush symptomatic horse positively (i.e., partially) and negatively, respectively, following gene-specific PCR detections. However, the availability of non-*F. necrophorum* bacteria in horse thrush has been unexplored to date. The present study explored thrush prevalence and its associated bacteria in rodeo horses. A total of 16 separate swabbed and non-swabbed (i.e., scraped frog skin) samples were randomly acquired from rodeo horses of three independent open pen raisers between September 2022 and May 2023. Hoof's indigenous bacterial suspensions were cultivated anaerobically and aerobically, and isolated colonies were separately streaked for purification. Bacterial genomes were extracted using the bead collision method and identified using 16S rRNA gene sequencing. Of 16 hoof samples, 11 were healthy and five were sickened, and the latter were more prevalent at moderate temperature (i.e., 75-85 °F, 67%) or combined moderate and dry conditions (67%). 41 phenotypically-variant bacteria were differentially detected in healthy (30) and sickened (11) hoofs. Of 18 bacterial DNAs analyzed, six (33%) *lkt*A positive isolates, presumptive of *F. necrophorum* availability, were from healthy (5) and sickened (1) hoofs. Subsequently, 16S rRNA bacterial identification exhibited non-*F.*

ISSN: 2455-6939

Volume: 10, Issue: 03 "May-June 2024"

necrophorum bacteria, including animal/human pathogens. The present comparative study reveals potential positive correlations (i.e., odds ratio>1) between thrush prevalence and ambient conditions (i.e., temperature and humidity). This discovery could improve currently emerging thrush management knowledge and prevent infection-associated performance and prolonged chemical treatment complications.

Keywords: Thrush-causing bacteria, *Fusobacterium necrophorum*, Rodeo Horse, Leukotoxin (*lkt*A) Gene, Temperature, Humidity, Pathogen, Correlation

1. INTRODUCTION

1.1 Thrush disease, prevalence, and the causing agent: Thrush, a microbial health-threatening disease (i.e., lameness) of hoof disorder, if left careless, is common in field animals, including food and gaming animals, and is attributed to environmental conditions such as wet floors, flax bedding, and poor horn quality (1). Both bacteria and yeast could contribute to thrush development in food and gaming animals. These animals include cattle (2), swine (3), sheep (4), and horse (5) hosts. Infection of animals (i.e., non-horse animals) could be attributed to combined bacterial infections (4) or infections with a single bacterial genus and species (5). *Dichelobacter nodosus* and *Fusobacterium necrophorum* are Gram-negative, anaerobic footrot-causing bacteria in food animals, causing hoof disorder and eventually leading to lameness and decreased productivity if left untreated (2,4). Remarkably, *F. necrophorum* has been reported in human infections with multiple manifestations, such as ontogenic, leg, throat, and bacteremia complications (Lemierre's syndrome). These conditions could lead to hemiparesis (i.e., semi-paralysis), hearing damage, and life-threatening disease (i.e., death) (6). Hence, *F. necrophorum* could possess multi-mammalian cross-infectivity (i.e., including humans and animals).

1.2 Thrush-causing bacteria in the horse: Recently, Petrov and Dicks (5) reported the exclusive contribution of *F. necrophorum* to horse thrush, as opposed to infectious bacteria mixtures detected in other lame animals (2,4). Previous work by Shinjo *et al.* (7) discovered that this bacterium causes in-vitro and in-vivo hemolysis. The disease could readily be notable phenotypically by the infected hoof's blackening (8) and rotten odor (9) symptoms.

1.3 Detection of *F. necrophorum*: To date, both molecular (i.e., gene-specific PCR) (4,5) and culture-based (10-12) methods are available for detecting and isolating *F. necrophorum*, respectively. Gene-specific detection polymerase chain reaction (PCR), namely leukotoxin A gene (*lkt*A), helps accelerate the detection of *F. necrophorum* in animals with thrush disorder (4,5), by which an amplicon size of ~400 bases signifies the bacterium prevalence. Culture-based detection/isolation is feasible with commercially available agar base media, including Brain Heart Infusion agar (12), Fusobacterium Egg York Agar (FEA) (10), and JVN Agar (11), in

ISSN: 2455-6939

Volume: 10, Issue: 03 "May-June 2024"

which the two latter media are supplemented with Brucella Agar base and egg yolk and Fastidious Anaerobe Agar base (FAA), respectively. These agar media are supplemented with antibiotics (10,11), animal erythrocytes (10,11), and anaerobic incubation at 37 °C. Additionally, the subspecies of this bacterium, such as *F. necrophorum* subsp. *Necrophorum* and *F. necrophorum* subsp. *funduliforme*, could be readily determined with their differential hemolytic activities (7).

1.4 Predictive model: Metadata of microbial communities from various ecosystems is broadly available and has allowed the development and application of predictive model frameworks (13).Predictive food microbiology has earned interest in the food industry due to its simple protocol to generate valuable predictions and analyses (14) necessary to preserve food safety and quality (15). Additionally, predictive microbial ecology is used for interspecies relationship predictions (16), enabling bacterial infection and pathogenesis studies. Holzhauer *et al.* (1) documented thrush-conducive environmental conditions, including wet floor, straw bedding, permanent pasturing (no indoor shelter) housing strategy, and poor horn quality, with no regard for predictive model, temperature and/or bacteria (7) role(s).

1.5 Present study: The horse thrush-causing bacterium *F. necrophorum* was previously detected using *lkt*A gene-specific PCR exclusively, rendering mischaracterization of other horse-associated facultatively anaerobic infectious bacteria (i.e., lesion causing bacteria), including *Corynebacterium* sp. (17) and *Staphylococcus* sp. (18). The present study comparatively analyzed horse hoofs for thrush, *lkt*A gene, *F. necrophorum*, and other bacteria availabilities. Rodeo horse hoofs were randomly separately collected at various time points from three independent local sources, cultivated, and isolated bacteria were analyzed for *lkt*A gene availability. Further, presumptive correlations were established between thrush and environment conditions, including temperature, humidity, *lkt*A gene availability, and hoof-associated bacteria (i.e., confirmed by 16S rRNA bacterial identification).

2. MATERIALS AND METHODS

2.1 Sample collection: A total of 16 visibly healthy (11) and sickened (5) hoofs (Figure 1) were randomly separately collected (i.e., swabbed or scraped) at sixtime-points between September 2022 and May 2023 from three independent groups (i.e., JT, 11; Raven, 3; Kade Goemer, 2) of rodeo horses raised in open pens in Alabama(i.e., JT and Kade Goemer) and Missouri (i.e., Raven), pre- and post-removing of dirt embedded within the central and collateral frogs (Table 1). Environmental conditions, including temperature (i.e., mildly, 65-75 °F; moderately, 75-85 °F; extreme, 85-95 °F) and humidity (i.e., dry, wet) were recorded (Table 1). Temperature ranges (°F) on sampling days were aggregations of two-week temperatures (i.e., 7-day before and after

ISSN: 2455-6939

Volume: 10, Issue: 03 "May-June 2024"

the day of sampling) acquired online from Accuweather.com (i.e., 2023 samples) and weatherspark.com (i.e., 2022 samples).

2.2 Classification criteria for healthy and sickened hoofs: Healthy and sickened horse hoofs from thrush infections were dictated by sensorial means (i.e., according to the justification of the horse raisers), including visibility of frog's blackening formation (Figure 1) and a notable thrush-mediated odor. Detectable (i.e., notable frog blackening and odor) and non-detectable (i.e., non-notable frog blackening and odor) thrush was classified as healthy and sickened hoofs, respectively (Figure 1).

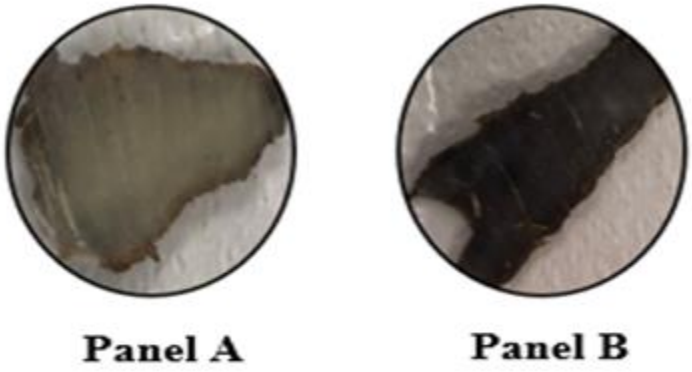

Fig. 1: Healthy (Panel A) and diseased hoof (Panel B) with no visible thrush-infected symptom and with visible thrush-infected symptoms (i.e., blackening of hoof and notable smell), respectively.

Table 1: Hoofsamples, temperature, and humidity.

Sample ID[a]	Source[b]	Health[c]	Mild[d]	Moderate[d]	Extreme[d]	Humidity[e]
1	JT	H	–	–	√	D
2	JT	S	–	–	√	D
3	JT	H	–	–	√	D
4	JT	H	–	–	√	D
5	R	S	–	√	–	D
6	R	S	–	√	–	D
7	JT	S	√	–	–	W
8	JT	S	√	–	–	W
9	JT	H	√	–	–	W
10	JT	H	√	–	–	W
11	R	H	–	√	–	D

ISSN: 2455-6939

Volume: 10, Issue: 03 "May-June 2024"

12	KG	H	√	–	–	W
13	KG	H	√	–	–	W
14	JT	H	√	–	–	D
15	JT	H	√	–	–	D
16	JT	H	√	–	–	D

[a] The ID is numerically chronologically assigned according to sample collection and processing time (i.e., September'22, samples 1-4; October'22, samples 5-8 and 11; November'22, samples 9 and 10; May'23, samples 12-16).

[b] Three sample suppliers, including JT, Raven (R), and Kade Goemer (KG)

[c] H, healthy hoofs (i.e., horse thrush were not visibly or odorly detectable); S, sickened hoofs (i.e., horse thrush were visibly and odorly detectable)

[d] √, Ambient temperature during which horse hoof was collected. Mild, 65-75 °F; moderate, 75-85 °F; extreme, 85-95 °F

[e] D, dry condition during which horse hoof was collected; W, wet condition during which horse hoof was collected

2.3 Hoof bacterial isolation, culture and storage conditions: Inoculated swabs or frog skin scrapes containing hoof bacteria were homogenized in buffered peptone water (BPW, 0.1% *w/v*, pH 7.4), the bacterial solution dilutions were inoculated on a complex medium, Brain Heart infusion agar (BHI, Difco, Detroit, MI, USA) (12), and aerobic and anaerobic (i.e., anaerobic gas pak was used) (AnaeroGen^{TM} 2.5L, ThermoFisher, Waltham, MA, USA) incubation were conducted at 35 °C for 48 h or until visible bacterial colonies formed. Bacterial colonies with visibly distinctive phenotypes were streaked for purification on fresh BHI agar, and isolated bacterial colonies were cultured in fresh BHI broth (35 °C, 16 h) followed by pelleted cells (12k RPM, 3 min) resuspended in fresh BHI supplemented with 10% sterile glycerol prior to long term storage at -70 °C. Prior to experimental analyses, thawed bacterial cultures were inoculated into sterile fresh BHI broth (inoculant dilution 1/100) and incubated at 35 °C for 16 h followed by another subculture in sterile fresh BHI broth (inoculant dilution 1/100).Bacterial isolates of horse hoofs were identified following one-directional sequencing (i.e., primer 515F) (Table 2) in an Applied Biosystems 3730 DNA analyzer (ThermoFisher, Waltham, MA, USA), DNA nucleotide chromatogram clean-up (i.e., MEGA X, https://www.megasoftware.net/), and the Blastn identification (i.e., megablast) of National Center for Biotechnology Information (NCBI).

ISSN: 2455-6939

Volume: 10, Issue: 03 "May-June 2024"

Table 2: Primers used in this study

Gene ID	Primer pair	Amplicon size (bp)	Melting temp. (°C)	Annealing temp. (°C)	Source
16S-515	F-GTGCCAGCMGCCGCGGTAA	900	65.2	56	(4)
16S-1391	R-GACGGGCGGTGTGTRCA		59.8	56	
*lkt*A	F-ACAATCGGAGTAGTAGGTTC	400	51	59	(1)
	R-ATTTGGTAACTGCCACTGC		53.4	59	

2.4 DNA extraction, gene-specific PCR, and electrophoresis conditions: Genomes of fresh bacterial cultures (16 h) were extracted in accordance to Tiong *et al.* (19). Briefly, bacterial pellets were washed twice with sterile deionized water prior to resuspension in Tris buffer solution (10 mM, pH 7.4) containing glass beads (500 µm) and subject to bead-collision cell lysis and DNA unleash following vortex collision (max speed, 1 min), cooling (4 °C, 2 min), repeat vortex collision, final cooling (4 °C, 4 min), and centrifugation (12K RPM, 2 min). After centrifugation, lysate supernatants were aspirated and kept at -70 °C. PCR was conducted according to the gene-specific conditions described by Bennett *et al.* (i.e., *lkt*A gene) (4) and Tanner *et al.* (i.e., 16S rRNA gene) (20) (Table 2). PCR reactions containing forward and reverse primers (0.4 µM) (Table 2) were prepared according to the GoTaq Flexi DNA Polymerase's instructions (Promega, Madison, WI, USA) and conducted in a thermal cycler (Veriti Thermal Cycler, Applied Biosystems, ThermoFisher, Waltham, MA USA) in accordance to Tiong *et al.* (19) PCR cycle step 1, 1 cycle of DNA denaturation at 95 °C for 5 min; step 2, 40 cycles of sequential denaturation at 95 °C for 1 min, annealing at primer-specific temperature (Table 2) for 40 s, and extension at 72 °C for amplicon size-specific time; step 3, 1 cycle of extended extension at 70 °C for 10 min followed by infinite holding at 4 °C. Amplicon availabilities were dictated on a DNA electrolyzed agarose gel (1.6%) pre-stained with a GelStarTM Nucleic Acid Gel Stain (stain dilution 0.005/50) (Lonza Walkersville Inc., Walkersville, MD, USA) following UV transillumination.

2.5 Hoof health and ambient condition correlation: A sample size of 16 horse frogs was estimated (21) to statistically evaluate thrush prevalence and the correlation between thrush and temperature and between thrush and humidity, respectively using descriptive statistics and binary logistic regression analysis (22) with 5% (i.e., $P < 0.05$) level of significance.

The horse hooves were recorded as healthy or sickened. Temperature was classified into three categories: mild, moderate, and extreme. Humidity was recorded as wet or dry. Since the response variable healthy/sickened status is binary and the predictors variables temperature and humidity are categorical, binary logistic regression analysis was used to estimate the correlation

ISSN: 2455-6939

Volume: 10, Issue: 03 "May-June 2024"

between healthy/sickened status and temperature and humidity, respectively. Utilizing binary logistic regression, the Maximum Likelihood Estimates (MLEs) for the regression parameters was determined. Subsequently, the probabilities associated with the horse's healthy or sickened status based on the specified environmental conditions were deduced. Minitab Statistical Software was used for the validation and analysis of the data.

3. RESULTS AND DISCUSSION

3.1 Thrush prevalence in rodeo horses: Holzhauer *et al*. (1) reported that thrush prevalence could be attributed to environmental conditions. Horse hoofs were randomly analyzed for thrush symptoms, including hoof blackening (8) and notable rotten odor (9), as noted previously. Of 16 samples evaluated, five indiscriminately possessed positive thrush symptoms in all conditions (i.e., temperature, 65-95 °F; humidity, dry or wet) examined (Table 1), as confirmed by the availability of frog blackening (Figure 1) and rotten odor, in which three and two were from dry (i.e., 3/10, 30%) and wet (i.e., 2/6, 33%) environments, respectively (Table 1), and two, two, and one were from 65-75 °F (i.e., mild temperature, 2/9, 22%), 75-85 °F (i.e., moderate temperature, 2/3, 67%), and 85-95 °F (i.e., extreme temperature, 1/4, 25%), respectively. Combined temperature and humidity, such as mild/wet, mild/dry, moderate/dry, and extreme/dry exhibited two (i.e., 2/6, 33%), zero (0/3, 0%), two (2/3, 67%), and one (1/4, 25%) sickened hoofs, respectively (Table 1), thereby suggesting a positive correlation between moderate temperature or combined moderate temperature/wet conditions and thrush acquisition in rodeo horses which conforms with bacterial temperature-dependent reactivity documented by Tiong and Muriana (23).

3.2 Prevalence of thrush-causing bacteria. Prior work documented the exclusive association between the thrush-causing bacterium *F. necrophorum* and hoof thrush availability and that it could be readily indicated by the *lkt*A gene availability (5). Subsequently, both healthy and sickened horse hoofs were analyzed for thrush-associated bacteria. A total of 41 phenotypically distinctive bacteria isolates (i.e., 36 anaerobic; 5 aerobic) were differentially acquired from 11 healthy (32) and five sick (10) hoofs (Table 3).

ISSN: 2455-6939

Volume: 10, Issue: 03 "May-June 2024"

Table 3: Hoof bacteria isolated in this study

Strain ID[a]	16S ID	Sample ID	Health[b]	T[c]	H[d]	*lkt*A[e]	Sampling[f]	Culture[g]
THT1	*Klebsiella* sp.	1	H	E	D	X	SS	A
THT2	*Streptococcus* sp.	1	H	E	D	√	SS	A
THT3	*Streptococcus* sp.	1	H	E	D	X	SS	A
THT4	*Lactococcus* sp.	2	S	E	D	X	SS	A
THT5	*Streptococcus* sp.	2	S	E	D	√	SS	A
THT6	*Streptococcus* sp.	1	H	E	D	X	SS	ANA
THT7	*Streptococcus* sp.	1	H	E	D	√	SS	ANA
THT8	*Streptococcus* sp.	2	S	E	D	X	SS	ANA
THT9	*Staphylococcus* sp.	2	S	E	D	X	SS	ANA
THT10	*Raoultella* sp.	3	H	E	D	√	S	ANA
THT11	*Enterobacter* sp.	3	H	E	D	X	S	ANA
THT12	*Klebsiella* sp.	3	H	E	D	X	S	ANA
THT13	*Klebsiella* sp.	4	H	E	D	√	S	ANA
THT14	*Enterobacter* sp.	4	H	E	D	X	S	ANA
THT15	*Streptococcus* sp.	4	H	E	D	X	S	ANA
THT16	*Streptococcus* sp.	4	H	E	D	√	S	ANA
THT17	*Staphylococcus* sp.	4	H	E	D	X	S	ANA
THT18	*Staphylococcus* sp.	4	H	E	D	X	S	ANA
THT19	*Vagococcus* sp.	5	S	M	D	ND	S	ANA
THT20	*Vagococcus* sp.	5	S	M	D	ND	S	ANA
THT21	*Vagococcus* sp.	6	S	M	D	ND	S	ANA
THT22	*Vagococcus* sp.	6	S	M	D	ND	S	ANA
THT23	*Vagococcus* sp.	7	S	Mi	W	ND	S	ANA
THT24	*Vagococcus* sp.	7	S	Mi	W	ND	S	ANA
THT26	*Corynebacterium* sp.	8	S	Mi	W	ND	S	ANA
THT27	*Lactococcus* sp.	9	H	Mi	W	ND	S	ANA
THT28	*Priestia* sp.	9	H	Mi	W	ND	S	ANA
THT29	*Bacillus* sp.	9	H	Mi	W	ND	S	ANA
THT30	*Bacillus* sp.	10	H	Mi	W	ND	S	ANA
THT31	*Brevibacterium* sp.	11	H	M	D	ND	S	ANA
THT32	*Rhodococcus* sp.	11	H	M	D	ND	S	ANA
THT33	*Bacillus* sp.	12	H	Mi	W	ND	S	ANA
THT34	*Bacillus* sp.	12	H	Mi	W	ND	S	ANA
THT35	*Bacillus* sp.	13	H	Mi	W	ND	S	ANA
THT36	*Bacillus* sp.	13	H	Mi	W	ND	S	ANA

ISSN: 2455-6939

Volume: 10, Issue: 03 "May-June 2024"

THT37	*Bacillus* sp.	14	H	Mi	D	ND	S	ANA
THT38	*Bacillus* sp.	14	H	Mi	D	ND	S	ANA
THT39	Enterococcus sp.	15	H	Mi	D	ND	S	ANA
THT40	*Lactococcus* sp.	15	H	Mi	D	ND	S	ANA
THT41	*Bacillus* sp.	16	H	Mi	D	ND	S	ANA
THT42	*Bacillus* sp.	16	H	Mi	D	ND	S	ANA

[a] THT1 – THT5, bacteria isolated using aerobic plate count; THT6 – THT42, bacteria isolated using anaerobic jars

[b] H, healthy hoofs (i.e., horse thrush were not visibly or odorly detectable); S, sickened hoofs (i.e., horse thrush were visibly and odorly detectable)

[c]Ambient temperature (T) recorded during sample collection. Mild (Mi; 65-75 °F), moderate (M; 75-85 °F), extreme (E; 85-95 °F) temperatures.

[d] Ambient humidity (H) recorded during sample collection. D, dry; W, wet

[e]√, *lkt*A-positive bacterial strain; X, *lkt*A-negative bacterial strain. ND, not determined.

[f]Horse hoof bacteria were acquired either by swabbing (S) or frog skin scraping (SS) following dirt cleaning out.

[g]Bacterial isolates were cultivated either aerobically (A) or anaerobically (ANA).

3.2 Prevalence of *lkt*A gene: Gene-specific amplification of 18 bacterial genomes (i.e., THT1-THT18) (Tables 2,4) revealed six *lkt*A positive isolates (Figure 2) from healthy (i.e., THT2, THT7, THT10, THT13, THT16) and sickened hoofs (i.e., THT5) (Table 4), suggesting the presumptive availability of *F. necrophorum* (5) and *Staphylococcus*sp. (24) as they were previously evident to possess the gene, including the equine thrush-causing bacterium *F. necrophorum* (5).

Table 4: *Ikt*A PCR amplicon availability in bacteria (THT1-18) isolated from healthy and sickened horse hoofs

Bacterial ID	*lkt*A[a]	Sample ID	Health[b]
THT1	X	1	H
THT2	√	1	H
THT3	X	1	H
THT4	X	2	S
THT5	√	2	S
THT6	X	1	H
THT7	√	1	H
THT8	X	2	S
THT9	X	2	S
THT10	√	3	H
THT11	X	3	H
THT12	X	3	H
THT13	√	4	H

ISSN: 2455-6939

Volume: 10, Issue: 03 "May-June 2024"

THT14	X	4	H
THT15	X	4	H
THT16	√	4	H
THT17	X	4	H
THT18	X	4	H
THT18	X	4	H

[a] √, *lkt*A-positive bacterial strain; X, *lkt*A-negative bacterial strain

[b] H, healthy hoofs (i.e., horse thrush were not visibly or odorly detectable); S, sickened hoofs (i.e., horse thrush were visibly and odorly detectable)

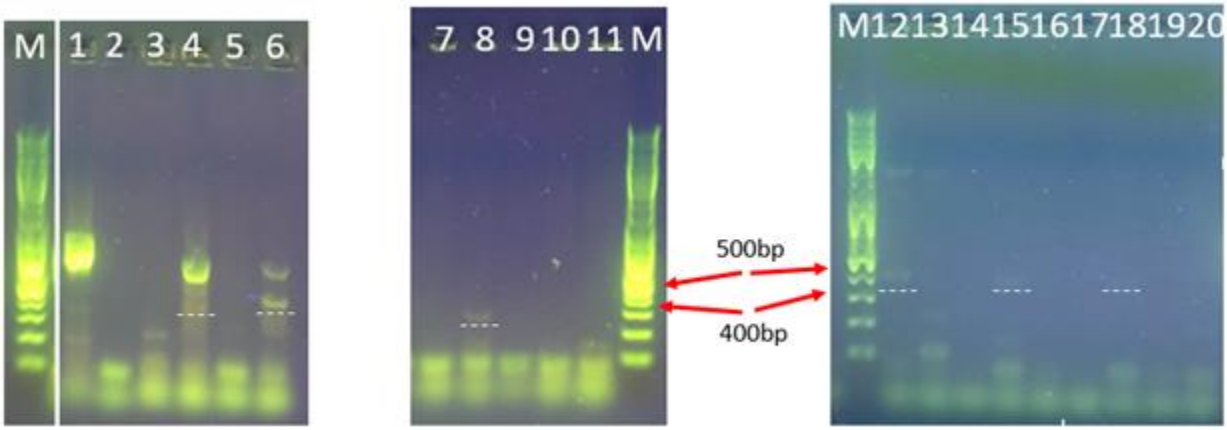

Fig. 2: Gene-specific PCR detection of *lkt*A in THT1-18 isolates. Lanes 1, "+" control (i.e., non-specific amplicon of *lkt*A primers); 2, "-" control; 3, THT1; 4, THT2; 5, THT6; 6, THT7; 7, THT4; 8, THT5; 9, THT8; 10, THT9; 11, THT3; 12, THT10; 13, THT11; 14, THT12; 15, THT13; 16, THT14; 17, THT15; 18, THT16; 19, THT17; 20, THT18; M, 1Kb DNA ladder. The dashed lines represent *lkt*A amplicons (i.e., *lkt*A-positive isolates)

3.3 Confirmed bacterial identity: Subsequently, 16S rRNA gene bacterial identification revealed that none of the bacterial isolates (i.e., 41 isolates) (Table 3), including the *lkt*A-positive isolates (i.e., THT2, THT5, THT7, THT10, THT13, and THT16) (Table 4), was *F. necrophorum*or *lkt*A-positive *Staphylococcus* sp. (i.e., VHT9, VHT17, and VHT18 were *lkt*A-negative *Staphylococcus* sp.) (Table 3). In fact, *lkt*A gene was differentially detected in *Streptococcus* sp. (i.e., 4 of 8 isolates exhibited detectable *lkt*A amplicons), *Raoultella* sp. (i.e., 1 isolate), and *Klebsiella* sp. (i.e., 1 of 3 isolates exhibited detectable *lkt*A amplicons) isolates (Tables 3,4), thereby agreeing with Narayanan *et al.* (25) report on the availability of other *lkt*A-carrying bacteria and suggesting the presence of truncated or altered *lkt*A gene sequence as previously observed in other bacteria species studied by Tiong and Muriana (23). Ludlam *et al.* (26) revealed the source-dependent *lktA* availability (i.e., *lkt*A-positive or -negative) in other bacterium (i.e., *F. necrophorum*) which could elucidate the absence of the gene in the isolates

ISSN: 2455-6939

Volume: 10, Issue: 03 "May-June 2024"

(i.e., *Streptococcus* sp., *Klebsiella* sp., and *Staphylococcus* sp.) and its non-viability essential role, as demonstrated in the viable *lkt*A-negative strains (i.e., *Streptococcus* sp. and *Klebsiella* sp.) (Tables 3,4) by PCR (Figure 2) in which only 50% and 33% of the isolates were *lkt*A positive (Tables 3,4), respectively.

Of 41 bacterial isolates (Table 3), 13 bacteria species with diverse prevalences (Figure 3) and functions (Table 5), including animal pathogens, human pathogens, plant growth promoting, antimicrobial, probiotic, animal and human symbionts, were identified (in this study), in which historically known animal and human pathogens (Table 5), *Vagococcus* sp. (27,28) and *Corynebacterium* sp. (29,30), were differentially exclusively recovered from sickened hoofs (Figure 3); thereby suggesting for the first time the presumptive association of hoof thrush in rodeo horse and mammalian infectious lesion- (27,28), cytotoxic-causing (29) *Vagococcus* sp. and *Corynebacterium* sp. bacteria. *Klebsiella* sp., *Raoultella* sp., *Enterobacter* sp., *Bacillus* sp., *Priestia* sp., *Brevibacterium* sp., *Rhodococcus* sp., and *Enterococcus* sp. were exclusively in healthy hoofs (Figure 3), whereas *Streptococcus* sp., *Staphylococcus* sp., and *Lactococcus* sp. were differentially acquired in both healthy and sickened hoofs (Figure 3). Interestingly, the *lktA*-positive isolates (i.e., THT10, *Raoultella* sp.; THT13, *Klebsiella* sp.) (Table 4) were exclusively acquired from healthy hoofs (Figure 3), thereby confirming its indirect contribution to thrush infections (26).

It is worth noting that the present bacterial work (Figure 3; Table 5) demonstrated the presence of horse symbiont and lesion-causing bacteria, including *Enterococcus* sp., (i.e., symbiont) (31), *Rhodococcus* sp. (i.e., symbiont) (32), *Corynebacterium* sp. (i.e., causes lesion) (17), and *Staphylococcus* sp. (i.e., causes lesion) (18), as documented previously; thereby confirming the legitimacy of bacterial culture data (in this study) from the sample size restriction (i.e., 16 hoof samples examined). However, the latter lesion-causing bacterium (i.e., *Staphylococcus* sp.) was acquired in both healthy (i.e., no visible thrush symptoms) and sickened (i.e., with visible thrush symptoms) hoofs (Figure 3; Tables 3,5), which could be attributed to bacterial infectivity- (33), disease onset time- and host immunity status-dependent symptom development (34). Further, the availability of an obligate aerobic bacterium (i.e., *Priestia* sp., *Brevibacterium* sp., and *Rhodococcus* sp.; presence) (Table 5) and *F. necrophorum* (i.e., absence) in this study implyfor the first time the availability of an anaerobic *Brevibacterium* sp. in a mammalian host (i.e., horse) and other thrush-causing agents (i.e., *Vagococcus* sp. and *Corynebacterium* sp.), respectively (Figure 3; Tables 3,5), attributed to the anaerobic cultivation method adopted in this study (i.e., BHI agar base, ~37 °C, anaerobic) (12). Additionally, the availability of common bacterial species in both acquisition methods (i.e., swabbing vs scraping) (Table 3) demonstrated for the first time the effectiveness of swabbing method as opposed to the counterpart, as demonstrated by the number of bacterial species isolated (13 species, swabbing; 4 species,

ISSN: 2455-6939

Volume: 10, Issue: 03 "May-June 2024"

scraping) (Table 3). This could be attributed to the immense contact areas of the bacterial acquisition of the swabbing method.

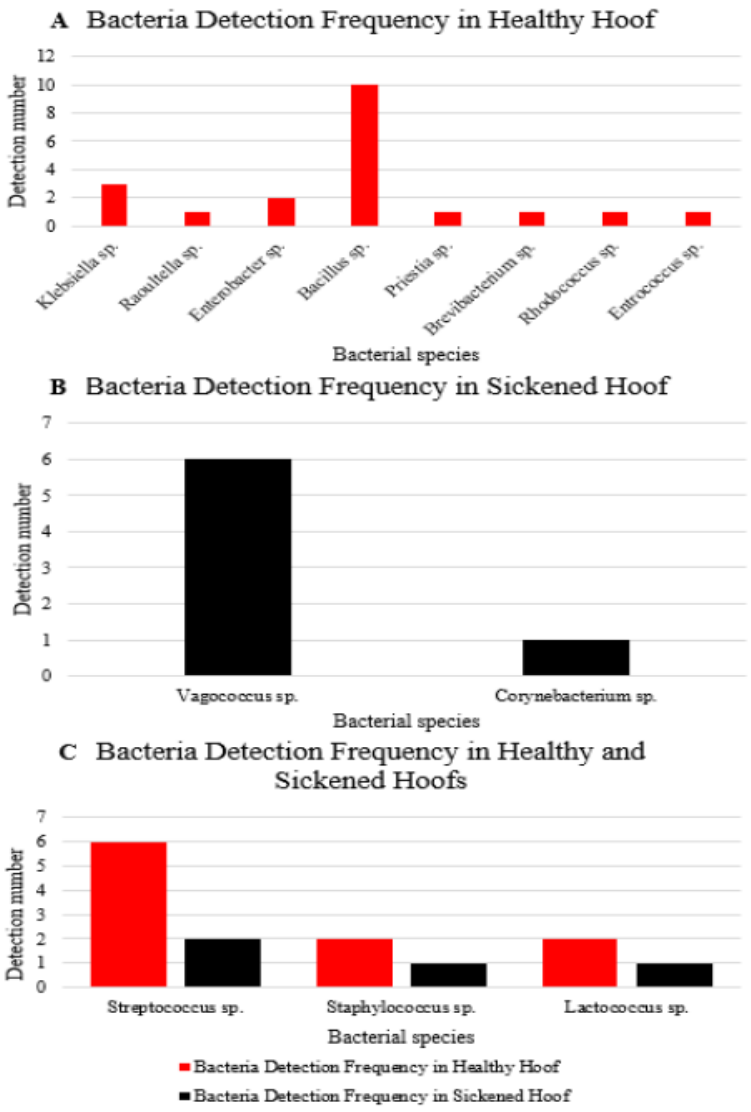

Fig. 3: Distribution and frequency of bacterial species detected in healthy, sickened, and both healthy and sickened hoofs

ISSN: 2455-6939

Volume: 10, Issue: 03 "May-June 2024"

Table 5: Bacterial isolate ID, function, and prevalence

16S ID	Function[a]	Ox[b]	Health[c]	#[d]
Klebsiella sp.	AP, HP (35)	FB (36)	H	3
Streptococcus sp.	AP, HP (37)	FB (38)	H, S	8
Lactococcus sp.	AP (39)	FB (40)	H, S	3
Staphylococcus sp.	AP, HP (18)	FB (41)	H, S	3
Raoultella sp.	AP, HP (42)	FB (43)	H	1
Enterobacter sp.	HP (44)	FB (44)	H	2
Vagococcus sp.	AP (27), HP (28)	FB (27)	S	6
Corynebacterium sp.	AP (17,29), HP (30)	FB (45)	S	1
Priestia sp.	PGP (46), AN (47,48)	AB (49)	H	1
Bacillus sp.	PR (50), AP, HP (51)	AB/FB (52)	H	10
Brevibacterium sp.	HP (35)	AB (35)	H	1
Rhodococcus sp.	AP, HP (54), AS (32)	AB (32)	H	1
Enterococcus sp.	AS (31), HS (55), AP (56), HP (55), AN (57)	FB (56)	H	1

[a] Bacterial function(s) reported according to literatures. AP, animal pathogen; HP, human pathogen; PGP, plant growth promoting; AN, antimicrobial; PR, Probiotic; AS, animal symbiont; HS, human symbiont

[b] Bacterial oxygen requirement. AB, obligate aerobic bacterium; FB, facultative anaerobic bacterium

[c] H, healthy hoofs (i.e., horse thrush were not visibly or odorly detectable); S, sickened hoofs (i.e., horse thrush were visibly and odorly detectable)

[d] #, number of detections of each bacterial species in 41 bacterial isolates

3.4 Thrush and the causing factors relatedness: Hoof thrush and the presumptive causing factors were evaluated for environment-thrush prevalence relatedness's, including temperature vs. symptomatic thrush (i.e., sickened hoof) and humidity vs. symptomatic thrush (i.e., sickened hoof). For the regression for healthy or sickened status and temperature (Table 1), with Extreme being the reference level, the *P*-values for the moderate temperature (M) and mild temperature (Mi) were 0.287 and 0.913, respectively (Table 6). Since the *P*-values are greater than the level significance 0.05, they are not statistically significant. The odds ratios for M relative to E was 6.0000 which indicates sickened hoof is more likely when it is M relative to E. The odds ratio for Mi relative to E and for Mi relative to M were 0.8571 and 0.1429, respectively, which indicates sickened hoof is less likely at Mi relative to E and also less likely at Mi relative to M. For the regression for healthy or sickened status and humidity (Table 1), with dry being the reference level, the *P*-value for wet is 0.889 (i.e., >0.05) which is not statistically significant. The odds ratio for wet relative to dry was 1.1667 which indicates that the sickened hoof is more likely when it is wet, conforming with Holzhauer *et al.* (i.e., non-rodeo horses) (1) report.

ISSN: 2455-6939

Volume: 10, Issue: 03 "May-June 2024"

Table 6: Hoof health status and environmental condition relatedness/correlation

#	Conditions correlated	*P*-value	Odds ratio
1	M/E	0.287[a]	6
2	Mi/E	0.913[a]	0.8571
3	Mi/M	NA[a]	0.1429
4	Wet/Dry	0.889[b]	1.1667

[a] Significant relatedness, $P<0.05$, was determined with reference to E
[b] Significant relatedness, $P<0.05$, was determined with reference to Dry
Mi, mild temperature; M, moderate temperature; E, extreme temperature
NA, not applicable (due to its absence in all samples studied in this study)

Collectively, symptomatic thrush infections (i.e., sickened hoofs) are differentially prevalent in both ambient conditions (i.e., temperature and humidity) examined (Table 1), and could be negatively correlated to *lkt*A (Figure 2; Tables 3,4) and *F. Necrophorum* bacterium (i.e., not detected in this study) (Table 4) which their positive correlation was established by Bennett *et al.* (4) and Petrov and Dicks (5), respectively. It is noteworthy that thrush-positive prevalence could closely be prompted by moderate temperature (i.e., 75-85 °F, 67% thrush prevalence; odds ratio 6, M/E vs. <1, Mi/E or Mi/M) (Tables 1,6) or combined moderate temperature/dry conditions (i.e., 67% thrush prevalence) (Table 1), that environmental humidity does not seem to differentially play a major role in causing thrush prevalence (i.e., dry, 30%; wet, 33%; odds ratio 1.1667, Wet/Dry) (Tables 1,6), and that *Vagococcus* sp. (27,28) and *Corynebacterium* sp. (29,30) could be non-*lkt*A thrush causing bacteria in rodeo horses as they were exclusively isolated in sickened hoofs (i.e., thrush hoofs) in this study (Figures 2,3; Tables 3-5) and possess skin lesion (27,28) or cytotoxic (29) implications in mammals, including animals, fish, and humans (Table 5).

4. CONCLUSION

Attributed to the natural co-inhabitant of both horse and thrush-causing bacteria, bacterial horse thrush is inevitable. The present study reveals for the first time bacterial horse thrush prevalence in rodeo horses (Table 1), its negative association with *lkt*A(Table 4) and *F. Necrophorum* bacterium (Table 3), and that *Vagococcus* sp. and *Corynebacterium* sp. could be novel thrush-causing bacteria (Figure 3; Table 5), as their pathogenesis were previously implicated in environmental non-horse animals (27-29) (Table 5). It is worth noting that the present study correlating environmental temperature and humidity (Table 6) with thrush prevalence could improve the thrush control guidelines of Holzhauer *et al.* (1). Further work involving large

ISSN: 2455-6939

Volume: 10, Issue: 03 "May-June 2024"

sample sizes of hoofs' bacteria, especially sickened hoofs, could warrant the relatedness of bacteria (i.e., non-*F. necrophorum*), horse thrush, and environmental conditions, and this information could improve or develop a predictive model for thrush preventive management and mitigate horse thrush-attributed performance complications (1,5).

ACKNOWLEDGEMENTS

The authors are thankful to the University of West Alabama (UWA) Office of Sponsored Programs and Research (OSPR) for funding the project through the faculty seed grants. The following participants are recognized for volunteering in manuscript construction and finalizing endeavors. Culture media preparation and bench-work, Ms. Batrina Reid (UWA undergraduate senior student), Ms. Zaria Gulley (UWA undergraduate junior student), Mr. Ryan Williams(UWA undergraduate junior student); full scientific literature copy distribution, Ms. Pei Jia Ng, M.S. (Oklahoma State University, Stillwater, Oklahoma, USA); manuscript review, Dr. Kevin Morse (Biology associate professor, UWA).

REFERENCES

[1] Holzhauer, M., Bremer, R., Santman-Berends, I., Smink, O., Janssens, I., & Back, W. (2017). Cross-sectional study of the prevalence of and risk factors for hoof disorders in horses in The Netherlands. *Preventive veterinary medicine*, *140*, 53–59. https://doi.org/10.1016/j.prevetmed.2017.02.013

[2] Bennett, G., Hickford, J.G., Zhou, H., Laporte, J., & Gibbs, J. (2009a). Detection of *Fusobacterium necrophorum* and *Dichelobacternodosus* in lame cattle on dairy farms in New Zealand. *Research in veterinary science, 87 3*, 413-5.https://doi.org/10.1016/j.rvsc.2009.04.001

[3] Zhou, H., Dobbinson, S., &Hickford, J. G. (2010). *Fusobacterium necrophorum* variants present on the hooves of lame pigs. *Veterinary microbiology*, *141*(3-4), 390. https://doi.org/10.1016/j.vetmic.2009.09.014

[4] Bennett, G., Hickford, J., Sedcole, R., & Zhou, H. (2009b). *Dichelobacternodosus*, *Fusobacterium necrophorum* and the epidemiology of footrot. *Anaerobe*, *15*(4), 173–176. https://doi.org/10.1016/j.anaerobe.2009.02.002

[5] Petrov, K. K., & Dicks, L. M. (2013). *Fusobacterium necrophorum*, and not *Dichelobacternodosus*, is associated with equine hoof thrush. *Veterinary microbiology*, *161*(3-4), 350–352. https://doi.org/10.1016/j.vetmic.2012.07.037

[6] Creemers-Schild, D., Gronthoud, F., Spanjaard, L., Visser, L. G., Brouwer, C. N., &Kuijper, E. J. (2014). *Fusobacterium necrophorum*, an emerging pathogen of otogenic and paranasal infections? *New microbes and new infections*, *2*(3), 52–57. https://doi.org/10.1002/nmi2.39

[7] Shinjo, T., Misawa, N., & Goto, Y. (1996). Comparison of haemolytic activity between *Fusobacterium necrophorum* subsp. *necrophorum* and *Fusobacterium necrophorum* subsp. *funduliforme* in vitro and in vivo. *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica*, *104*(1), 75–78. https://doi.org/10.1111/j.1699-0463.1996.tb00689.x

[8] American Farriers Journal. How to prevent and treat thrush in horse hooves. Available online: https://www.americanfarriers.com/articles/13214-how-to-prevent-and-treat-thrush-in-horse-hooves (accessed on 12 March 2024)

[9] Vetericyn. Horse thrush symptoms, treatment, and more. Available online: https://vetericyn.com/blog/horse-thrush-symptoms/#:~:text=Thrush%20is%20typically%20easy%20to,with%20a%20distinctive%20rotten%20smell.&text=Other%20horse%20thrush%20symptoms%20include,necrotic%20areas%20around%20the%20frog (accessed on 12 March 2024)

[10] Morgenstein, A. A., Citron, D. M., & Finegold, S. M. (1981). New medium selective for *Fusobacterium* species and differential for *Fusobacterium necrophorum*. *Journal of clinical microbiology*, *13*(4), 666–669. https://doi.org/10.1128/jcm.13.4.666-669.1981

[11] Brazier, J. S., Citron, D. M., & Goldstein, E. J. (1991). A selective medium for *Fusobacterium* spp. *The Journal of applied bacteriology*, *71*(4), 343–346. https://doi.org/10.1111/j.1365-2672.1991.tb03798.x

[12] Tan, Z. L., Nagaraja, T. G., &Chengappa, M. M. (1992). Factors affecting the leukotoxin activity of *Fusobacterium necrophorum*. *Veterinary microbiology*, *32*(1), 15–28. https://doi.org/10.1016/0378-1135(92)90003-c

[13] Hallin S. Environmental microbiology going computational-Predictive ecology and unpredicted discoveries. (2023). *Environmental microbiology*, *25(1)*, 111-114. https://doi:10.1111/1462-2920

[14] Ross, T. & McMeekin, T.A. (1994). Predictive microbiology. *International journal of food microbiology*, *23(3-4)*, 241-264. https://doi:10.1016/0168-1605(94)90155-4

[15] Ross, T., Dalgaard, P., &Tienungoon, S. (2000). Predictive modelling of the growth and survival of *Listeria* in fishery products. *International journal of food microbiology*, *62(3)*, 231-245. https://doi:10.1016/s0168-1605(00)00340-8

[16] Otwell, A.E., López García de Lomana, A., Gibbons, S.M., Orellana, M.V., &Baliga, N.S. (2018). Systems biology approaches towards predictive microbial ecology. *Environmental microbiology*, *20(12)*, 4197-4209. https://doi:10.1111/1462-2920.14378

[17] Merck manual. Pathogenesis and clinical findings of lymphangitis of horses and cattle. Available online https://www.merckvetmanual.com/circulatory-system/lymphadenitis-and-lymphangitis-of-large-animals/lymphangitis-of-horses-and-cattle (accessed 21 March 2024)

[18] Haag, A. F., Fitzgerald, J. R., &Penadés, J. R. (2019). *Staphylococcus aureus* in Animals. *Microbiologyspectrum*, *7*:10.1128/microbiolspec.gpp3-0060-2019. https://doi.org/10.1128/microbiolspec.gpp3-0060-2019

[19] Tiong, H. K., Walker, A., Wei, X. N., & Meza, G. (2023). Prevalence of heat-resistant *Vibrio parahaemolyticus* in retail seafood, *International journal of agriculture and environmental research*, Malwa international journals publication, *9*(5).

[20] Tanner, M. A., Shoskes, D., Shahed, A., & Pace, N. R. (1999). Prevalence of corynebacterial 16S rRNA sequences in patients with bacterial and "nonbacterial" prostatitis. *Journal of clinical microbiology*, *37*(6), 1863–1870. https://doi.org/10.1128/JCM.37.6.1863-1870.1999

[21] Haynes, W. (2013). Maximum Likelihood Estimation. In: Dubitzky, W., Wolkenhauer, O., Cho, KH., Yokota, H. (eds) Encyclopedia of Systems Biology. Springer, New York, NY. https://doi.org/10.1007/978-1-4419-9863-7_1235

[22] Mukaka M. M. (2012). Statistics corner: A guide to appropriate use of correlation coefficient in medical research. *Malawi medical journal: the journal of Medical Association of Malawi*, *24*(3), 69–71.

[23] Tiong, H. K., & Muriana, P. M. (2016). RT-qPCR analysis of 15 genes encoding putative surface proteins involved in adherence of *Listeria monocytogenes*. *Pathogens (Basel, Switzerland)*, *5*(4), 60. https://doi.org/10.3390/pathogens5040060

[24] Srikumaran S. (2020). Leukotoxins. *Toxins*, *12*(4), 231. https://doi.org/10.3390/toxins12040231

[25] Narayanan, S. K., Nagaraja, T. G., Chengappa, M. M., & Stewart, G. C. (2002). Leukotoxins of gram-negative bacteria. *Veterinary microbiology*, *84*(4), 337–356. https://doi.org/10.1016/s0378-1135(01)00467-9

[26] Ludlam, H. A., Milner, N. J., Brazier, J. S., Davies, I. H., Perry, K., Marriott, R. K., Donachie, L., & Curran, M. D. (2009). *lkt*A-encoded leukotoxin is not a universal virulence factor in invasive *Fusobacterium necrophorum* infections in animals and man. *Journal of medical microbiology*, *58*(Pt 4), 529–530. https://doi.org/10.1099/jmm.0.005231-0

[27] Fu, S., Xia, W., Wang, Q., Rahman, M. M., Hao, J., Ye, S., Liu, Y., & Li, R. (2020). Genomic characterization and pathogenicity analysis of the probiotic *Vagococcuslutrae*strain VL-18 causing severe skin lesions in warm-blooded animals. *Aquaculture*, *523*. https://doi.org/10.1016/j.aquaculture.2020.735166

[28] Asfar, M, &Antoine-Reid, T. (2023). First US Case of *Vagococcuslutrae* infection in a human: A case report. *American Journal of Clinical Pathology*, *160*(1), S84. https://doi.org/10.1093/ajcp/aqad150.186

[29] Möller, J., Busch, A., Berens, C., Hotzel, H., &Burkovski, A. (2021). Newly Isolated

Animal Pathogen *Corynebacterium silvaticum* Is Cytotoxic to Human Epithelial Cells. *International journal of molecular sciences*, *22*(7), 3549. https://doi.org/10.3390/ijms22073549

[30] Mangutov, E. O., Kharseeva, G. G. ,&Alutina, E. L. (2021). *Corynebacterium* spp. - problematic pathogens of the human respiratory tract (review of literature). *Clinical Laboratory Diagnostics*,*66*(8), 502-508. https://doi:10.51620/0869-2084-2021-66-8-502-508

[31] Willis, A. T., Magdesian, K. G., Byrne, B. A., & Edman, J. M. (2019). *Enterococcus* infections in foals. *Veterinary journal (London, England : 1997)*, *248*, 42–47. https://doi.org/10.1016/j.tvjl.2019.04.005

[32] Ayoade, F., Vaqar, S., &Alam, M. U. *RhodococcusEqui*. [Updated 2024 Jan 25]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available online: https://www.ncbi.nlm.nih.gov/books/NBK441978/(accessed 12April 2024)

[33] Tadepalli, S., Stewart, G. C., Nagaraja, T. G., Jang, S. S., & Narayanan, S. K. (2008). *Fusobacterium equinum* possesses a leukotoxin gene and exhibits leukotoxin activity. *Veterinary microbiology*, *127*(1-2), 89–96. https://doi.org/10.1016/j.vetmic.2007.08.027

[34] Doron, S., & Gorbach, S. L. (2008). Bacterial infections: Overview. *International encyclopedia of public health*, 273–282. https://doi.org/10.1016/B978-012373960-5.00596-7

[35] Ribeiro, M. G., de Morais, A. B. C., Alves, A. C., Bolaños, C. A. D., de Paula, C. L., Portilho, F. V. R., de Nardi Júnior, G., Lara, G. H. B., de Souza Araújo Martins, L., Moraes, L. S., Risseti, R. M., Guerra, S. T., Bello, T. S., Siqueira, A. K., Bertolini, A. B., Rodrigues, C. A., Paschoal, N. R., de Almeida, B. O., Listoni, F. J. P., Sánchez, L. F. G., … Paes, A. C. (2022). *Klebsiella*-induced infections in domestic species: a case-series study in 697 animals (1997-2019). *Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology]*, *53*(1), 455–464. https://doi.org/10.1007/s42770-021-00667-0

[36] Goldberg, R. B., & Hanau, R. (1980). Regulation of *Klebsiella pneumoniae* hut operons by oxygen. *Journal of bacteriology*, *141*(2), 745–750. https://doi.org/10.1128/jb.141.2.745-750.1980

[37] Vela, A. I., Villalón, P., Sáez-Nieto, J. A., Chacón, G., Domínguez, L., & Fernández-Garayzábal, J. F. (2017). Characterization of *Streptococcus pyogenes* from Animal Clinical Specimens, Spain. *Emerging infectious diseases*, *23*(12), 2013–2016. https://doi.org/10.3201/eid2312.151146

[38] Hasan Yesilkaya, Vahid FarshchiAndisi, Peter W. Andrew, Jetta J.E. Bijlsma (2013).*Streptococcus pneumoniae* and reactive oxygen species: an unusual approach to living with radicals. Trends in Microbiology (ISSN: 0966-842X), 21(4), 187-

195.https://doi.org/10.1016/j.tim.2013.01.004

[39] Ortega, C., Irgang, R., Valladares-Carranza, B., Collarte, C., & Avendaño-Herrera, R. (2020). First identification and characterization of *Lactococcus garvieae* isolated from rainbow trout (*Oncorhynchus mykiss*) cultured in Mexico. *Animals : an open access journal from MDPI*, *10*(9), 1609. https://doi.org/10.3390/ani10091609

[40] Gaudu, P., Vido, K., Cesselin, B., Kulakaukas, S., Tremblay, J., Rezaiki, L., Lamberet, G., Sourice, S., Duwat, P., & Gruss, A. (2002). Respiration capacity and consequences in *Lactococcus lactis*. *Antonie Van Leeuwenhoek*,*82*, 263–269. https://doi.org/10.1023/A:1020635600343

[41] Fuchs, S., Pané-Farré, J., Kohler, C., Hecker, M., & Engelmann, S. (2007). Anaerobic gene expression in Staphylococcus aureus. *Journal of bacteriology*, *189*(11), 4275–4289. https://doi.org/10.1128/JB.00081-07

[42] Pas, M. L., Vanneste, K., Bokma, J., Van Driessche, L., De Keersmaecker, S. C. J., Roosens, N. H., Haesebrouck, F., Boyen, F., & Pardon, B. (2021). Case report: Multidrug resistant *Raoultellaornithinolytica* in a septicemic calf. *Frontiers in veterinary science*, *8*, 631716. https://doi.org/10.3389/fvets.2021.631716

[43] Bustamante, D., Segarra, S., Montesinos, A.,Tortajada, M., Ramón, D.,&Rojas, A. (2019). Improved *Raoultellaplanticola* strains for the production of 2,3-butanediol from glycerol. *Fermentation*, *5*, 11. https://doi.org/10.3390/fermentation5010011

[44] Ramirez D.,& Giron M.*Enterobacter*infections. [Updated 2023 Jun 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available online: https://www.ncbi.nlm.nih.gov/books/NBK559296/ (accessed on 12 April 2024)

[45] Michel, A., Koch-Koerfges, A., Krumbach, K., Brocker, M., & Bott, M. (2015). Anaerobic growth of *Corynebacterium glutamicum* via mixed-acid fermentation. *Applied and environmental microbiology*, *81*(21), 7496–7508. https://doi.org/10.1128/AEM.02413-15

[46] Lin, X. R., Chen, H. B., Li, Y. X., Zhou, Z. H., Li, J. B., Wang, Y. Q.. Zhang, H., Zhang, Y., Han, Y. H., &Wang, S. S. (2022). *Priestia* sp. LWS1 is a selenium-resistant plant growth-promoting bacterium that can enhance plant growth and selenium accumulation in *Oryza sativa* L. *Agronomy*, *12*, 1301. https://doi.org/10.3390/agronomy12061301

[47] Liu, J. M., Liang, Y. T., Wang, S. S., Jin, N., Sun, J., Lu, C., Sun, Y. F., Li, S. Y., Fan, B., & Wang, F. Z. (2023). Antimicrobial activity and comparative metabolomic analysis of *Priestia megaterium* strains derived from potato and dendrobium. *Scientific reports*, *13*(1), 5272. https://doi.org/10.1038/s41598-023-32337-6

[48] Cui, Z., Hu, L., Zeng, L., Meng, W., Guo, D., & Sun, L. (2023). Isolation and characterization of *Priestia megaterium* KD7 for the biological control of pear fire blight. *Frontiers in microbiology*, *14*, 1099664.

https://doi.org/10.3389/fmicb.2023.1099664

[49] BacDive. *Priestia megaterium* CIP 51.17 is an obligate aerobe, spore-forming, mesophilic bacterium that was isolated from environment, soil. Available online https://bacdive.dsmz.de/strain/138171#:~:text=Priestia%20megaterium%20CIP%2051.17%20is,was%20isolated%20from%20Environment%2C%20Soil (accessed 10 April 2024

[50] Luise, D., Bosi, P., Raff, L., Amatucci, L., Virdis, S., &Trevisi, P. (2022). *Bacillus* spp. probiotic strains as a potential tool for limiting the use of antibiotics, and improving the growth and health of pigs and chickens. *Frontiers in microbiology*, *13*, 801827. https://doi.org/10.3389/fmicb.2022.801827

[51] Calvigioni, M., Cara, A., Celandroni, F., Mazzantini, D., Panattoni, A., Tirloni, E., Bernardi, C., Pinotti, L., Stella, S., &Ghelardi, E. (2022). Characterization of a *Bacillus cereus* strain associated with a large feed-related outbreak of severe infection in pigs. *Journal of applied microbiology*, *133*(2), 1078–1088. https://doi.org/10.1111/jam.15636

[52] Bocchi, M. B., Cianni, L., Perna, A., Vitiello, R., Greco, T., Maccauro, G., &Perisano, C. (2020). A rare case of *Bacillus megaterium* soft tissues infection. *Acta bio-medica: Atenei Parmensis*, *91*(14-S), e2020013. https://doi.org/10.23750/abm.v91i14-S.10849

[53] Asai, N., Suematsu, H., Yamada, A., Watanabe, H., Nishiyama, N., Sakanashi, D., Kato, H., Shiota, A., Hagihara, M., Koizumi, Y., Yamagishi, Y., &Mikamo, H. (2019). *Brevibacteriumpaucivorans* bacteremia: case report and review of the literature. *BMC infectious diseases*, *19*(1), 344. https://doi.org/10.1186/s12879-019-3962-y

[54] Prescott J. F. (1991). Rhodococcusequi: an animal and human pathogen. *Clinical microbiology reviews*, *4*(1), 20–34. https://doi.org/10.1128/CMR.4.1.20

[55] Hammerum A. M. (2012). Enterococci of animal origin and their significance for public health. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, *18*(7), 619–625. https://doi.org/10.1111/j.1469-0691.2012.03829.x

[56] Stępień-Pyśniak, D., Bertelloni, F., Dec, M., Cagnoli, G., Pietras-Ożga, D., Urban-Chmiel, R., & Ebani, V. V. (2021). Characterization and Comparison of *Enterococcus* spp. Isolates from Feces of Healthy Dogs and Urine of Dogs with UTIs. *Animals : an open access journal from MDPI*, *11*(10), 2845. https://doi.org/10.3390/ani11102845

[57] Hanchi, H., Mottawea, W., Sebei, K., & Hammami, R. (2018). The genus *Enterococcus*: between probiotic potential and safety concerns-an update. *Frontiers in microbiology*, *9*, 1791. https://doi.org/10.3389/fmicb.2018.01791

