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CLINICAL STUDY

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An outbreak of ischaemic teat necrosis in a dairy herd in Taranaki, New Zealand

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ABSTRACT

Case History: In spring 2021, on a seasonally calving, pastorally based, Taranaki dairy farm, 12 first-calving heifers (≤ 30 days post-calving) developed similar dry, red to black, crusting lesions on the medial aspect of the teat udder junction extending down the medial teat. Some cows had multiple teats affected. Treatment was initially unrewarding and did not slow the progression of the disease. Overall, 8/12 cows recovered, and 4/12 cows were culled, with three of the cows culled after a teat sloughed and the fourth after surgical amputation of a teat. Outbreaks of the same condition, on the same farm but affecting fewer animals, occurred in spring 2022 (n = 6) and spring 2023 (n = 3).

Clinical Findings: An initial scab-like or crusting lesion progressed to resemble a thick eschar consisting of very dry and hard dead tissue. The unaffected areas of the teat felt normal but immediately under the dead tissue, there was a warm, firmer area consistent with an inflammatory reaction. Removing the scab led to profuse bleeding, with no visible bed of granulation underneath the scab. There was no leaking of milk in those cows that lost a teat, and no smell to the lesions themselves. Serology and virology ruled out the involvement of bovine alphaherpesvirus (BoHV-2) bovine gammaherpesvirus (BoHV-4), orthopoxviruses (cowpox) and parapoxviruses (pseudocowpox). Histopathology of an affected and surgically amputated teat showed multifocal erosion and ulceration of the epidermis, covered by a thick serocellular crust. In areas of ulceration, there were numerous neutrophils, and the dermis was expanded by granulation tissue with variable numbers of neutrophils, eosinophils, and lymphocytes around small blood vessels.

Diagnosis: Based on the similarity of the history, presentation, and histopathological changes to those described for a novel disease reported in the UK, a diagnosis of ischaemic teat necrosis (ITN) was made.

Clinical Relevance: If ITN is an emerging condition in New Zealand and becomes as prevalent as it has in the UK, clinicians will be confronted with a significant new welfare problem in dairy cows. Anecdotally, there have been reports of other ITN outbreaks in New Zealand, and the Ministry for Primary Industries would be interested in collating reports from other New Zealand veterinarians.

Abbreviations: BoHV-2: Bovine alphaherpesvirus; BoHV-4: Bovine gammaherpesvirus; DD: Digital dermatitis; ITN: Ischaemic teat necrosis; MPI: Ministry for Primary Industries; UCD: Udder cleft dermatitis

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Introduction

Ischaemic teat necrosis (ITN) is an emerging disease mostly affecting first-calved heifers in the UK, with animals developing a focally extensive, red-to-black, crusting lesion, frequently on the medial aspect of the teat udder junction, which is initially dry, and can later extend down the teat (Baines and Hillerton 2004; Blowey 2004; Crosby-Durrani 2023). The lesions may have a fetid smell (Clegg *et al*. 2016) and can progress rapidly, causing pain and discomfort (Crosby-Durrani *et al*. 2022a). Cows with ITN have been observed aggressively licking their affected teats. The affected teat itself may be lost, either through sloughing (after extensive necrosis), self-trauma, or surgical amputation to control the spread of disease (Crosby-Durrani 2023). ITN is estimated to have occurred on half of UK farms, with some farms losing up to 20% of early lactation, first-calved heifers (Blowey 2015). Even if the teat is not lost and the lesion heals, it can become firm and difficult to milk, with nearly 25% of affected animals culled in the first 100 days of lactation (Crosby-Durrani *et al*. 2022b). The lesions usually develop within 90 days of calving, and most afflicted animals will have one or two affected teats. There is no teat predilection for lesions, with all teats equally likely to be affected or to slough (Crosby-Durrani *et al*. 2022a). In half of

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affected cows, the lesions will spread onto the haired area of the udder.

A similar condition to ITN has been reported in milking buffalo (*Bubalis bubalus*) from India, where it is known as "tenacious teat ulcer" (Dhillon *et al*. 2005; Sharma and Singh 2006), in the Netherlands (Sol *et al*. 2005), where it is also called "teat necrosis," and in the USA, where it is known as "seasonal teat lesion syndrome" and is described as a type of dry gangrene (Moriello *et al*. 1993). The Dutch report describes two presentations for teat necrosis, the first of which resembles the UK description of ITN where the skin of the teat becomes hardened and purplish, and the second in which a vesicle at the teat udder junction ruptures, causing a small fissure. This fissure may then erode into the teat, leading to sloughing if it progresses deeply enough or encircles the teat. The presence of udder cleft dermatitis (UCD), along with chapped teats, has been positively associated with the presence of ITN within a herd (Crosby-Durrani *et al*. 2022b). UCD is a common skin disease of dairy cows worldwide, where mild to severe dermatitis and ulcers develop in the fold between the front quarters of the udder or in the fold at the junction of the anterior udder and the abdomen (Waller *et al*. 2014). The aetiology of UCD is unknown and is likely multifactorial (Ekman *et al*. 2021; Van Engelen *et al*. 2021).

Digital dermatitis (DD)-associated treponemes have been suggested as a cause of both ITN (Clegg *et al*. 2016) and UCD (Evans *et al*. 2010). However, Crosby-Durrani (2023) found little evidence to support an aetiological role for DD-associated treponemes in ITN, although their involvement as a bacterial contaminant could not be ruled out. Likewise, the involvement of bovine herpes mammillitis (bovine alphaherpesvirus (BoHV-2) and bovine gammaherpesvirus (BoHV-4)), cowpox (orthopoxvirus) and pseudocowpox (parapoxvirus) in the aetiology of ITN has been mostly ruled out by researchers in the UK, the Netherlands, and the USA (Moriello *et al*. 1993; Sol *et al*. 2005; Crosby-Durrani 2023), although an association between tenacious teat ulcer and BoHV-2 in India is proposed (Sharma and Singh 2006).

There is no known treatment for ITN. Blowey (2004) commented that the response to non-steroidal anti-inflammatory drugs was poor, but two treatments have been suggested for tenacious teat ulcer in buffalo. The first is a mixture of zinc oxide, boric acid and kaolin in a 1:1:1 ratio, dusted onto the teat lesion after cleaning with methylated spirit, which is reported to give noticeable improvement after 4 days of treatment (Dhillon *et al*. 2005). The second is the use of a post-milking teat dip containing povidone-iodine and glycerine in a 4:1 ratio, which resolves lesions in 25–35 days (Sharma and Singh 2006).

To the authors' knowledge, ITN has not previously been reported in New Zealand, although a suspected case of ITN was reported to the Ministry for Primary Industries (MPI) exotic pest and disease hotline in spring 2018 but ruled out after consultation with an MPI exotic disease incursion investigator (Bingham 2019). The present report describes the first confirmed outbreak of ITN in New Zealand.

Case history

The disease outbreak occurred in spring 2021 in a 720-head (including 170 heifers), seasonally calving, closed dairy herd (no replacement cows or breeding bulls had been purchased or leased for 2 years) from Taranaki. The planned start of calving was 15 July, with the planned start of mating 12 weeks later on 6 October. The herd was milking twice daily, mainly Friesian, and Friesian-cross cows through a 50-bale rotary shed. The milking liners were changed twice yearly, in June or July and in December, and an annual milking machine check was carried out in November or December. The teat spray was iodine-based, with added emollient, and was used for the whole season. Teat spraying was automatic at cups-off.

The first five cases of teat necrosis were found sequentially on 9, 11, 25, 26 and 27 August, with three further cases observed between 27 August and 1 September. By the time of the last case, on 21 September, 12 cows had been affected, all first-calved heifers and all within 20–30 days of calving. The first veterinary visit was on 27 August, the second on 1 September, and the last on 22 September.

Clinical findings

At the first veterinary visit, there were five affected first-calved heifers, of which two (40%) had completely sloughed a teat and one (20%) had partially sloughed a teat. While in the collecting yard prior to examination, the affected first-calved heifers were seen aggressively licking their teats by the attending veterinarian (see video in online Supplementary Information). In affected animals, the earliest observed lesion was a small scab at the base of the teat, usually on the medial aspect (Figure 1A). The lesions grew rapidly, doubling in size within 2 days and progressed either to a crusting lesion on the medial aspect of the teat udder junction, with dry, red to black lesions extending down the medial teat (Figure 1B), or to partial or complete sloughing of the teat (Figure 1C). If the teat was not lost, the early-stage scab could develop, within 6–8 weeks, into a thick eschar of dead tissue that felt very dry and hard (Figure 1D).

The remaining areas of an affected teat felt like normal teat tissue, but there was a warm, firmer area, consistent with an inflammatory reaction, immediately under the dead tissue. When the scab from a lesion (Figure 1D) was removed, there was profuse bleeding with no granulation tissue present underneath the

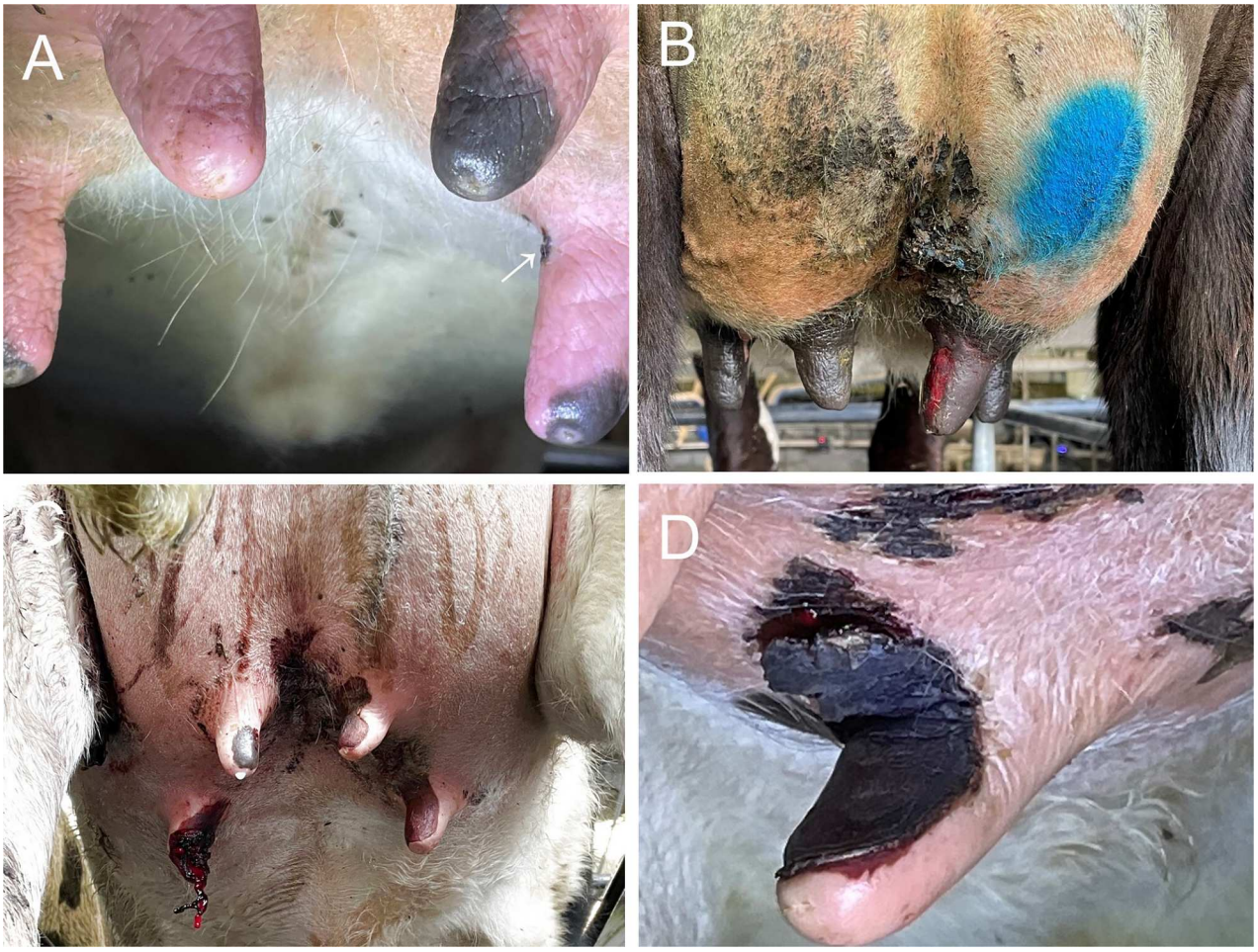

Figure 1. Photographs of the udders and teats of first-calved milking heifers affected with ischaemic teat necrosis on a Taranaki dairy farm in New Zealand, showing lesion progression. (A) earliest observable lesion: a small dark scab near the right fore teat base (white arrow). (B) 36 hours after first presenting, right rear teat showing crusting at the teat udder junction and red discolouration on the medial aspect. (C) More advanced ischaemic teat necrosis lesions showing severe necrosis and partial sloughing of the left fore teat and diffuse red to black discolouration of the medial aspect of the right fore and right hind teats. (D) Surface of the lesion on the right fore teat (same teat as in (c) but 5 weeks later) preparing to slough.

scab. There was no leaking of milk in cows that lost a teat, even after surgical amputation, and there was no odour associated with the lesions. Mastitis was not a feature of the outbreak other than in one cow that developed mastitis after the lesion involved the tip of the teat. All the affected cows maintained their body condition score and no decrease in appetite was observed.

There was no history of digital dermatitis on the farm and there were no clinical signs of digital dermatitis or photosensitisation observed in either the affected or unaffected cows.

Pathological and laboratory findings

At the first veterinary visit on 27 August 2021, with ITN as a possible differential diagnosis, the farm veterinarian (author KC) called the MPI exotic pest and disease hotline and discussed the case with the duty incursion investigator (author JO). As well as the investigation of reports of suspect exotic disease the MPI incursion investigation team directive includes the investigation of suspect new and emerging diseases.

A sampling plan was agreed. On 1 September, an affected teat from the most recently identified case was surgically amputated (by KC) under a regional nerve block, with half submitted to a commercial laboratory (Awanui Veterinary, Palmerston North, NZ) for histopathology and the remainder to the Animal Health Laboratory, (Upper Hutt, NZ) for virus isolation and molecular diagnostics. These diagnostics included two passages of teat tissue through bovine lung and monkey kidney (Vero) cell lines, a generic conventional PCR test for herpesvirus based on the method described by VanDevanter *et al*. (1996), a conventional PCR test for pan-poxvirus (subfamily *Chordopoxvirinae*) based on the method described by Li *et al*. (2010), and a real-time, quantitative PCR test for BoHV-2 based on the method described by Lanave *et al*. (2020). The half of the teat submitted to the commercial diagnostic laboratory was fixed in 10% buffered formalin, routinely processed for histopathology, and slides were stained with H&E.

On the same day as the teat amputation, and again 3 weeks later (22 September) blood samples were

taken from eight affected first-calved heifers, eight unaffected first-calved heifers, and eight unaffected mixed-age cows from the affected herd. Paired sera were submitted to the United States Department of Agriculture National Veterinary Services Laboratories (Ames, IA, USA) where antibody concentrations against BoHV-2 and BoHV-4 were measured using virus neutralisation and immunofluorescence antibody testing, respectively.

Histopathology

Over the medial surface of the teat, there was multifocal erosion and ulceration of the epidermis, covered by a thick serocellular crust containing occasional bacterial colonies (Figure 2A). Large numbers of neutrophils and fewer eosinophils were present in the areas of ulceration and occasionally formed intracorneal pustules in the adjacent intact epidermis. Multifocally, keratinocytes exhibited cytoplasmic vacuolation (ballooning degeneration) and there was occasional vesicle formation within the superficial dermis, with these vesicles containing eosinophilic fluid or erythrocytes. Rare keratinocytes had marginated chromatin and small, indistinct, lightly basophilic structures within the nucleus, which were reported as possible inclusion bodies (Figure 2B). In the areas of erosion, and adjacent to the areas of ulceration, the epidermis was frequently hyperplastic (Figure 2C). The underlying dermis was expanded by immature granulation

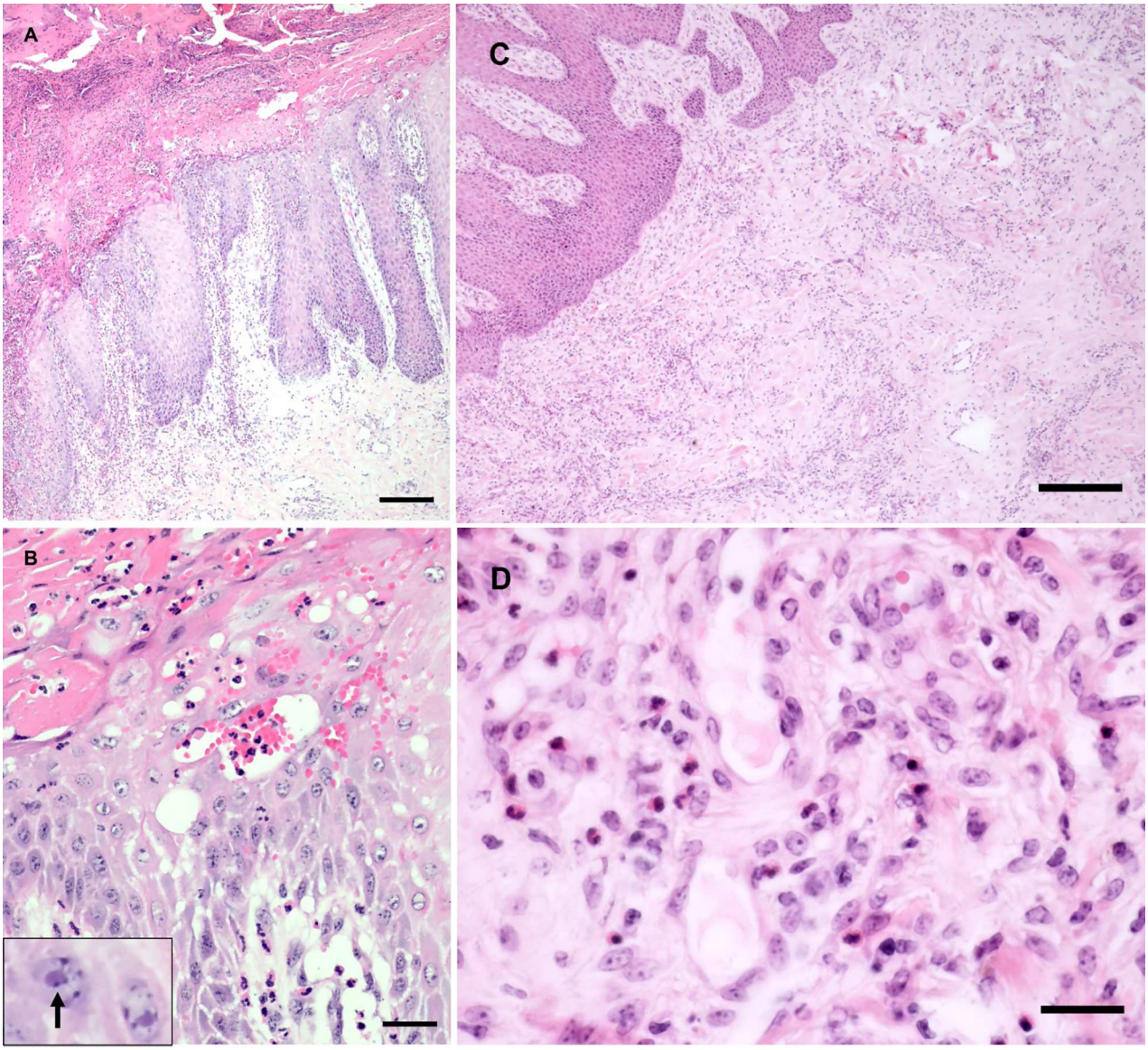

Figure 2. Histological sections from the amputated teat of a first-calved milking heifer affected with ischaemic teat necrosis on a Taranaki dairy farm in New Zealand showing: (A) erosion of the superficial teat epidermis, which is covered by a serocellular crust with hyperplasia of the basal layers of the epidermis (bar = 60 µm); (B) ballooning degeneration of the superficial epithelial cells with neutrophils and eosinophils (bar = 30 µm), and at higher magnification (inset), a squamous epithelial cell with vesicular chromatin and a circular, lightly basophilic inclusion within (arrow); (C) inflammation and fibrosis within the dermis of the teat (bar = 120 µm); and (D) a higher magnification of the dermis, with eosinophils, neutrophils, and lymphocytes around small blood vessels (bar = 30 µm). All H&E.

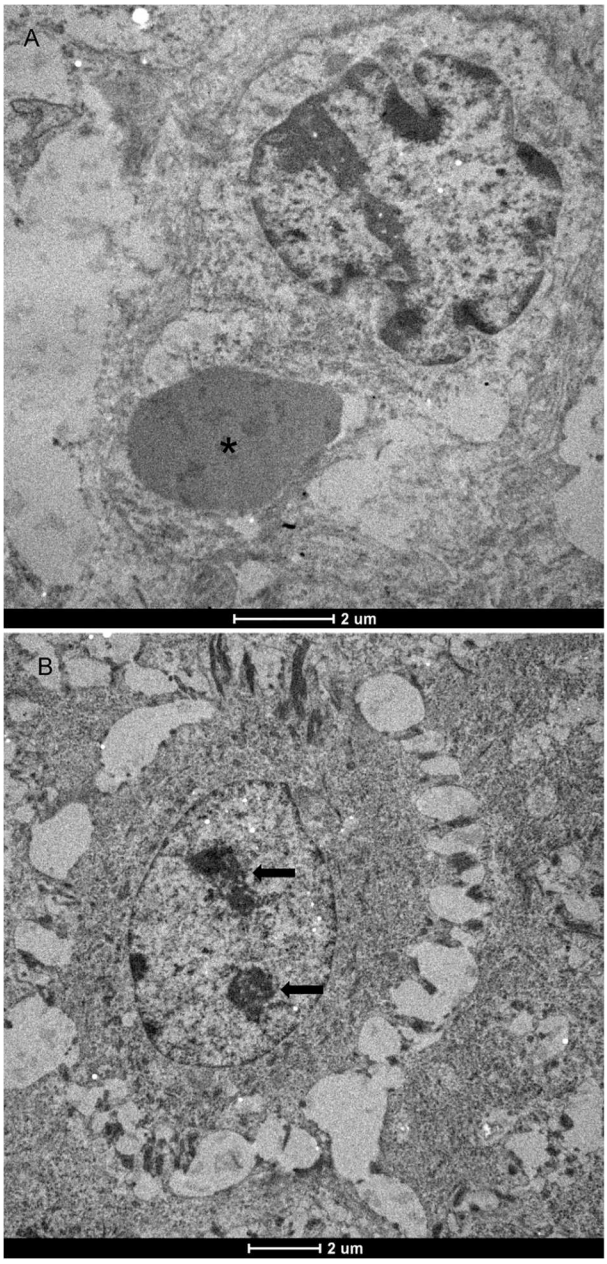

Figure 3. Transmission electron microscopy of sections from the teat of a first-calved milking heifer affected with ischaemic teat necrosis on a Taranaki dairy farm in New Zealand showing (A) infolding of the nuclear membrane with condensation of the underlying chromatin, and aggregates of keratin within the cytoplasm (*); and (B) dark areas within the nucleus consistent with nucleoli (black arrows). No viral structures are visible.

tissue and fibrosis, infiltrated by low numbers of perivascular lymphocytes, macrophages, eosinophils, and neutrophils (Figure 2D). The teat sinus was normal.

A histological diagnosis of severe, multifocal, subacute, ulcerative mammillitis was made. Furthermore, it was suggested to have an "outside in" pathogenesis, where something from the external environment causes epithelial damage that leads to underlying tissue damage, in contrast to an "inside out" pathogenesis where a systemic agent reaches the tissue via the circulatory system and causes damage to deeper layers and subsequently the epithelium.

Electron microscopy

Using a scalpel blade, a 2-mm cube of tissue in the region of the suspect intranuclear inclusions was removed from the paraffin block used to prepare the histological sections. The paraffin was melted by briefly placing the cube in a 70°C oven, then the tissue cube was processed through four changes of xylene before being moved through decreasing concentrations of ethanol and finally placed in a buffer, as described by Graham and Orenstein (2007). The cube was post-fixed in osmium tetroxide and embedded into epoxy resin. Thin sections from resin blocks were stained using lead citrate/uranyl acetate and viewed using a Tecnai G2 Biotwin transmission electron microscope (FEI, Hillsboro, OR, USA) at the Manawatū Microscopy and Imaging Centre (Massey University, Palmerston North, NZ).

Squamous cells in the epidermis occasionally demonstrated infolding of the nuclear membrane (cytoplasmic invaginations) with condensation of chromatin adjacent to the areas of infolding (Figure 3A). Homogeneous grey structures, consistent with aggregates of keratin, were also present in the cytoplasm of some cells. Multifocal dark areas were present in the nucleus of many cells (Figure 3B), consistent with nucleoli, and no viral structures were observed in the sections examined.

Virus isolation and molecular diagnostics

Following two passages of teat tissue in bovine lung and monkey kidney (Vero) cells, no cytopathogenic effect was observed that would suggest the presence of viruses. The molecular tests for herpesvirus, both generic and specific for BoHV-2, orthopoxviruses and parapoxviruses, were also all negative.

Serology

All eight unaffected first-calved heifers were seronegative on both sampling occasions. Of the affected first-calved heifers, one was seropositive (1:2,000) for

Table 1. Serology results for bovine alphaherpesvirus (BoHV-2) and bovine gammaherpesvirus (BoHV-4) from affected and unaffected milking cows during (1 September 2021) and after (22 September 2021) an outbreak of ischaemic teat necrosis (ITN) on a Taranaki dairy farm in New Zealand.

	1 September 2021		22 September 2021	
	BoHV-2[a]	BoHV-4[b]	BoHV-2[a]	BoHV-4[b]
Affected first-calved heifers (n = 8)	0	0	0	1
Unaffected first-calved heifers (n = 8)	0	0	0	0
Unaffected mixed-age cows (n = 8)	0	2	0	2

[a]Virus neutralisation.
[b]Immunofluorescence antibody testing.

BoHV-4 at the second sampling. Two of the unaffected mixed-age cows were seropositive (1:200) for BoHV-4 at both sampling events (Table 1).

Based on the similarity of the history, presentation, and histopathological changes to those described for a novel disease presentation reported in the UK, a diagnosis of ischaemic teat necrosis was made.

On-farm management

Initial management

The farm staff had been treating the teat lesions with a topical antibiotic spray (Alamycin Aerosol, containing 35.8 mg/g oxytetracycline hydrochloride complexed with magnesium chloride and polyvinylpyrrolidine; Norbrook NZ Ltd., Auckland, NZ). There was no evidence that this treatment had been efficacious. All affected animals were still milked twice a day, and if possible, at the end of milking to limit spread.

Subsequent management

Since the first outbreak in spring 2021, treatment evolved by trial and error to involve dressing the lesion at alternate milkings with a topical antibiotic spray (Tetravet spray, containing 25 mg/mL oxytetracycline hydrochloride, 2 mg/mL gentian violet and methanol; Bayer New Zealand Ltd., Auckland, NZ) and an iodine-containing teat cream (FIL Active Teat Cream, containing 0.2% iodine with mixed emollients; FIL, Mount Maunganui, NZ). At the morning milking, once an affected cow had been milked, Tetravet spray was applied (2–3 pumps) to the teat lesions, and at the afternoon milking, a thick layer of FIL teat cream containing iodine was applied to the teat lesions once milking had finished. Prior to the application of topical treatments, any scab that was only lightly attached was removed; however, over time, this practice became discretionary and was not carried out if fresh blood or a pain response in the heifer was observed. Affected heifers with full or partial teat loss were treated as above but only milked in the unaffected quarters. The response to treatment was not improved by bandaging teats, and so these were left uncovered.

This treatment regime appeared to be quite effective; the impression of farm staff was that it stopped the progression of the lesion until the skin healed and the "scab" dropped off.

Importantly, if started early enough, the affected first-calved heifers could continue to be milked and were tolerant of twice-a-day milking. However, it cannot be stressed strongly enough that if treatment was not started early enough then the response was generally, although not universally, poor.

Clinical outcomes

At the end of the outbreak in spring 2021, 8/12 (67%) affected first-calved heifers had recovered and were retained in the herd, and 4/12 (33%) were culled, three of which had sloughed teats and one after a teat was amputated. In spring 2022, there were six cases of ITN in the herd with one cow culled, and in spring 2023 there were three cases with no cows culled. Affected cows were considered to have "recovered" if all affected teats on an individual affected animal healed, and the cow carried on milking in the herd. Remarkably, in recovered animals, there were no discernible teat deformities post-recovery. Any animal that lost a part or whole teat (i.e. did not have four intact teats) was dried off and considered "non-recovered." These animals were culled once the udder defect had sufficiently healed.

Discussion

The clinical and microscopic features of the teat lesions in the cows in this case study are consistent with those described in ITN in the UK (Crosby-Durrani *et al*. 2022b). The important clinical similarities were that the outbreak was restricted to first-calved heifers, that each heifer had red to black, focal, extensive necrosis of one or more teats, and that 3/12 (25%) heifers had a teat slough. Microscopically there was serocellular crusting, ulceration, hyperplasia, ballooning degeneration of the superficial epithelial cells, and eosinophilic infiltration. ITN has not been previously reported in New Zealand but may be underrecognised, as the authors are aware of anecdotal reports of other cases from 2022 and 2023.

The consistent presentation of ITN on the medial aspect of affected teats in the New Zealand outbreak is by itself quite extraordinary and should, in our opinion, be an important clue to the aetiology of this unusual condition. In the first published UK description of ITN, Blowey (2004) hypothesised that ITN could be due to a vascular constriction of the erectile plexus at the base of the teat, and although this suggestion has not been specifically investigated, some thrombi have been identified in the blood vessels of the lamina propria in about 20% of UK cases where histological examination was carried out (Crosby-Durrani *et al*. 2022a). However, no thrombi were observed in the New Zealand case examined histologically.

The earliest abnormality noted by the attending veterinarian (KC) was a small, scabbed lesion at the medial teat udder junction. The UK observational study found a strong association between chapped teats and ITN, based on farmer diagnosis (Crosby-Durrani *et al*. 2022b). It is possible that the UK farmers were mis-identifying the same lesion identified by KC as a teat chap, as they could easily be confused.

The absence of important viral agents that affect the bovine udder, and the suggestion by NZ pathologists that this is of "outside in" rather than "inside out" pathogenesis, supports an alternative hypothesis. We believe that the progression of this condition is largely through self-trauma, by licking. Furthermore, from UK teats affected with ITN, metagenomics has identified five named *Mannheimia* spp. (*M. varigena*, *M. granulomatis*, *M. ruminalis*, *M. haemolytica* and *M. glucosida)* (Crosby-Durrani 2023). *Mannheimia* species are commensal organisms of the oropharynx, and it is highly likely that licking has caused bacterial contamination of the ITN lesions. However, it cannot be entirely ruled out that the *Mannheimia* spp. could have a role in ITN either as an opportunistic infectious agent, secondary to mechanical damage, or even as the primary aetiological agent, given that there is no evidence for viral involvement at this point. If we accept that licking may have a role in lesion contamination and that DD treponemes may be associated with UCD, then this could explain why DD treponemes have also sometimes been associated with ITN, if the affected cow has concurrent UCD.

Based on our clinical observations, we suggest that the small lesion seen at the medial teat-udder junction possibly provides the initial irritation, potentially being intensely pruritic, which stimulates the cow to aggressively lick the lesion and teat. This causes the lesion to progress down the medial aspect of the teat or can even lead to tearing the teat off the udder. This could explain why this condition is almost exclusively seen in first-calved heifers and why it has a medial presentation. First-calved heifers are still supple enough to reach the udder (which is suspended much higher and has not dropped as in more mature cows) and, as the video in Supplementary Information shows, the affected animals lick from the medial side of the teat, looping their tongue around the teat base. The presence of eosinophils in the histology from the amputated teat in this study supports the suggestion that this lesion would be intensely irritating, as granule proteins and cytokines in eosinophils are known to stimulate pruritis (Radonjic-Hoesli *et al*. 2021). Eosinophils are part of the innate immune system and have a role against bacterial, viral, and fungal diseases, as well as helminth infections (Gaur *et al*. 2022), and they are also often a feature of hypersensitivity responses. Therefore, the presence of eosinophils may serve as a proxy for an unidentified aetiological agent or suggest an underlying hypersensitivity component to the condition.

Although tenacious teat ulcer in Indian buffalo could be a similar condition to ITN, the inability of Indian buffalo to lick their own teats and the proposed association between tenacious teat ulcer and BoHV-2 possibly suggests that it is a different disease. In addition, an aetiological role in ITN for the suspect viral inclusion bodies seen in the histopathology sections from these New Zealand cases was subsequently ruled out from the use of electron microscopy, supporting the results of molecular and serology diagnostics completed here, and decreasing the likelihood of a viral agent.

Interestingly, the treatment adapted by the staff and veterinarians on the affected farm was similar to that advocated to control BoHV-2 (Petrovski 2005). This raises the question of whether the treatment was having an antiviral effect, or whether it had an unpleasant taste that would deter further licking. This may explain why the topical antibiotic spray containing gentian violet, which has a bitter taste, appeared more effective. If it is the latter, then using something like bitter apple spray on the udder may be equally effective.

Conclusion

From the New Zealand perspective, the development of ITN appears to include tissue damage and excoriation as a result of excessive self-trauma from licking, possibly in response to a localised hypersensitivity. However, the aetiological cause of the precipitating small scab-like lesion at the medial teat udder junction remains unknown. As such, the condition shares many similarities to the one described by Dutch and UK researchers (Sol *et al*. 2005; Crosby-Durrani 2023). Future work should concentrate on sampling the initiating lesion, and again ruling out a viral or bacterial agent.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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