RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume 45 Issue 3: 508-516 (June 2026)

Clinical and Molecular Phylogenetic Detection of *Trueperella pyogenes* in Abscessed-wounds of Cattle

Tamadhir A. Al-Hamed[1], Hawraa F.H. Al-Abedi[2], Noor K.H. Al-Quraishy[3], Israa M. Essa[4], Hasanain A.J. Gharban[5]

10.18805/ajdfr.DRF-627

ABSTRACT

Background: Abscessed wounds in cattle represent a significant veterinary concern, often leading considerable economic losses due to decreased productivity, treatment cost, potential culling and even death. This study aims to investigate the incidence rate of *Trueperella pyogenes* in abscessed wounds of cattle using molecular technique with phylogenetic analysis of study isolates and estimation the relationship of infection to animal risk factors.

Methods: A total of 271 cattle of different ages and sexes admitted to private veterinarian clinics or being visited to their farms in Wasit province (Iraq) were diagnosed clinically with abscessed wounds. Then, samples of abscess swab were collected and examined molecularly by the conventional PCR. Finally, the positive *T. pyogenes* isolates were sequenced, submitted in the NCBI database and analysed phylogenetically.

Result: Findings of clinical examination revealed a significant increase in abscessed wounds at abdomen but decreased in neck and back when compared to other body regions; head, forelimbs, hindlimbs and pelvis. Targeting of *16S rRNA* gene, 7.38% from abscessed wounds of study cattle were positively infected with *T. pyogenes*. Phylogenetic analysis of the study *T. pyogenes* isolates revealed its identity to NCBI-BLAST USA *T. pyogenes* isolate (KX592206.1) at rates of similarity and mutation/changes ranged 98.47-99.86% and 0.0003-0.001%, respectively. Relation to risk factors, incidence and risk of positivity in body parts were elevated significantly in abdomen but decreased in neck and back, head, forelimbs and hindlimbs when compared to pelvis. Concerning age, although insignificant variation in incidence of positivity was seen among age groups of study animals, cattle aged 1-4 years and >4 years were appeared at higher risk of infection than those of <1 year. For sex, also insignificant differences in incidence rate of positivity was detected between females and males; however, females were detected at higher risk of infection than males.

Key words: Bovine bacterial infections, National centers for biotechnology center (NCBI), Polymerase chain reaction (PCR), Risk factors, Skin injury.

INTRODUCTION

The complexity of wound healing in cattle as in other terrestrial vertebrates involves intricate biological processes influenced by both animal-related factors in addition to nutrition and husbandry practices which play a critical role in facilitating optimal wound healing in cattle; while conversely, disruptions and infections can impair and complicate it (Lux, 2022; Singh *et al.*, 2024). Bacterial pathogens represent the main cause of wound infections which result mostly in various clinical forms that ranged from localized abscesses and folliculitis to more diffuse cellulitis, each presenting unique challenges in diagnosis and management following the prevalence and specific types of bacteria responsible for these purulent conditions that varied geographically and depending on the farm-specific hygiene practices (Malone and Schultz, 2022; Mohammad *et al.*, 2022; Al-Eodawee *et al.*, 2024). *Trueperella pyogenes* consider one of the most neglected opportunistic pathogens which found usually in mucous membranes and skin of various animals including cattle, pigs and other livestock (Rzewuska *et al.*, 2019). This bacterium can lead to purulent infections in lung, udder, genital tract, liver and

[1]Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Basrah, Basra, Iraq.
[2]Department of Biology, Collage of Education for Pure Sciences, University of Al-Hamdaniya, Al-Hamdaniya, Nineveh, Iraq.
[3]Department of Anatomy and Histology, College of Veterinary Medicine, University of Wasit, Wasit, Iraq.
[4]Department of Public Health, College of Veterinary Medicine, University of Basrah, Basra, Iraq.
[5]Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Wasit, Wasit, Iraq.

Corresponding Author: Hasanain A.J. Gharban, Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Wasit, Wasit, Iraq.
Email: hghirban@uowasit.edu.iq
ORCIDs: 0000-0002-6294-3683, 0000-0003-1693-0424, 0009-0000-3143-7852, 0000-0002-7379-7503, 0000-0002-6438-1450

How to cite this article: Al-Hamed, T.A., Al-Abedi, H.F.H., Al-Quraishy, N.K.H., Essa, I.M. and Gharban, H.A.J. (2026). Clinical and Molecular Phylogenetic Detection of *Trueperella pyogenes* in Abscessed-wounds of Cattle. *Asian Journal of Dairy and Food Research*. **45(3):** 508-516. doi: 10.18805/ajdfr.DRF-627.

Submitted: 27-11-2025 **Accepted:** 21-01-2026 **Online:** 04-02-2026

skin such as pneumonia, mastitis, metritis as well as liver and skin abscesses (Nagaraja, 2022). Abscesses caused by *T. pyogenes* can vary in size and location, to resulting sometimes in severe complications like fistulas and arthritis (Rzewuska *et al.*, 2019).

Trueperella pyogenes was discovered and classified generally as *Bacillus pyogenes* in 1893, then to *Arcanobacterium pyogenes* and recently renamed in honor of the German microbiologist, Hans Georg Trüper. Today, *T. pyogenes* is classified in the genus of *Trueperella* that belongs to the family *Actinomycetaceae* in the order Actinomycetales of the class Actinobacteria (Ashur *et al.*, 2019; Menon, 2024). The bacterium is a Gram-positive, non-motile, non-sporulating, non-capsulated and short rod-shaped facultative anaerobic organism which grown on blood agar as very small, white, opaque and glistening colonies surrounded by a zone of b-hemolysis (Carr, 2017; Saad *et al.*, 2023). Due to limitations of culture methods (fastidious nature, long incubation time due to slow growing, potentially mixed infections and difficulties in identifying), molecular techniques like PCR (polymerase chain reaction) are often used for the detection and identification of *T. pyogenes* as a rapid, reliable, highly sensitive and specific tool (Mobed *et al.*, 2019; Al-Graibawi *et al.*, 2021; Stefañska *et al.*, 2022; Ehmud *et al*., 2025). Sequencing methods provide additional advantages in accurate identification, characterization and even detection of antimicrobial resistance by determining specific genes (Boolchandani *et al.*, 2019; Bahloul *et al*., 2024; Magossi *et al.*, 2025).

In Iraq, although *T. pyogenes* were isolated from the synovial fluid obtained from the knee joint of sheep diagnosed clinically with suppurative arthritis in Mosul (Arslan *et al.*, 2009), milk samples of subclinical mastitic she-camels in middle Euphrates (AL-Tofaily and Al-Roddan, 2011), as well as urine from the urinary balder samples of slaughtered buffaloes (Al-Iraqi *et al.*, 2016) and slaughtered goats (Sadoon, 2021) in Mosul abattoirs. Hence, this study aims to investigate the incidence of *T. pyogenes* in abscessed wounds of cattle molecularly using conventional PCR assay, sequencing and phylogenetic analysis of study *T. pyogenes* isolates to determine its identity to the global NCBI-BLAST isolates/strains and estimate the association of infection to animal risk factors including body site of wound, age and sex.

MATERIALS AND METHODS

Samples

This study was conducted during September (2024) to May (2025) on totally 271 cattle of different ages and sexes which attended to the private veterinarian clinics or visited at their farms in Wasit province (Iraq) with abscessed wounds in their bodies. Swab samples were collected from each abscessed wounds of each study animal using the rayon-budded swabs into the ready-to-use-PrimeStore MTM transport tubes (EKF, Germany). All tubes were transported to the Clinical Pathology Lab. (College of Veterinary Medicine/University of Wasit) under cooled conditions and kept frozen until be used for molecular examination. Animal risk factors (body site of wound, age and sex) were reported, also (Almaliky *et al.*, 2024; Jiheel *et al.*, 2025).

Molecular examination

After preparation of swab samples at room temperature, manufacturer instructions of the Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan) were followed. After extraction, all samples of DNAs were checked for purity and concentration by the Nanodrop system (Thermo-scientific, UK) and served for preparation the MasterMix tubes at a final volume 25 µl using the AccuPower® PCR PreMix Kit (Bioneer, Korea) and the designated primer for this study (F: 5′-TTT TGG ATG GGG ATG GGC TC-3′ and R: 5′-TGG CAC ATC GCA GTG TAT GT-3′) based on the NCBI-GenBank *T. pyogenes* strain (ID: KT191136.1). In the Thermal Cycler system (Bio Rad, USA), the MasterMix tubes were subjected to the conditions of the DNAs amplification as following: 1 cycle for initial denaturation (95°C/7 min), 35 cycles for denaturation (95°C/30 sec), annealing (54°C/30 sec) and extension (72°C/45 sec) and 1 cycle for final extension (72°C/7 min). Electrophoresis for Agarose-gel (1.5%) stained with ethidium bromide was done at 100 Volts and 80Am for 90 min and the bands size of positive PCR products was visualized under the UV transilluminator (Clinx Science, China) at an approximately ≈ 782 bp.

Sequencing and phylogenetic analysis

The positive *T. pyogenes* isolates were sequenced at the Macrogen Company (South Korea) and the received data were submitted in the NCBI-GenBank to get specific access numbers. Then, ClustralW alignment, multiple sequence alignment and phylogenetic tree analysis was performed using the MEGA-11 Software.

Statistical analysis

One-Way ANOVA and *t*-test in the GraphPad Prism Software version 8.0.2 in addition to the odds ratio (OR) and relative risks (RR) in the MedCalc Statistical Software were applied to detect significant differences between the obtained values at $p<0.05$ and 95% confidence interval (95%CI), (Al-Abedi *et al.*, 2018).

RESULTS AND DISCUSSION

Clinical examination

The findings of clinical examination revealed that the abscessed wounds were increased significantly ($p<0.008$; 95%CI: 5.116 to 23.46) in abdomen [34.69% (94/271)] but decreased significantly ($p<0.05$) in neck [4.06% (11/271)] and back [8.49% (23/271)] when compared to other body regions; head [14.02% (38/271)], forelimbs [11.07% (30/271)], hind-limbs [10.33% (28/271)] and pelvis [17.34%

(47/271)], (Fig 1). In cattle, skin wounds occur most commonly due to various physical injuries including trauma from fencing, kicks, or falls as well as injuries from other animals or sharp aspects in their environments and can have complications and infections (Wheeler, 2019; Valkova *et al.*, 2021; Faccin *et al.*, 2023). In female, improper milking techniques and mastitis can lead to teat damage and subsequent viral and bacterial infections (Abd-El-Hady, 2015; Sharun *et al.*, 2021); while in males especially adults, warts as the most etiology and other causes (penile hematomas, hair rings and viral infections) can lead to penile lesions and ulcers (Paul, 2024).

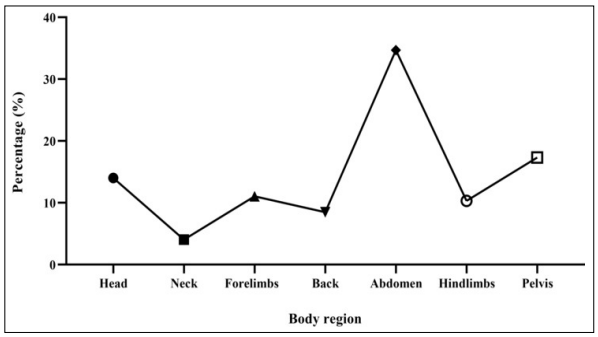

Fig 1: Incidence of abscessed wounds in various bodily parts of study animals.

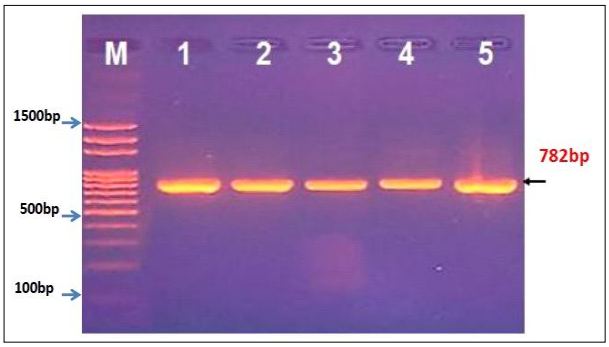

Fig 2: Agarose-gel electrophoresis of some positive PCR products to *T. pyogenes* isolates.

Molecular phylogeny

Targeting the *16S rRNA* gene, 7.38% (20/271) from abscessed wounds of study cattle were positively infected with *T. pyogenes* (Fig 2). The sequencing data of 20 positive *T. pyogenes* isolates were submitted in the NCBI database (Cattle-Hass1-Cattle-Hass20). Phylogenetic analysis of the study *T. pyogenes* isolates revealed its significant identity to the NCBI-BLAST USA *T. pyogenes* isolate (KX592206.1) at a similarity ranged 98.47-99.86% and mutation/changes ranged 0.0003-0.001% (Fig 3-5, Table 1). In comparison to other studies, Ertaş *et al.* (2005) investigated the presence of *T. pyogenes* in abscessed kidney samples of 500 cattle and found that 40% of study samples were positive by PCR. In a study conducted by Liu *et al.* (2009), 23.5% of endometritic cows were shown a positive reactivity by the PCR assay. Petit *et al.* (2009) isolated *T. pyogenes* from the genital tract of 41.3% cows with and without abortion; while, Santos *et al.* (2010) isolated *T. pyogenes* from the 86.1% of uterine fluid of Holstein dairy cows. Zastempowska and Lassa (2012) detect the presence of 89 *T. pyogenes* isolates from the inflamed secretions of 89 dairy cows with clinical mastitis. Ishiyama *et al.* (2017)

Table 1: Homology Sequence identity for local and NCBI-BLAST *T. pyogenes* isolates.

Local isolate		NCBI isolate			
Name	Access no.	Isolate	Host	Access no.	Identity (%)
Cattle-Hass1	PV208511.1	B53-006	Ruminants	KX592206.1	99.72
Cattle-Hass2	PV208512.1	B53-006	Ruminants	KX592206.1	99.25
Cattle-Hass3	PV208513.1	B53-006	Ruminants	KX592206.1	99.31
Cattle-Hass4	PV208514.1	B53-006	Ruminants	KX592206.1	99.86
Cattle-Hass5	PV208515.1	B53-006	Ruminants	KX592206.1	98.47
Cattle-Hass6	PV208516.1	B53-006	Ruminants	KX592206.1	98.87
Cattle-Hass7	PV208517.1	B53-006	Ruminants	KX592206.1	98.99
Cattle-Hass8	PV208518.1	B53-006	Ruminants	KX592206.1	98.54
Cattle-Hass9	PV208519.1	B53-006	Ruminants	KX592206.1	99.12
Cattle-Hass10	PV208520.1	B53-006	Ruminants	KX592206.1	98.96
Cattle-Hass11	PV208521.1	B53-006	Ruminants	KX592206.1	99.06
Cattle-Hass12	PV208522.1	B53-006	Ruminants	KX592206.1	99.71
Cattle-Hass13	PV208523.1	B53-006	Ruminants	KX592206.1	99.46
Cattle-Hass14	PV208524.1	B53-006	Ruminants	KX592206.1	98.86
Cattle-Hass15	PV208525.1	B53-006	Ruminants	KX592206.1	99.67
Cattle-Hass16	PV208526.1	B53-006	Ruminants	KX592206.1	99.73
Cattle-Hass17	PV208527.1	B53-006	Ruminants	KX592206.1	99.12
Cattle-Hass18	PV208528.1	B53-006	Ruminants	KX592206.1	98.93
Cattle-Hass19	PV208529.1	B53-006	Ruminants	KX592206.1	99.04
Cattle-Hass20	PV208530.1	B53-006	Ruminants	KX592206.1	99.22

detected that the occurrence of *T. pyogenes* in mastitis cases of 81 Holstein cows was 16.61%. Rezanejad *et al.* (2019) tested 226 bovine mastitic milk and 172 uterine swabs; in which, positive *T. pyogenes* isolates were detected in 14.15% and 23.83% samples, respectively. Saad *et al.* (2023) found that the occurrence of *T. pyogenes* in raw milk samples of dairy farms was 60% in both cows and buffaloes and 63.33% in milk of farmer houses. Tamai *et al.* (2023) isolated 65 *T. pyogenes* strains (453%) from 150 postpartum cattle with clinical abscess symptoms on 22 farms around Tehran (Iran). Wente *et al.* (2024) detected a total of 151 *T. pyogenes* isolates among 16 herds with an overall 41 out of 124 isolates (25%) were delivered by one herd. In a recent study, *T. pyogenes* was isolated from multiple abscesses in spleen of cattle suggesting its role in infections of visceral organs (Hamedi *et al.*, 2025).

Targeting of *16S rRNA* gene, phylogenetic analysis revealed that the study *T. pyogenes* isolates are close-related to the NCBI-BLAST USA *T. pyogenes* isolate (KX592206.1) which conducted by Rogovskyy *et al.* (2018)

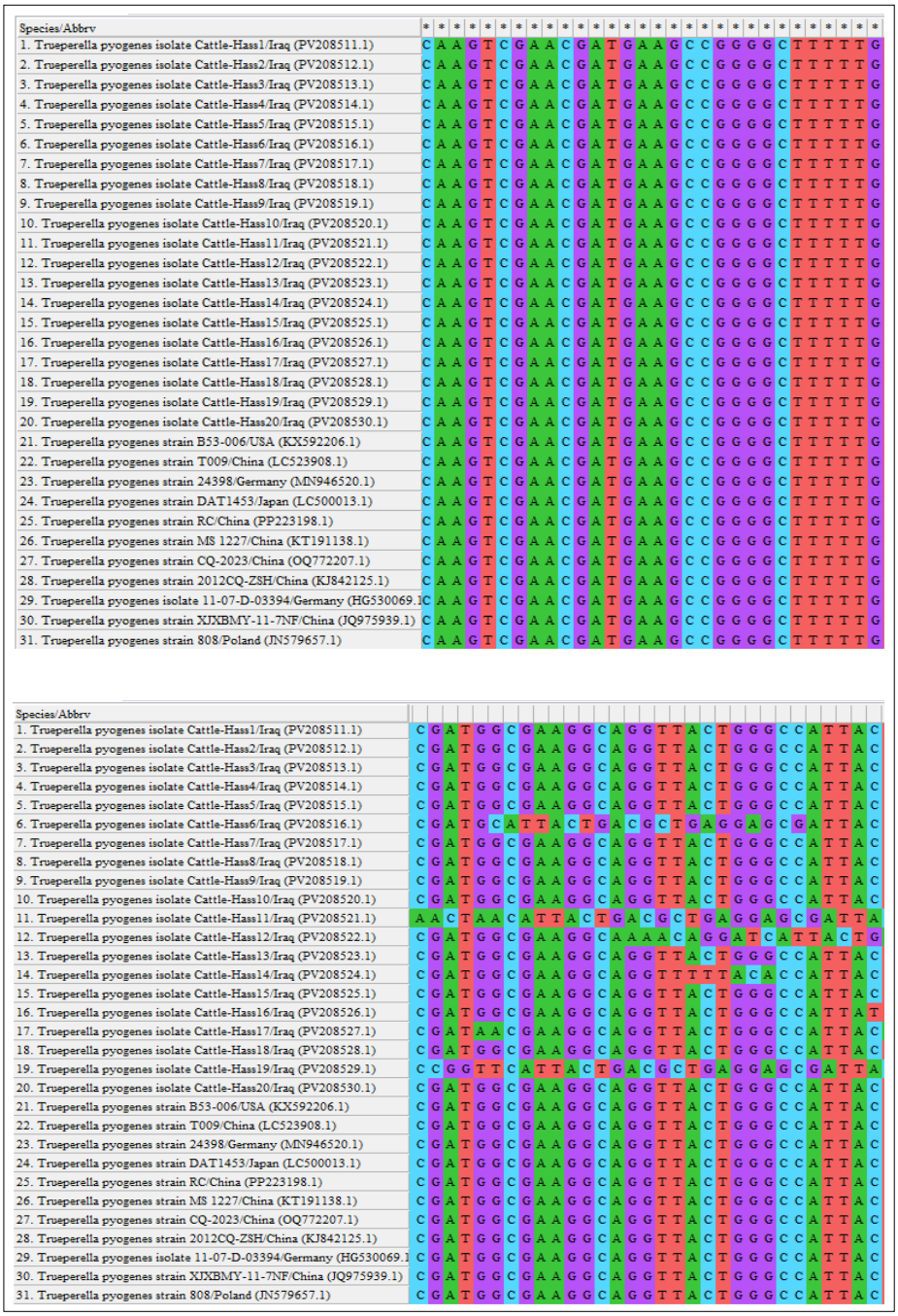

Fig 3: Multiple sequence alignment using the MEGA-11 software.

and recovered from 35 ruminants including 25 cattle, 8 goats and 2 sheep. These data indicate that the local *T. pyogenes* isolates might geographically widespread, potentially transmitted pathways and having similar virulence factors. Thus, the *16S rRNA* gene is a powerful tool for bacterial detection and identification of genetic diversity and can enable researchers to identify *T. pyogenes* in various samples and environments (Gharban and Yousif, 2021; Kwiecień *et al.*, 2024; Saifudeen *et al.*, 2024).

Risk factors

In the current study, the distribution of positive *T. pyogenes* infections were varied significantly ($p<0.05$) among the groups of each factor (Table 2). For body part, incidence of positive *T. pyogenes* infections as well as values of OR

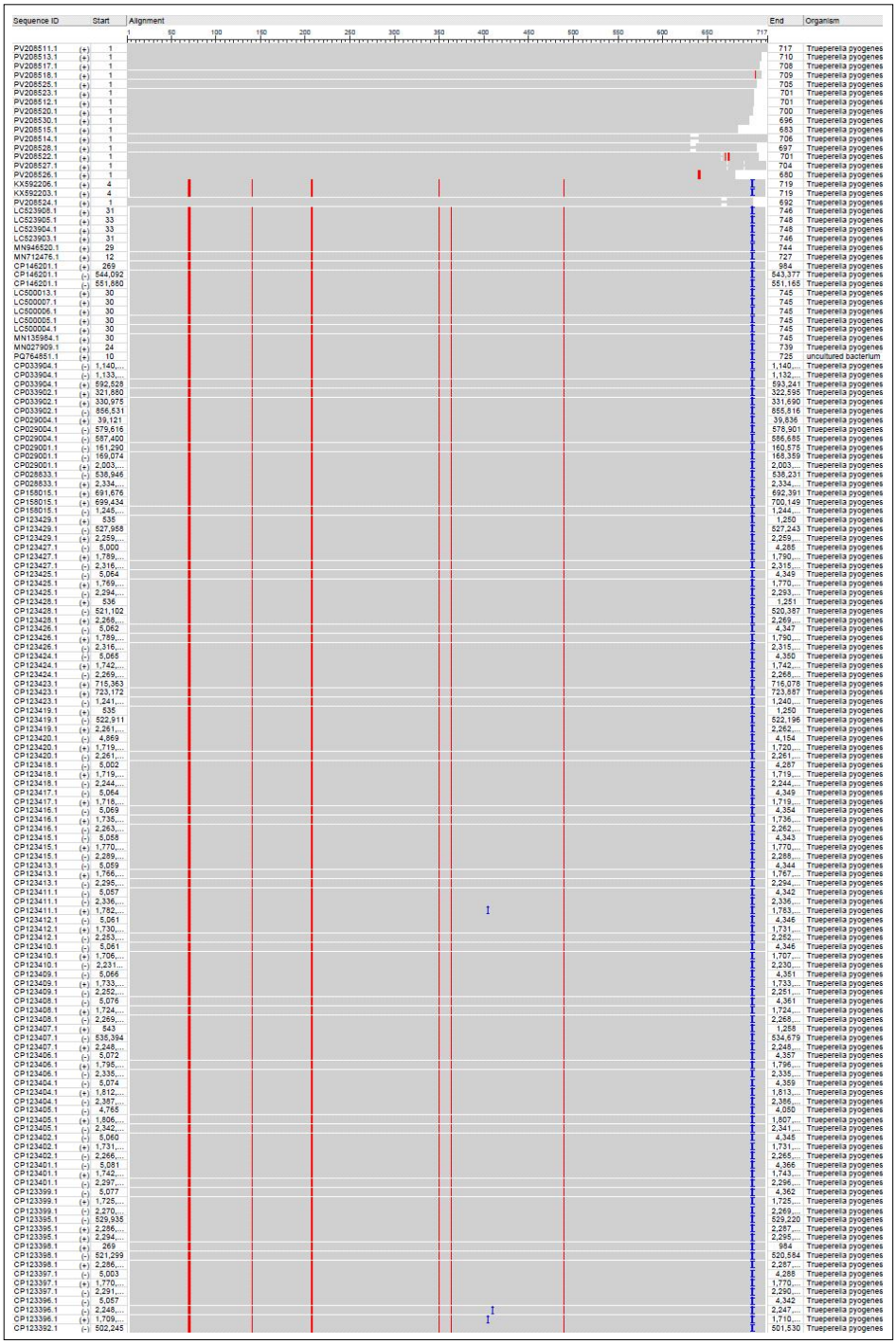

Fig 4: Multiple sequence alignment using the NCBI-MSA viewer.

and RR were elevated significantly (p<0.0487, p<0.0001 and p<0.0001, respectively) in abdomen (13.83%, 3.902 and 3.485, respectively) but decreased significantly in neck and back (0%, 0 and 0, respectively), head (2.63%, 0.329 and 0.317, respectively), forelimbs (3.33%, 0.4 and 0.418, respectively) and hindlimbs (3.57%, 0.435 and 0.462, respectively) when compared to pelvis (8.51%, 1.208, 1.197, respectively). Concerning age, although insignificant variation (p<0.0755) was seen in incidence of positive *T. pyogenes* infections among age groups of study animals, cattle aged 1-4 years and >4 years were reported a significant (p<0.0001) higher values of OR (1.399 and 1.397, respectively) and RR (1.367 and 1.348, respectively) than those of <1 year (0.57 and 0.6). Regarding sex, insignificant differences (p<0.0562) was detected between the incidence rate of positive *T. pyogenes* infections among females (8.81%) and males (3.85%); however, significant (p<0.0001) higher risk of infection (OR and RR) was observed in females (2.415 and 2.316, respectively) than males (0.414 and 0.432, respectively). Although, there was

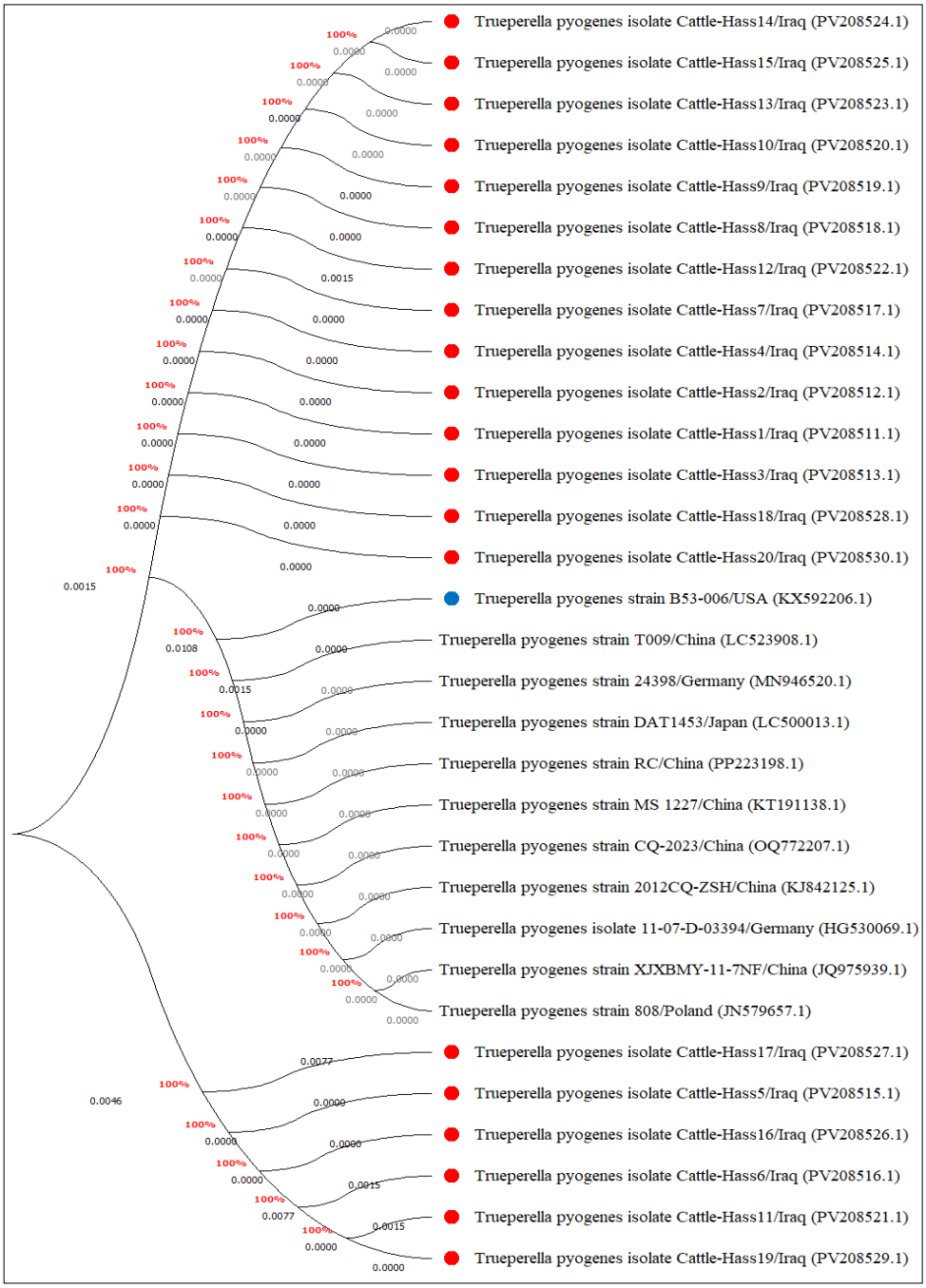

Fig 5: Phylogenetic tree analysis of study and NCBI-BLAST *T. pyogenes* isolates/strains.

Table 2: Distribution of positive *T. pyogenes* among groups of animals' risk factors.

Factor	Total no.	Positive	OR	RR
Body part				
Head	38	1 (2.63%)	0.329	0.317
Neck	11	0 (0%)	0	0
Forelimbs	30	1 (3.33%)	0.4	0.418
Back	23	0 (0%)	0	0
Abdomen	94	13 (13.83%) *	3.902 ****	3.485 ****
Hindlimbs	28	1 (3.57%)	0.435	0.462
Pelvis	47	4 (8.51%)	1.208	1.197
p-value		0.0487	0.0001	0.0001
95% CI		0.06119 to 9.167	0.3852 to 2.178	0.3009 to 1.981
Age (Year)				
<1	124	7 (5.65%)	0.57	0.6
1-4	96	9 (9.38%)	1.399 ****	1.367 ****
>4	51	5 (9.8%)	1.397 ****	1.348 ****
p-value		0.0755	0.0001	0.0001
95% CI		-2.602 to 13.95	0.1078 to 2.416	0.01832 to 2.192
Sex				
Female	193	17 (8.81%)	2.415 ****	2.316 ****
Male	78	3 (3.85%)	0.414	0.432
p-value	0.0562	0.0001	0.0001	
95% CI		-25.18 to 37.84	11.30 to 41.13	10.60 to 33.34

no clear explanation for this case, we thought that there were problems with inappropriate environments or handling methods, high stocking densities and tying the animal too tightly (Alam *et al.*, 2010). In addition, the increasing incidence of mastitis in adult cows and warts in both males and females might play a role in increasing the risk and occurrence of abscessed wounds (Gharban *et al.*, 2023).

Although no significant association was reported between the incidence of *T. pyogenes* age of study animals, there was a significant elevation in risk of infection in cattle aged 1-4 years and >4 years more than those of <1 years. The relationship between age and infection risk can be complex; however with advancing age, increased exposure to several pathogens and waning of immunity might explain our results (Vlasova and Saif, 2021). Our results showed that female cattle were at a higher risk of *T. pyogenes* infection than males. This might be attributed to anatomical and physiological differences since skin of males is thicker particularly in dermis and may have higher density of collagen and sebaceous glands than females (Yang *et al.*, 2017; Tarique *et al.*, 2021). Also, female cattle experienced more skin wounds due to mastitis that leading to increase susceptibility and skin damage; as well as during pregnancy that increase pressure on abdomen from the growing fetus (Zhao and Lacasse, 2008; More *et al.*, 2017).

CONCLUSION

This might represent the first Iraqi study indicates the incidence of *T. pyogenes* in cattle suggesting the need to furthermore studies in other bovine infections as well as in other animals' infection. Molecular phylogeny demonstrates a high reliability in detection of the bacterium in the swabs of abscess wounds; therefore, utilization of molecular techniques can support the cost, time and effort in detection of such infections. However, effective wound management necessitates a comprehensive evaluation of the wound itself, coupled with a systemic examination of the animal's overall health and environmental condition. Also, successful treatment could be obtained by regular draining of pus along with daily antiseptic dressing and parenteral administration of antibiotics.

Conflict of interest

All authors declare that they have no conflict of interest.

REFERENCES

Abd-El-Hady, A.A.A. (2015). Clinical observations on some surgical udder and teat affections in cattle and buffaloes. *Scholars Journal of Agriculture and Veterinary Sciences*. **2:** 270-281. https://doi.org/10.36347/sjavs.2015.v02i04.001.

Al-Abedi, G.J., Sray, A.H., Hussein, A.J. and Gharban, H.A. (2018). Molecular detection and blood profiles evaluation of naturally infected camels with subclinical *Trypanosoma evansi*, Iraq. *Annals of Tropical Medicine and Public Health*. **23:** SP232243. https://doi.org/10.36295/asro.2020.232243.

Alam, M.R., Gregory, N.G., Jabbar, M.A., Uddin, M.S., Kibria, A.S.M.G. and Silva, F.A. (2010). Skin injuries identified in cattle and water buffaloes at livestock markets in Bangladesh. *Veterinary Record*. **167:** 415-419. https://doi.org/10.1136/vr.c3301.

Al-Eodawee, E.M., Essa, I.M., Aggar, O.A. and Gharban, H.A. (2024). Molecular, hematological and biochemical investigation of *Trypanosoma* spp. in sheep. *Asian Journal of Dairy and Food Research*. **43(4):** 736-742. doi: 10.18805/ajdfr.DRF-412.

Al-Graibawi, M.A., Yousif, A.A., Gharban, H.A. and Zinsstag, J. (2021). First serodetection and molecular phylogenetic documentation of *Coxiella burnetii* isolates from female camels in Wasit governorate, Iraq. *Iraqi Journal of Veterinary Sciences*. **35:** 47-52. https://doi.org/10.33899/ijvs.2021.130888.1890.

Al-Iraqi, O.M., Shareef, A.Y. and Dhahir, S.H. (2016). Urinary tract bacterial infection of local Iraqi buffaloes (*Bubalus bubalis*) in Mosul city. *The Iraqi Journal of Veterinary Medicine*. **40:** 124-130. https://doi.org/10.30539/iraqijvm.v40i2.123.

AL-Tofaily, Y.I.K. and Al-Roddan, M.A. (2011). Epidemiological and bacteriological study on subclinical mastitis in she-camels (*Camelus dromedarius*) in some areas of middle euphrates in Iraq. *Kufa Journal for Veterinary medical Sciences*. **2:** 106-172. https://doi.org/10.29079/vol10iss2art156.

Almaliky, N.K., Al-Sari, U.A., AL-Shaeli, S.J. and Gharban, H.A. (2024). Insights for possible association and impact of thyroidectomy to osteoarthritis. *Beni-Suef University Journal of Basic and Applied Sciences*. **13:** 99. https://doi.org/10.1186/s43088-024-00554-1.

Alrafas, H.R., Alahmed, J.A.S., Essa, I.M., Kadhim, S.Z., Al-Tameemi, H.M., Abduljaleel, M.R. and Al-Hejjaj, M.Y. (2023). Role of anti-inflammatory interleukin 10 in asymptomatic heartworm infection (Dirofilariasis) in dogs. *Advancements in Life Sciences*. **10:** 412-417. https://doi.org/10.62940/als.v10i3.1817.

Arslan, S.H., Al-Jammly, M.M.H. and Khaleel, Q.J. (2009). Diagnosis of causes of suppurative arthritis in sheep in mosul, Iraq. *Iraqi Journal of Veterinary Sciences*. **23:** 115-119.

Ashur, A.A., Yakimova, E.A., Burlakova, G.I., Kapustin, A.V. and Laishevtsev, A.I. (2019). Prevalence and etiological significance of *Trueperella* (*Arcanobacterium*) *pyogenes*. *Bulletin of the Kursk State Agricultural Academy*. **7:** 120-129.

Bahloul, H.A., Bekenniche, N., Zeriouh, I.F., Hadadji, M. and Kihal, M. (2024). Identification of indigenous *lactobacillus fermentum* by 16S rRNA gene sequencing isolated from two different ecosystems: Newborn infants and bee's intestine. *Asian Journal of Dairy and Food Research*. **43(4):** 702-707. doi: 10.18805/ajdfr.DRF-346.

Boolchandani, M., D'Souza, A.W. and Dantas, G. (2019). Sequencing-based methods and resources to study antimicrobial resistance. *Nature Reviews Genetics*. **20:** 356-370. https://doi.org/10.1038/s41576-019-0108-4.

Carr, F.J. (2017). Microbiology: A fundamental introduction. *EC Microbiology*. **8:** 123-183.

Ertaş, H.B., Kiliç, A., Özbey, G. and Muz, A. (2005). Isolation of *Arcanobacterium* (*Actinomyces*) *pyogenes* from abscessed cattle kidney and identification by PCR. *Turkish Journal of Veterinary and Animal Sciences*. **29:** 455-459.

Ehmud, M.A., Mteran, A.S., Al-Furaiji, M.H. and Hassoon, G.S. (2025). Identification and characterization of *Staphylococcus aureus* 16s rRNA gene isolate from environmental and clinical sources. *Asian Journal of Dairy and Food Research*. **44(5):** 807-814. doi: 10.18805/ajdfr.DRF-506.

Faccin, M., Wiener, D.J., Rech, R.R., Santoro, D. and Hoffmann, A.R. (2023). Common superficial and deep cutaneous bacterial infections in domestic animals: A review. *Veterinary Pathology*. **60:** 796-811. https://doi.org/10.1177/03009858231176558.

Gharban, H.A.J. and Yousif, A.A. (2021). First isolation and molecular phylogenetic analysis of *Coxiella burnetii* in lactating cows, Iraq. *Bulgarian Journal of Veterinary Medicine*. **24:** 508-519. https://doi.org/10.15547/bjvm.2322.

Gharban, H.A., Al-Shaeli, S.J. and Hussen, T.J. (2023). Molecular genotyping, histopathological and immunohistochemical studies of bovine papillomatosis. *Open Veterinary Journal*. **13:** 26-41. https://doi.org/10.5455/ovj.2023.v13.i1.4.

Hamedi, M., Esmaeili, H., Tamai, I.A., Moradbakhsh, M.R. and Davoudi, M. (2025). *Trueperella pyogenes*; A cause of spleen multi abscesses in cattle. *Journal of Medical Bacteriology*. **2025:** 28-32. https://doi.org/10.18502/jmb.v13i1.18044.

Ishiyama, D., Mizomoto, T., Ueda, C., Takagi, N., Shimizu, N., Matsuura, Y. and Kawai, K. (2017). Factors affecting the incidence and outcome of *Trueperella pyogenes* mastitis in cows. *Journal of Veterinary Medical Science*. **79:** 626-631. https://doi.org/10.1292/jvms.16-0401.

Jiheel, M.J., Qushawi, A.A., Latif, A.D. and Gharban, H.A. (2025). Physiological and histological ameliorative effects of walnuts-pulp extract on kidney function of rats treated with citalopram. *Asian Journal of Dairy and Food Research*. **44(5):** 753-760. doi: 10.18805/ajdfr.DRF-537.

Kwiecień, E., Stefańska, I., Kizerwetter-Świda, M., Chrobak-Chmiel, D., Czopowicz, M., Moroz-Fik, A. and Rzewuska, M. (2024). Genetic diversity and virulence properties of caprine *Trueperella pyogenes* isolates. *BMC Veterinary Research*. **20:** 395-407. https://doi.org/10.1186/s12917-024-04262-x.

Liu, M.C., Wu, C.M., Liu, Y.C., Zhao, J.C., Yang, Y.L. and Shen, J.Z. (2009). Identification, susceptibility and detection of integron-gene cassettes of *Arcanobacterium pyogenes* in bovine endometritis. *Journal of Dairy Science*. **92:** 3659-3666. https://doi.org/10.3168/jds.2008-1756.

Lux, C.N. (2022). Wound healing in animals: A review of physiology and clinical evaluation. *Veterinary Dermatology*. **33:** 19-27. https://doi.org/10.1111/vde.13032.

Magossi, G., Gzyl, K.E., Holman, D.B., Nagaraja, T.G., Amachawadi, R. and Amat, S. (2025). Genomic and metabolic characterization of *Trueperella pyogenes* isolated from domestic and wild animals. *Applied and Environmental Microbiology*. **91:** 17-24. https://doi.org/10.1101/2024.09.09.612034.

Malone, M. and Schultz, G. (2022). Challenges in the diagnosis and management of wound infection. *British Journal of Dermatology*. **187:** 159-166. https://doi.org/10.1111/bjd.21612.

Menon, T. (2024). Uncommon pathogens causing infective endocarditis. *Indian Journal of Thoracic and Cardiovascular Surgery*. **40:** 8-15. https://doi.org/10.1007/s12055-023-01627-z.

Mobed, A., Baradaran, B., de la Guardia, M., Agazadeh, M., Hasanzadeh, M., Rezaee, M.A. and Hamblin, M.R. (2019). Advances in detection of fastidious bacteria: from microscopic observation to molecular biosensors. *TrAC Trends in Analytical Chemistry*. **113:** 157-171. https://doi.org/10.1016/j.trac.2019.02.012.

Mohammad, H.A., Ajaj, E.A. and Gharban, H.A. (2022). The first study on confirmation and risk factors of acute and chronic canine distemper in stray dogs in Wasit Province, Iraq, using enzyme-linked immunosorbent assay and reverse transcription-polymerase chain reaction. *Veterinary World*. **15:** 968-974. https://doi.org/10.14202/vetworld.2022.968-974.

More, S., Bicout, D., Botner, A., Butterworth, A., Calistri, P. and Winckler, C. (2017). Animal welfare aspects in respect of the slaughter or killing of pregnant livestock animals (cattle, pigs, sheep, goats, horses). *EFSA Journal*. **15:** 47-52. https://doi.org/10.2903/j.efsa.2017.4782.

Nagaraja, T.G. (2022). Trueperella. Veterinary Microbiology. pp. 252-256. https://doi.org/10.1002/9781119650836.ch27.

Paul, J. (2024). Skin Infections. In: Disease Causing Microbes. Cham: Springer International Publishing. pp. 65-98. https://doi.org/10.1007/978-3-031-28567-7_2.

Petit, T., Spergser, J., Rosengarten, R. and Aurich, J. (2009). Prevalence of potentially pathogenic bacteria as genital pathogens in dairy cattle. *Reproduction in Domestic Animals*. **44:** 88-91. https://doi.org/10.1111/j.1439-0531.2007.01002.x.

Rezanejad, M., Karimi, S. and Momtaz, H. (2019). Phenotypic and molecular characterization of antimicrobial resistance in *Trueperella pyogenes* strains isolated from bovine mastitis and metritis. *BMC Microbiology*. **19:** 1-17. https://doi.org/10.1186/s12866-019-1630-4.

Rogovskyy, A.S., Lawhon, S., Kuczmanski, K., Gillis, D.C., Wu, J., Hurley, H. and Duncan, K. (2018). Phenotypic and genotypic characteristics of *Trueperella pyogenes* isolated from ruminants. *Journal of Veterinary Diagnostic Investigation*. **30:** 348-353. https://doi.org/10.1177/1040638718762479.

Rzewuska, M., Kwiecień, E., Chrobak-Chmiel, D., Kizerwetter-Świda, M., Stefańska, I. and Gieryńska, M. (2019). Pathogenicity and virulence of *Trueperella pyogenes*: A review. *International Journal of Molecular Sciences*. **20:** 2737-2751. https://doi.org/10.3390/ijms20112737.

Saad, N.M., Kamal, S. and Abo-Sagher, A.G. (2023). Incidence of *Trueperella pyogenes* in raw milk. *Assiut Veterinary Medical Journal*. **69:** 123-133. https://doi.org/10.21608/avmj.2023.221296.1163.

Sadoon, A.S. (2021). Study of bacterial infections in the urinary system of goats in Mosul City. *Basrah Journal of Veterinary Research*. **20:** 172-191. https://doi.org/10.23975/bjvetr.2031.175866.

Saifudeen, S.M., Anilkumar, K., Aravindakshan, T.V., Valsalan, J., Ally, K. and Gleeja, V.L. (2024). Evaluation of rumen microbes for milk fat yield in cattle based on 16S rRNa amplicon metagenomic sequencing. *Asian Journal of Dairy and Food Research*. doi: 10.18805/ajdfr.DR-2176.

Santos, T.M.A., Caixeta, L.S., Machado, V.S., Rauf, A.K., Gilbert, R.O. and Bicalho, R.C. (2010). Antimicrobial resistance and presence of virulence factor genes in *Arcanobacterium pyogenes* isolated from the uterus of postpartum dairy cows. *Veterinary Microbiology*. **145:** 84-89. https://doi.org/10.1016/j.vetmic.2010.03.001.

Sharun, K., Dhama, K., Tiwari, R., Gugjoo, M.B., Iqbal Yatoo, M., Patel, S.K. and Chaicumpa, W. (2021). Advances in therapeutic and managemental approaches of bovine mastitis: A comprehensive review. *Veterinary Quarterly*. **41:** 107-136. https://doi.org/10.1080/01652176.2021.1882713.

Singh, S., Sharma, P., Pal, N., Sarma, D.K., Tiwari, R. and Kumar, M. (2024). Holistic one health surveillance framework: Synergizing environmental, animal and human determinants for enhanced infectious disease management. *ACS Infectious Diseases*. **10:** 808-826. https://doi.org/10.1021/acsinfecdis.3c00625.

Stefańska, I., Kwiecień, E., Górzyńska, M., Sałamaszyńska-Guz, A. and Rzewuska, M. (2022). RAPD-PCR-based fingerprinting method as a tool for epidemiological analysis of *Trueperella pyogenes* infections. *Pathogens*. **11:** 562-573. https://doi.org/10.3390/pathogens11050562.

Tamai, I.A., Mohammadzadeh, A., Mahmoodi, P., Pakbin, B. and Salehi, T.Z. (2023). Antimicrobial susceptibility, virulence genes and genomic characterization of *Trueperella pyogenes* isolated from abscesses in dairy cattle. *Research in Veterinary Science*. **154:** 29-36. https://doi.org/10.1016/j.rvsc.2022.10.018.

Tarique, I., Shah, M.G., Kalhoro, I.B., Nizamani, Z.A., Tariq, M., Gandahi, J.A. and Sahito, B. (2021). Morphometric analysis of skin in different body regions of young and adult red Sindhi cattle *Bos indicus*. *Pakistan Journal of Zoology*. **53:** 1603-2000. https://doi.org/10.17582/journal.pjz/20191020141047.

Valkova, L., Vecerek, V., Voslarova, E., Kaluza, M. and Takacova, D. (2021). The welfare of cattle, sheep, goats and pigs from the perspective of traumatic injuries detected at slaughterhouse postmortem inspection. *Animals*. **11:** 1-14. https://doi.org/10.3390/ani11051406.

Vlasova, A.N. and Saif, L.J. (2021). Bovine immunology: Implications for dairy cattle. *Frontiers in Immunology*. **12:** 643206. https://doi.org/10.3389/fimmu.2021.643206.

Wente, N., Leimbach, S., Woudstra, S. and Krömker, V. (2024). *Trueperella pyogenes*-strain diversity and occurrence in dairy herds. *Pathogens*. **13:** 534. https://doi.org/10.3390/pathogens13070534.

Wheeler, J.A. (2019). Cattle related trauma: Are we underestimating its severity? *Australasian Medical Journal*. **12:** 123-130. https://doi.org/10.35841/1836-1935.12.4.123-130.

Yang, X., Cui, Y., Yue, J., He, H., Yu, C., Liu, P. and Meng, Y. (2017). The histological characteristics, age-related thickness change of skin and expression of the HSPs in the skin during hair cycle in yak (*Bos grunniens*). *PLoS One*. **12:** e0176451. https://doi.org/10.1371/journal.pone.0176451.

Zastempowska, E. and Lassa, H. (2012). Genotypic characterization and evaluation of an antibiotic resistance of *Trueperella pyogenes* (*Arcanobacterium pyogenes*) isolated from milk of dairy cows with clinical mastitis. *Veterinary Microbiology*. **161:** 153-158. https://doi.org/10.1016/j.vetmic.2012.07.018.

Zhao, X. and Lacasse, P. (2008). Mammary tissue damage during bovine mastitis: Causes and control. *Journal of Animal Science*. **86:** 57-65. https://doi.org/10.2527/jas.2007-0302.