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Helcococcus ovis – AN EMERGING PATHOGEN IN NEW ZEALAND?

Helcococcus ovis is a slow-growing, facultatively anaerobic, gram-positive coccus of the Peptoniphilaceae family. It was first identified as a novel bacterial species in 1999 after being isolated in mixed culture from the lung, liver and spleen of an adult male sheep in Scotland and from the milk of a sheep in Spain with subclinical mastitis (Collins et al. 1999). Early reports queried the significance of this bacteria as it is commonly co-isolated with other bacteria such as *Trueperella pyogenes* and *Staphylococcus* spp.. However more recently, there has been growing evidence that it is capable of causing disease by itself and may be an under recognised pathogen in ruminants.

Overseas, *H. ovis* has been detected in cases of bacterial endocarditis, bronchopneumonia, septic arthritis, abortion, metritis and mastitis in cattle (Post et al. 2003, Kutzer et al. 2008, Bilk et al. 2011, Jost and Sickinger, AHVLA disease surveillance report 2014, Locatelli et al 2013, Cunha et al. 2018, Liu et al. 2014). Respiratory infections and endocarditis have also been reported in other species including sheep, goats, a horse and a rooster (Zhang et al. 2009, Garcia et al 2012, Rothschild et al 2004, Crispo et al. 2017). In one German study, the organism was cultured from 33% of hearts with bovine valvular endocarditis, in most instances in heavy pure growth (Kutzer et al. 2008).

The rise of genomic techniques in the last 15 years has also revealed a potential role in metritis in cows. Studies using PCR and gene sequencing have caused a complete reassessment of the bacteria involved in metritis by detecting species which would otherwise be challenging to isolate in traditional culture-based studies. Recent genomic studies have found *H. ovis* was the second most abundant species identified in uterine swabs from cows with metritis and cows with metritis were 18 times more likely to have this organism detected than healthy post-partum cattle (Cunha et al. 2018, Cunha et al. 2024). Interestingly in another study, *H. ovis* was not found to be part of the core flora of the vaginal tract but was a core bacteria detected in the blood from cows in the peripartum period. This study found correlations between the blood and uterine microbiomes, leading the authors to suggest blood may act as a transmission route for bacteria from the gut to the uterus in the peri-partum period (Jeon et al. 2017).

Figure 1: Cases of *Helcococcus ovis* recorded in New Zealand since 2018 including disease presentation and animal species involved (number of cases provided in brackets). In 71% of cases, *H. ovis* was isolated as a single pure growth. In the remaining cases, it was co-isolated with another pathogen, most commonly *Trueperella pyogenes*. No cases of *H. ovis* are recorded in the Awanui Veterinary database or appear in the MPI surveillance database prior to 2018. This may reflect changes in laboratory methods and the difficulty of isolating and identifying this organism previously rather than a true absence of clinical cases prior to 2018.

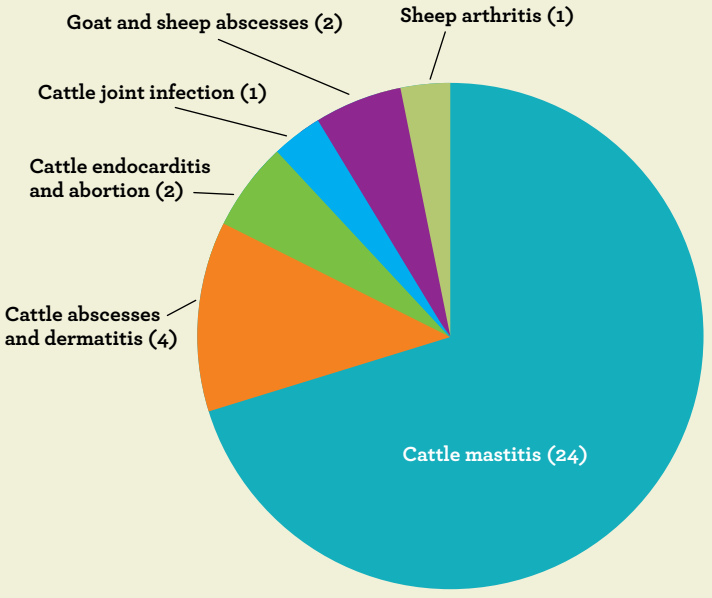
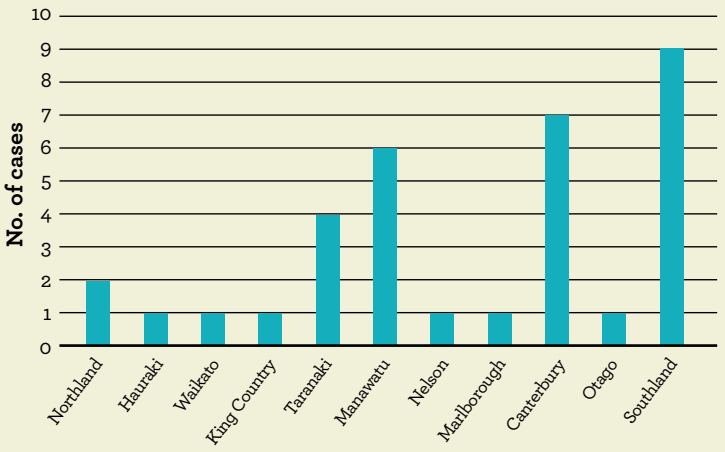


Figure 2: Regional distribution of *Helcococcus ovis* cases



Culture characteristics

Helcococcus ovis has a number of characteristics that make it difficult to both culture and identify. While it will grow on standard blood agar plates, it is slow growing taking up to 3 to 6 days for tiny grey colonies to appear. This means standard 48-hour cultures may miss its presence and it is also easily overgrown by other bacteria. Additionally, some isolates may only grow in the laboratory in the presence of other bacteria and may require plates to be streaked first with *Staphylococcus aureus* to be detectable. In these cases, *H. ovis* displays satellitism appearing as small grey colonies growing around the periphery of larger *S. aureus* colonies. This satellitism is believed to be due to a requirement for pyridoxal produced by the other bacteria although *H. ovis* often loses this nutritional dependency and is able to grow independently when subcultured (Kutzer et al. 2008, Rothschild et al. 2004). As plates streaked with *Staphylococcus* are not used routinely in diagnostic laboratories, this may further decrease the detection rate of *Helcococcus*.

Once cultured, *Helcococcus ovis* is not easy to identify due to its biochemical inertness. It may be non-haemolytic or alpha-haemolytic and can be misidentified as other gram-positive, catalase-negative cocci such as *Aerococcus* spp. and *Streptococcus* spp. when using routine biochemical methods (Almuzara et al. 2016). Additionally, some laboratory identification systems such as the Vitek system ((bioMérieux, Marcy l'Etoile, France) may not be able to adequately identify this organism (Cunha et al. 2024). Accurate identification therefore requires the use of MALDI-tof or PCR techniques.

Helcococcus in New Zealand

Over the past seven years, *H. ovis* has been isolated from 34 cases submitted to Awanui Veterinary laboratories (Figure 1). This represents all isolates that have been recorded in the Ministry for Primary Industries Animal Health Surveillance programme. These have occurred throughout the North and South Island (Figure 2). There are no reports of this organism prior to 2018, however this may reflect the difficulties of identifying this organism using traditional microbiological methods before the more recent regular use of MALDI-tof.

continued

Figure 3: Injection site infections post-vaccination in a mob of ewe hoggets in Southland (Kelly and Hughes, 2022). Approximately 9% of the mob became unwell with fore- or hind-limb lameness, severe cellulitis at the injection site or over the rump with subsequent sloughing of the skin (A). Some animals also developed oedema of the brisket with eight deaths recorded. Culture of fluid collected from the lesions at the first visit grew a heavy pure growth of *Helcococcus ovis* and suppurative bacterial myositis with numerous intra-lesional gram-positive cocci were observed on histopathology. Samples collected on a subsequent visit grew a mixed culture of *H. ovis* and *Trueperella pyogenes*. *Photo credit: K. Kelly.*

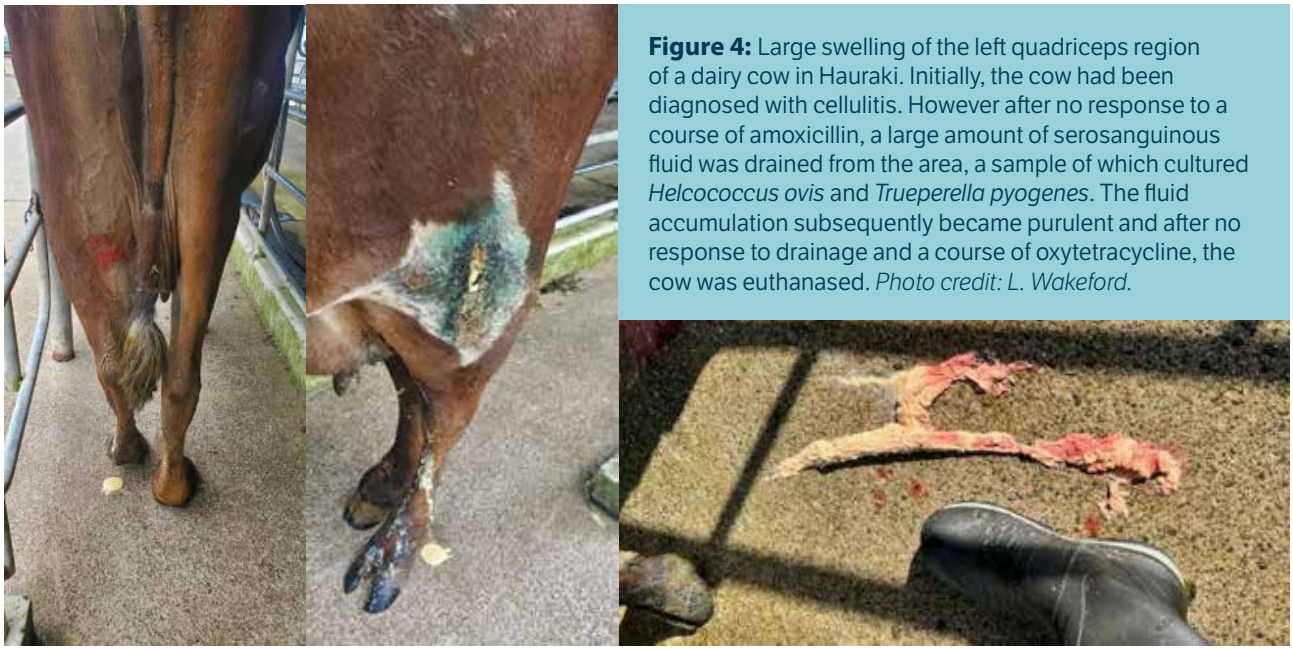


Figure 4: Large swelling of the left quadriceps region of a dairy cow in Hauraki. Initially, the cow had been diagnosed with cellulitis. However after no response to a course of amoxicillin, a large amount of serosanguinous fluid was drained from the area, a sample of which cultured *Helcococcus ovis* and *Trueperella pyogenes*. The fluid accumulation subsequently became purulent and after no response to drainage and a course of oxytetracycline, the cow was euthanased. *Photo credit: L. Wakeford.*

continued

The majority of cases diagnosed in New Zealand have been cases of mastitis in cattle. In mastitis cases, *H. ovis* was most often isolated in pure growth (16/24) although in 8 cases it was co-isolated with other organisms including *Trueperella pyogenes* (4 cases), *Staphylococcus aureus* (1 case) and *Proteus* sp. (1 case). The submissions often came with a history of treatment failure (9/24) following multiple courses of antibiotics including tetracyclines, tylosin, and systemic and intramammary penicillins. This was despite subsequent susceptibility testing indicating that the organism was often susceptible to all routinely tested antimicrobials (penicillin, amoxicillin-clavulanic acid, cephalothin, erythromycin, tetracycline). Tetracycline resistance was detected in three out of the 16 isolates and one isolate was resistant to erythromycin. Overseas, tetracycline resistance has been frequently identified in *H. ovis* isolated from cases of metritis, endocarditis and bronchopneumonia in cattle with erythromycin resistance detected less commonly (Bilk et al. 2011, Cunha et al. 2024). The results of in-clinic testing were not often reported with the New Zealand submissions but in three mastitis cases where *H. ovis* was isolated in heavy pure growth, one had been identified as Strep. dysgalactiae/agalactiae, one as Strep. uberis and one as E. coli/unspecified gram-negative on Mastatest.

Another common presentation has been subcutaneous abscesses or injection site infections, the most significant being in a flock of ewe hoggets following vaccination (Figure 3). An injection site infection involving *H. ovis* has also been diagnosed in a goat and there have been three cases of localised abscesses in dairy cattle including a large abscess on the hindquarter of an adult milking cow (Figure 4), an abdominal abscess, and abscesses on the chin and the angle of the mandible of an adult cow. In these cases, *Helcococcus* was often co-isolated with *T. pyogenes*.

The frequency Helcococcus is isolated alongside other bacterial species in veterinary cases indicate that there is synergism in animal infections as well.

Septic arthritis due to *H. ovis* has been diagnosed in 2-week-old lambs and in a separate case, the organism was isolated in pure growth from the joint fluid of an 18-month-old bull presenting with arthritis of the left and right carpi. *Helcococcus ovis* has also been isolated from an interdigital swab from a Jersey bull in Canterbury, which was part of a group of four out of 16 bulls that had severe interdigital dermatitis with granulating proud flesh extending up to the metacarpus. This case is interesting given *Helcoccocus* species in humans have been found to play an important synergistic role in chronic wound infections, particularly diabetic ulcers (Caliendo et al. 1995, Durand et al. 2024). The frequency *Helcococcus* is isolated alongside other bacterial species in veterinary cases indicate that there is synergism in animal infections as well. This has been demonstrated in one study using a mouse model of *H. ovis* isolated from bovine mastitis cases. In this study, more severe disease developed when *H. ovis* was co-innoculated with *T. pyogenes* (Liu et al. 2022). This has parallels with another member of the Peptoniphilaceae family, *Peptoniphilus indolicus*, and *T. pyogenes*, where more severe disease and more frequent systemic involvement develops when both organisms are present in cases of summer mastitis in cattle (Hillerton et al. 1989).

Finally, two cases of systemic infection in dairy cows have been diagnosed In New Zealand. In one case, *H. ovis* was isolated in moderate growth from the stomach contents of an aborted foetus. Histopathology of the foetus and placenta supported an infectious aetiology. The dam had not been well for a significant period before aborting and was in light body condition. A week after aborting, the cow was found to have a heart murmur and metritis. Vegetative endocarditis of the left atrioventricular (AV) heart valve was subsequently diagnosed on post-mortem. Unfortunately, culture of the valve yielded a heavy growth of post-mortem contaminants and *H. ovis* was not detected. The second case is presented in the following report:

Case study

A two-year-old Holstein heifer on a Southland farm was initially examined in October 2023 for two large abscesses in the right and left axillary region in the location of the axillary lymph nodes. The mob had a history of footrot over the winter and this heifer had been treated along with a number of others. While the footrot resolved, she continued to appear unwell. The axillary abscesses were lanced, flushed and treated with five days of penicillin G (Intracillin 300, Virbac) and meloxicam (Metacam 40, Boehringer Ingelheim). However, two months later in December, the heifer was re-examined due to continued weight loss.

Figure 5: Two large abscesses present in the subcutaneous tissues of the right lateral thigh and tibial region of a Southland heifer. A heavy pure growth of *Helcococcus ovis* was cultured from fresh tissue collected from the abscess.

Figure 5: Both the right and left lungs had areas of consolidation ventrally and were severely adhered to the ribcage. Histopathology revealed a severe chronic active suppurative bronchopneumonia with a pure heavy growth of *Helcococcus ovis* on culture of fresh left lung tissue.

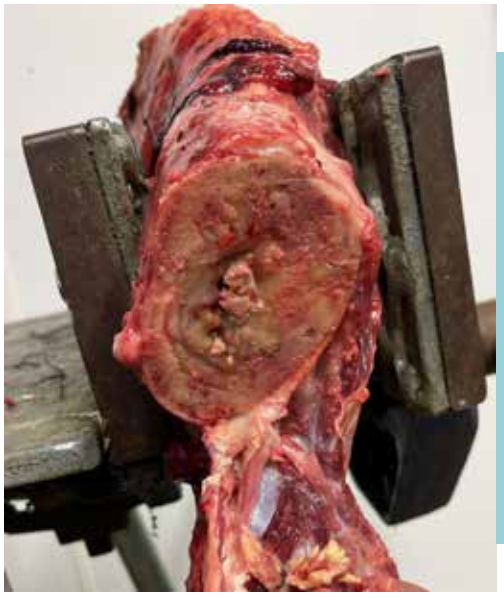


Figure 7: Cross-section of a large firm swelling of the fifth right rib revealing large amounts of caseous purulent material. The histopathology of the rib was consistent with a large abscess from which a heavy pure growth of *Helcococcus ovis* was cultured.

On re-examination, the heifer had harsh lung sounds and a grade 1/6 heart murmur. Small draining abscesses, 5-6 cm in diameter, were present in the right and left axilla. A large hot diffuse swelling approximately 15 cm in diameter was present over the lateral femoral region of the right hindleg with a smaller hot mass of 10 cm diameter ventral to this at the mid-tibia level. A small amount of purulent material was aspirated from both lesions. A hard 6 cm lump was also

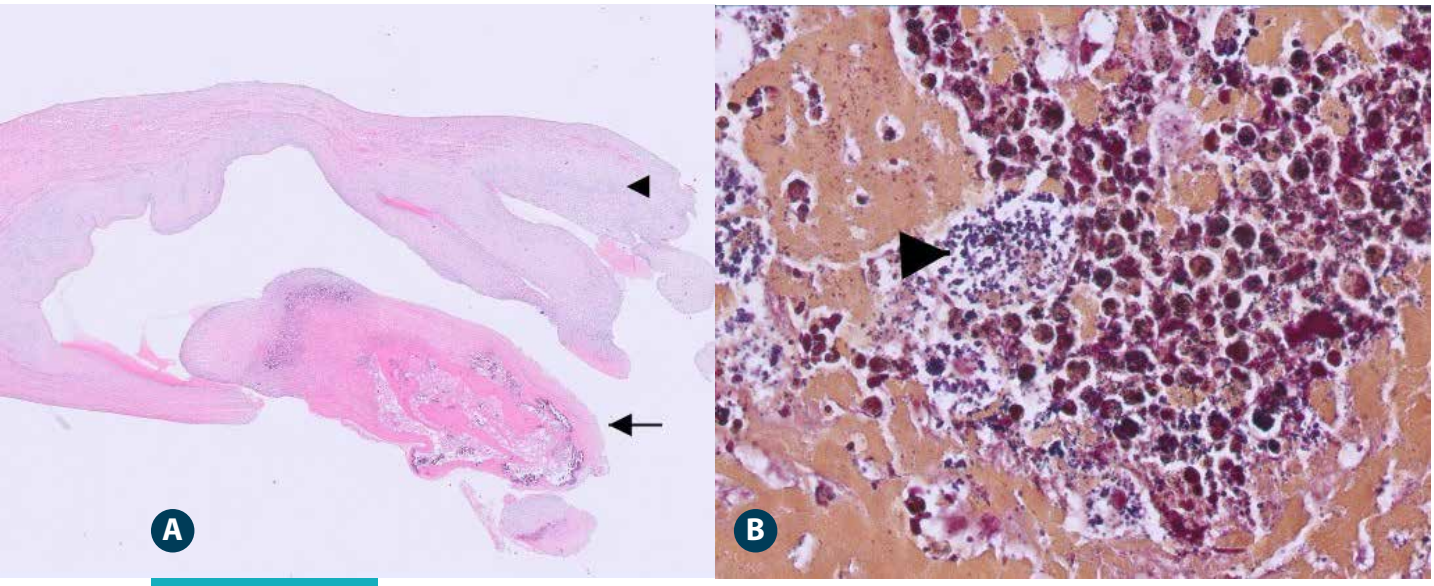


Figure 8: Histopathology of the right atrioventricular valve. (A) A large thrombus (arrow) composed of fibrin, degenerate neutrophils and macrophages is attached to the AV valve (arrowhead). (B) On a Gram-stained section of the thrombus, large colonies of gram-positive cocci (arrowhead) are visible amongst the fibrin and leukocytes.

continued

noted behind the right elbow involving the rib. Due to the progression of the disease and failure to respond to therapy, the heifer was euthanased and a post-mortem was conducted.

On post-mortem, a large multilobulated abscess was found on incision of the swelling on the right hindleg (Figure 5). Both lungs were consolidated ventrally with severe adhesions to the ventral ribcage (Figure 6). Hard swellings involving the left fourth rib and the right fifth rib were also found. On sectioning of the ribs, these swellings contained large amounts of caesous material (Figure 7). Mild endocarditis was noted on the right AV valve and a large pale depressed area was present in the left kidney suggesting a previous infarct.

A selection of fresh and fixed samples were submitted to the laboratory for histopathology and culture. Severe chronic-active suppurative bronchopneumonia was present in the lung. Histopathology also confirmed the presence of a large focal abscess in the rib and abscesses in the left axilla and the subcutis of the right hindleg. Chronic active fibrinosuppurative endocarditis was found in the right AV valve of the heart (Figure 8). At all these locations, multiple colonies of gram-positive cocci were observed within the lesions. Culture of fresh tissue from the right rib abscess, left axilla, right hindleg and left lung yielded heavy pure growths of *H. ovis* confirming this as the likely infecting organism. A moderate growth of *H. ovis* was also co-isolated with *Streptococcus uberis* from a swab of an intercostal lymph node. A light growth of *Streptococcus parauberis* was detected from the right AV valve. It is not certain whether these streptococci were involved in the disease or if they were post-mortem contaminants. The latter however seems more likely given they were not isolated from the other tissue locations.

An aerogenous pattern was seen in the histologic lesions of the lung suggesting this infection may have started as a primary bronchopneumonia. However, the history of footrot is interesting given the previous case of *H. ovis* isolated from an outbreak of interdigital dermatitis in Jersey bulls in the South Island.

In summary, it is clear that the spectrum of diseases associated with *H. ovis* overseas are also occurring in New Zealand. Given the difficulties of isolating and identifying of this organism, it is difficult to gauge the true prevalence of *H. ovis* infections in dairy cattle in New Zealand but it is worth keeping this organism in mind for non-responding mastitis where culture fails to isolate the usual culprits. Further research would be interesting to see whether it has wider involvement in other disease presentations such as metritis, endocarditis and interdigital dermatitis in this country.

References:

AHVLA disease surveillance report. (2014) Bovine abortions caused by *Helcococcus ovis*. *Vet. Rec.* 175:38–41.

Bilk, S., Nordhoff, M., Schulze, C., Wieler, L. H., and Kutzer, P. (2011) Antimicrobial susceptibilities and occurrence of resistance genes in bovine *Helcococcus ovis* isolates. *Vet. Microbiol.*, 149, 488–491.

Caliendo AM, Jordan CD, Ruoff KL. (1995) *Helcococcus*, a new genus of catalase-negative, gram-positive cocci isolated from clinical specimens. *J. Clin. Micro.* 33:1638-1639.

Collins MD, Falsen E, Foster G, Monasterio LR, Dominguez L, Fernandez-Garazabal JF. (1999) *Helcococcus ovis* sp. nov., a gram-positive organism from sheep. *Int. J. Syst. Bacteriol.*, 49:1429–1432.

Cunha F, Jeon SJ, Daetz R, Vieira-Neto A, Laporta J, Jeong KC, Barbet AF, Risco CA, Galvão KN. (2018) Quantifying known and emerging uterine pathogens, and evaluating their association with metritis and fever in dairy cows. *Theriogenology*. 114:25–33.

Cunha F, Zhai Y, Casaro S, Jones KL, Hernandez M, Bisinotto RS, Kariyawasam S, Brown MB, Phillips A, Jeong KC, Galvão KN. (2024) Pangenomic and biochemical analyses of *Helcococcus ovis* reveal widespread tetracycline resistance and a novel bacterial species, *Helcococcus* bovis. *Front. Microbiol*., 15:1456569. doi: 10.3389/fmicb.2024.1456569

Durand B, Daher R, Grenga L, Morsli M, Armengaud J, Lavingne J-P, Dunyach-Remy C. (2024) Interactions between *Helcococcus* kunzii and *Staphylococcus aureus:* How a commensal bacterium modulates the virulence and metabolism of a pathogen in a chronic wound in vitro model. *BMC Microbiol.* 24:406.

Garcia A, Risco D, Benitez JM, Martinez R, Garcia WL, Cuesta JM, Gomez L, Sanchez S. (2012) *Helcococcus ovis* isolated from a goat with purulent bronchopneumonia and pulmonary abscesses. *J. Vet. Diagn. Invest.* 24:235–237.

Hillerton, J. E., and A. J. Bramley. (1989) Infection following challenge of the lactating and dry udder of dairy cows with Actinomyces pyogenes and Peptostreptococcus indolicus. *Br. Vet. J.,* 145:148–158.

Jeon SJ, Cunha, Vieira-Neto FA, Bicalho RC, Lima, S, Bicalho ML, Galvão KN. (2017) Blood as a route of transmission of uterine pathogens from the gut to the uterus in cows. *Microbiome* 5:109.

Jost A, Sickinger M. *Helcococcus ovis* associated with septic arthritis and bursitis in calves – a case report. (2021) *BMC Vet. Res.* 17:291. https://doi.org/10.1186/s12917-021-02996-6

Kelly K, Hughes L. (2022) *Helcococcus ovis* – another (unwanted?) new first for New Zealand? Grazing Gazette, Issue 62:10-11.

Kutzer P, Schulze C, Engelhardt A, Wieler LH, Nordhoff M. (2008) *Helcococcus ovis*, an emerging pathogen in bovine valvular endocarditis. *J. Clin. Microbiol.* 46:3291–5.

Liu K, Deng Z, Zhang L, Gu X, Liu G, Liu Y, Chen P, Gao J, Han B and Qu W. (2022) Biological characteristics and pathogenicity of *Helcococcus ovis* isolated from clinical bovine mastitis in a chinese dairy herd. Front. Vet. Sci. 8:756438. doi: 10.3389/fvets.2021.756438.

Locatelli C, Scaccabarozzi L, Pisoni G, Bronzo V, Casula A, Testa F, et al. *Helcococcus* kunzii and *Helcococcus ovis* isolated in dairy cows with puerperal metritis. (2013) *J. Gen. Appl. Microbiol.* 2013;59:371–4.

Post WK, Rushton SD, Billington SJ. (2003) Valvular endocarditis associated with *Helcococcus ovis* infection in a bovine. *J. Vet. Diagn. Invest.* 15:473–475.

Rothschild CM, Oaks JL, Schaupp JK, Rurangirwa FR, Sellon DC, Hines MT. (2004) *Helcococcus ovis* isolated from a pulmonary abscess in a horse. *J. Clin. Microbiol.* 42:2224–6.

Zhang Y, Cui J, Parkinson A, Hayes J, Ott K, Byrum B. (2009) Isolation of *Helcococcus ovis* from sheep with pleuritis and bronchopneumonia. *J. Vet. Diagn. Invest.* 21:164-166.

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