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Clinical and Molecular Detections of Lumpy Skin Disease: Possibilities of Co-infection with Foot-and-Mouth Disease

Asmamaw Bihonegn[1] and Abdi Feyisa[2*]

[1]*School of Veterinary Medicine, College of Agriculture, Woldia University, Woldia, Ethiopia*
[2]*Department of Clinical Studies, College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia*
*Corresponding author's Email: abdi.feyisa@aau.edu.et

CASE REPORT
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ABSTRACT

Lumpy skin disease (LSD) and foot and mouth disease (FMD) are notable viral diseases of cattle. This report aimed to highlight the possibilities of an uncommon case of LSD and FMD co-infection. The report also presents the clinical and molecular detection of LSD virus in six crossbred calves and LSD and FMD virus co-infection in a heifer at small-scale dairy farms located in northern Ethiopia. Nasal swabs and tissue samples were collected following aseptic techniques from the six calves suspected of having LSD and a tissue sample from one heifer suspected of having LSD-FMD co-infection and submitted to the laboratory for cell culture and real-time polymerase chain reaction (PCR) tests. Different-sized, firm, painful skin nodules with necrotic centers were seen on different parts of the calves' body. Swelling of the prescapular and prefemoral lymph nodes, conjunctivitis, and corneal cloudiness were also observed. Uniquely, one heifer was seen with erosive lesions in the oral cavity and tongue, salivation, lameness, and skin nodules. Intracytoplasmic inclusion bodies, a distinctive feature of LSD virus, and the formation of syncytia, a characteristic of FMD virus, were observed in the cell lines. The heifer was diagnosed with a rare co-infection of LSDV and FMDV based on clinical signs, cell culture, and real-time PCR test results. The other six calves were diagnosed with the LSD virus. Treatment with broad-spectrum antibiotics, local wound cleansing, and anti-inflammatory drugs was initiated. Unfortunately, the heifer with LSD-FMD co-infection died while under treatment, and just three calves with LSD were recovered. It can be concluded that vaccination of animals against both diseases and promotion of bio-security protocols in farms is more helpful than treatment, and early case reporting is also warranted to avoid losses related to the diseases.

Keywords: Calves, Co-infection, Foot and mouth disease, Heifer, Lumpy skin disease

INTRODUCTION

Foot and mouth disease (FMD) is a severe, clinically acute, vesicular disease of both domestic and wild cloven-hoofed animals (Pacheco et al., 2010). The disease has the potential for rapid and widespread transmission within and between countries, as well as severe economic consequences (Sarker et al., 2011). The disease is distinguished by vesicular lesions on the feet, mouth, snout, and teats, as well as fever, lameness, loss of appetite, and salivation (Arzt et al., 2011). Furthermore, vesicles (blisters) on the tongue, dental pad, or another area of the skin that burst after 3 days leave shallow erosions (Tolawak and Pal, 2022). Despite the fact that FMD does not have a high mortality rate in adult animals, it has major implications such as weight loss, a drop in milk yield, reproductive problems, and a loss of draught ability, which leads to lower productivity (Knight-Jones and Rushton, 2013). In young animals, the virus causes heart degeneration, which might result in a high mortality rate (Sarker et al., 2011). The diagnosis of FMD depends on characteristic clinical signs and laboratory testing to determine the serotypes of the causal virus (Admassu et al., 2015). The detection and isolation of the FMD virus are required to confirm the FMD outbreak and design prevention strategies (Kitching et al., 2005). Because FMD is a viral disease, treatment focuses on preventing secondary bacterial complications using broad-spectrum antibiotics and other supportive care (Ole-Miaron, 2003).

Lumpy skin disease (LSD) is another devastating viral skin disease of cattle caused by the Lumpy skin disease virus (LSDV), a member of the *Poxviridae* family of the genus *Capripoxvirus* (Gupta et al., 2020). The virus can cause infection mainly in cattle and buffaloes; there are also reports in other wild ruminant species, such as giraffes, and springboks (Ratyotha et al., 2022). Lumpy skin disease is a notifiable disease by the World Organization for Animal Health (WOAH) because of the significant economic impact on the cattle industry related to decreased milk volume and meat production, abortions, infertility, and damaged hides (Whittle et al., 2023). It is the second most economically significant viral disease in cattle after FMD in Ethiopia (Tamire, 2022).

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Infected animals may exhibit a range of clinical indications, from asymptomatic to severe morbidity and mortality (Ratyotha et al., 2022). In natural infections, the incubation period of the disease lasts between 2 and 5 weeks, whereas in the experimental setting, it lasts between 7 and 14 days (Gupta et al., 2020). Lumpy skin disease can take three forms, including acute, subacute, and chronic (Salib and Osman, 2011). The mild form of LSD manifests as fever, naso-ocular discharge, and nodular lesions on the skin, skeletal, and mucosal tissue (Coetzer and Tuppurainen, 2004). Later, nodular lesions on the other body parts, particularly the skin of the muzzle, nares, back, legs, scrotum, perineum, eyelids, lower ear, nasal and oral mucosa, and tail, may be detected (Salib and Osman, 2011). Furthermore, necrotic plaques in the mucous membranes of the oral and nasal cavities can cause purulent or mucopurulent nasal discharge and excessive salivation (Salib and Osman, 2011). Ulcerative lesions in the cornea of one or both eyes can also occur, resulting in vision impairment and even blindness (Tuppurainen et al., 2021). Oedema of the limbs induced by lymphangitis and vasculitis can occur in calves (Ratyotha et al., 2022). Calves may also develop mucosal lesions within 23 and 48 hours following infection (Al-Salihi, 2014). The subsequent lesions can result in necrotic tissue and scarring, which may lead to complications, such as corneal opacity (keratitis), mastitis, lameness, pneumonia, and myiasis (Salib and Osman, 2011; Ratyotha et al., 2022; Whittle et al., 2023). Lumpy skin disease virus is primarily spread through mechanical transmission by arthropod vectors, mainly mosquitoes, ticks, and biting flies (Khan et al., 2021; Tuppurainen et al., 2021). In addition, indirect transmission through contaminated water and feeding through saliva or nasal discharge from infected animals can also occur (Sprygin et al., 2019).

Morbidity and mortality of LSD vary from 3 to 85% and 1 to 3% (up to 40% in heavy outbreaks), respectively (Ochwo et al., 2019). However, according to a recently published review document by Dubey et al. (2023), the mortality rate of LSD is around 10% (rarely 40% in severe outbreaks), and the morbidity rate ranges from 5% to 45% (occasionally even up to 100%). According to Dubey et al. (2023), these variations in severity were likely to be due to variations in cattle breed, age, immunological condition or health status, viral isolates, insect vectors involved in the transmission, and stage of development.

Lumpy skin disease can be diagnosed based on the characteristic clinical signs, which include circumscribed skin nodules and enlarged superficial lymph nodes, which will later be confirmed by laboratory tests (Tamire, 2022). Conventional and real-time polymerase chain reaction (PCR) have been developed for efficient and rapid molecular diagnosis of LSD (Orlova et al., 2006). However, it should be distinguished from pseudo-lumpy skin disease, urticaria, dermatophilosis, dermatophytosis, hypoderma bovis infection, photosensitization, bovine papular stomatitis, FMD, bovine viral diarrhea and malignant catarrhal fever (Abutarbush, 2017).

Co-infections in viral diseases of cattle were uncommon and/or less reported. A mixed infection of LSD with pseudocowpox was reported by Omoniwa et al. (2023). Based on common clinical symptoms and in accordance with the clinical hallmarks of both disorders, FMD and LSD were clinically diagnosed (Abas et al., 2021). However, the confirmed diagnosis of FMD and LSD co-infection was not reported so far. Therefore, this case report presents the clinical and molecular detection of LSD in six crossbreed calves and a rare co-infection of LSD and FMD in a heifer at two small-scale dairy farms located in Northern Ethiopia.

CASE REPORT

Ethical approval and consent to participate

This clinical casework was conducted following the World Organization for Animal Health's (WOAH) animal welfare guidelines. The farmers or owners of the animals were informed of the objectives of the work, and they were willing to allow sample collection from the animals.

Lumpy skin disease case description in calves

Six crossbred calves from a dairy farm located in Kombolcha City, Northern Ethiopia, were examined for skin nodules on different body parts, lesions on the hind limb, and eyes. The lesions were first seen in the two calves (one male and one female). The ages of two calves were 11 months and six to nine months for other calves. The calves were living with other dairy cattle of different ages of the same breed in an intensive farming system. Based on the owner's information, one male calf died within a week of infection. There had been a history of similar cases before two years at the farm, as well as complaints from another nearby farm before one month. All animals on the farm aged above six months, including two of the diseased calves, had been vaccinated against the LSD virus. In addition, the calves started treatment with short-acting oxytetracycline, diclofenac sodium, and multivitamin injections one day prior. On clinical examination, the calves had a rectal body temperature ranging from 38.7 to 40.5°C, a respiratory rate of 18 to 64 breaths per minute, and a pulse rate of 72 to 120 beats per minute. Variably-sized skin nodules were seen on the neck, ear, teat, perineum, face, back, muzzles, and scrotum. The nodules were firm, painful, raised above the surrounding skin, and sometimes separated by a narrow ring of hemorrhage with a characteristic intact central area. The findings of physical examinations, including the specific identification and clinical signs, are summarized in Table 1.

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Table 1. Identification, clinical signs, and treatment outcome of calves affected by Lumpy skin disease

No	Sex	Age (Month)	Observed clinical signs	Body temperature (°C)	Respiratory rate (breath/min)	Pulse rate (beat/min)	Vaccination history	Outcome
1	F	11	Skin nodules from the neck, face, ear, muzzle, back, perineum, and teat, ulcerative nodular lesions from the muzzle and external nose, ocular and nasal discharge, swollen lymph node and necrotic lesions over oedematous limb with skin peel off and lameness.	39.9	64	120	Yes	Died
2	M	9	Skin nodule from the neck, rectum, and scrotum, ocular discharge, swollen lymph node.	40	36	98	No	Recovered
3	F	6	Skin nodules on the neck, face, and ear.	38.9	28	104	No	Died
4	F	11	Skin nodules on the neck, face, ear, perineum, and teat, ocular discharge, conjunctivitis and corneal cloudiness, and salivation.	40.5	48	92	Yes	Killed by the owner
5	F	8	Skin nodules on the neck and face.	39.3	18	84	No	Recovered
6	F	6	Skin nodules on the neck, swollen lymph node	38.7	22	72	No	Recovered

No: Number

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The nodular skin lesions were identified at various stages of growth and in varying numbers and sites among afflicted calves. Typical ring-like ulcerative lesions from the muzzle and external nose (only two calves), ocular-nasal discharge that stains the face, swelling of the prescapular and prefemoral lymph nodes, conjunctivitis, and corneal cloudiness (both eyes of one calf) were also observed. Severe depression and oedematous swelling of the left hind limb with pits on palpation, large areas of necrotic lesions over the oedematous limb with skin peeling off, and lameness were observed in one calf. The skin lesions and clinical features of LSD in the affected calves are indicated in Figure 1. Therefore, based on history, observed clinical findings, and the endemic nature of the disease in the area, LSD was tentatively diagnosed, and appropriate nasal swabs and nodular skin biopsy samples were collected for further laboratory confirmation.

Figure 1. Characteristic skin lesions and clinical features of Lumpy skin diseasein crossbred calves: Typical ring-like ulcerative lesions from the muzzle and external nose (**A**), Raised nodules separated by a narrow ring of haemorrhage after the hair over the nodule was clipped off (**B**), Different sized and staged skin nodules on the neck and face (**C**), Necrotic lesions over an oedematous limb with skin peeling off (**D**), Nodules on the testicle (**E**), Conjunctivitis and corneal opacity (**F**), and the affected calves at day one (**G**).

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Lumpy skin disease and foot and mouth co-infection in heifer

A crossbred heifer (2-year-old) with history of reduced feed intake, oral lesions, lameness, skin nodules, and salivation was presented to the Kombolcha City Veterinary Clinic, Northern, Ethiopia. The heifer was kept intensively with three cows and an ox. The heifer was purchased from the South Gondar Zone at one month ago with unknown vaccination history, although the rest of the animals were treated with ivermectin and long-acting oxytetracycline upon arrival on the farm. Based on the owner's complaint, a farm visit was made, and physical examinations were conducted at the heifer's barn. Upon physical examination, the heifer was depressed and febrile, with a rectal body temperature of 40.5°C. Respiratory and heart rates were 56 breaths per minute and 120 beats per minute, respectively. The prefemoral and prescapular lymph nodes were swollen. There were typical multiple and different-sized nodular lesions, particularly on the skin of the neck, shoulder, teat, around the vulva, and on the face, including the ear and mouth. The heifer was depressed, and the left forelimb was swollen and pitted on palpation. There were extensively necrotized lesions on the exterior of the nose, ruptured tissue on the gum, erosive lesions on the dorsal epithelium of the tongue, drooling of saliva, ruptured vesicles on the digits, and swelling on the lower jaw. Ocular and nasal discharge, reddened ocular mucous membranes, and lameness were also observed. The clinical hallmarks are presented in Figure 2. Based on the history and typical clinical findings observed, lumpy skin disease and foot and mouth disease co-infections were suspected.

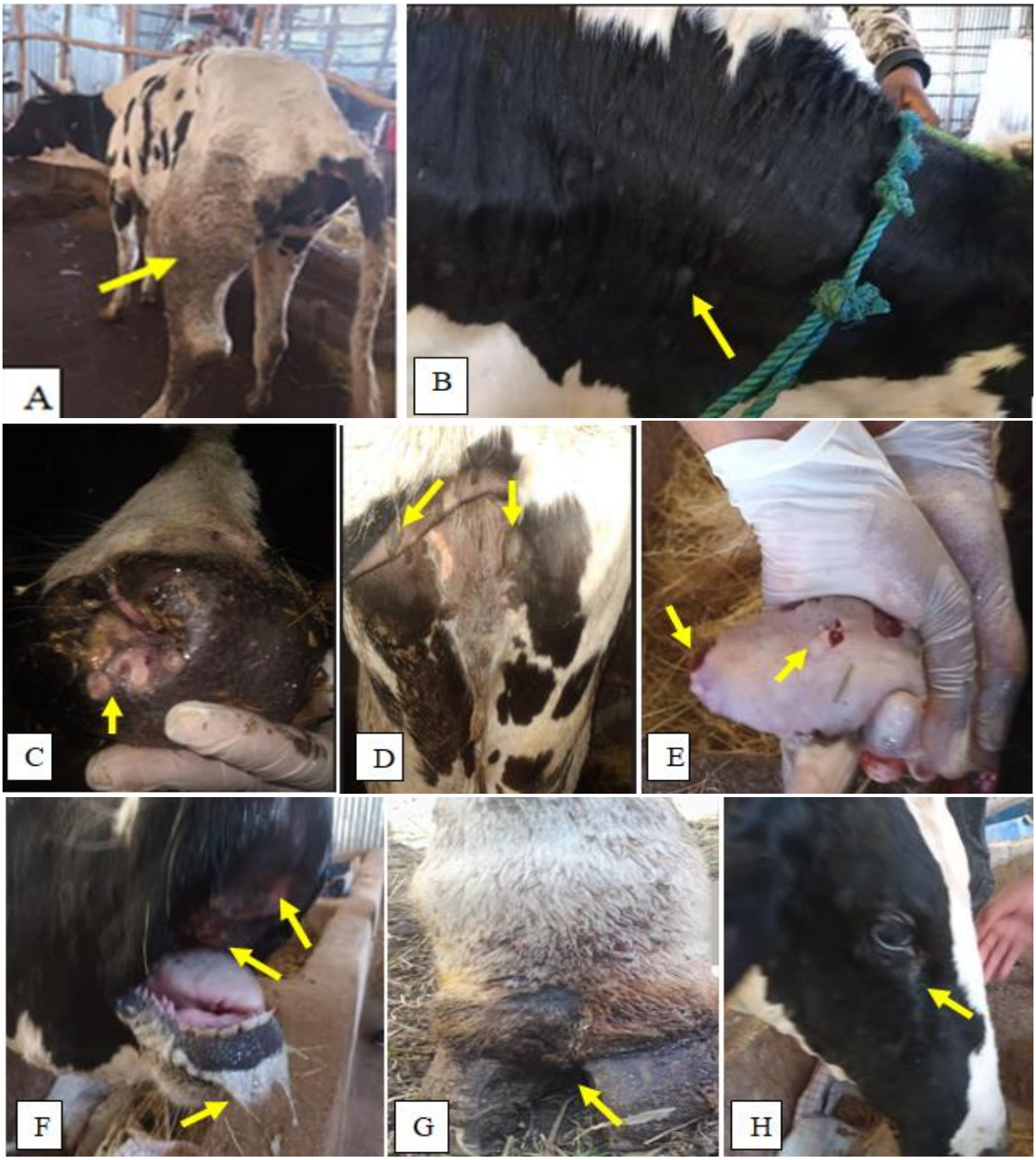

Figure 2. The different clinical indications of both Foot and mouth disease and LSD in crossbreed heifer. A heifer with a swollen left hind limb (**A**), Skin nodules around the neck (**B**), Nasal discharge and necrotized lesions on the exterior of the nose (**C**), Nodules around the perineum (**D**), Erosive lesions on the tongue (**E**), Lesions on the oral cavity with salivation (**F**), Lesion on the digit (**G**) and Lacrimation (**H**).

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Sample collection and laboratory findings

Nasal swabs and tissue samples were collected following aseptic techniques from the six calves suspected of having LSD and a tissue sample from one heifer suspected of having LSD-FMD co-infection and submitted to the laboratory for further testing. The animals were restrained properly before collecting the sample. Accordingly, for the laboratory diagnosis of LSD, a nasal swab and an excisional biopsy of cutaneous nodules were collected. The nasal swab samples were collected by vigorously rubbing the swab on the lining inside the nose. For cutaneous nodule collection, the selected nodule was prepared aseptically, and the hair over the nodule was clipped off. The area was anesthetized locally using 2% lidocaine, and the entire nodular biopsy was surgically removed and taken aseptically using scalpel blade number 24 and thumb forceps. The area was sutured and cleaned with 1% iodine. Similarly, for the laboratory diagnosis of FMD, about 1 g of epithelial tissue from the tongue was collected aseptically with the help of tissue forceps. After collecting the tissue samples, they were properly labeled with a history, including the provisional diagnosis, kept in sterile universal bottles that contain viral transport media, and then sent with a cold chain to the National Veterinary Institute (NVI), Ethiopia, for laboratory examination. Finally, the real-time PCR amplification result and melting curve analysis showed the presence of the LSD virus in both nasal swabs and tissue samples in both cases, as shown in Figure 3.

Further, the samples were then inoculated on the Embryonic Sheep Skin cell line (ESH-L), and the intracytoplasmic inclusion bodies, a distinctive feature of LSDV, were observed on the cell line, as indicated in Figure 4. The epithelial tissue sample from a suspected heifer was inoculated into the Baby Hamster Kidney (BHK-21) cell line, and syncytia formation, a characteristic cytopathic effect for FMDV, was observed (Figure 5). The test results confirmed LSD and FMD virus co-infection in the heifer.

Treatment and its outcomes

As LSD and FMD are viral diseases, the treatment in both cases was aimed at avoiding secondary bacterial infection by applying broad-spectrum antibiotics, dressing lesions with antiseptics, and practicing proper animal managment, reducing pain to the animal and losses due to the disease. Therefore, short-acting oxytetracycline (Herbei Hobe Harmony Pharmaceutical, China) at 10 mg/kg/day, intramuscular, for 5 days was prescribed. A multivitamin (Aether Centre [Beijing] Biology, China, 10-20 ml/animal, intramuscular) was given to improve the appetite. Diclofenac sodium (Reyong Pharmaceutical, China), at 2.5 mg/kg, intramuscular, was given once daily for three to five days. All the doses were prescribed based on manufacturer indications. The skin wound was cleaned and disinfected daily with an iodine solution. Topical eye ointment was applied to manage ocular lesions in the affected calf. The owners were advised to provide soft, palatable feed and isolate the animals from the others. Unfortunately, the heifer died on the third day of therapy while under treatment. Three calves among the LSD-affected were fully recovered after two months from the last treatment (Figure 5).

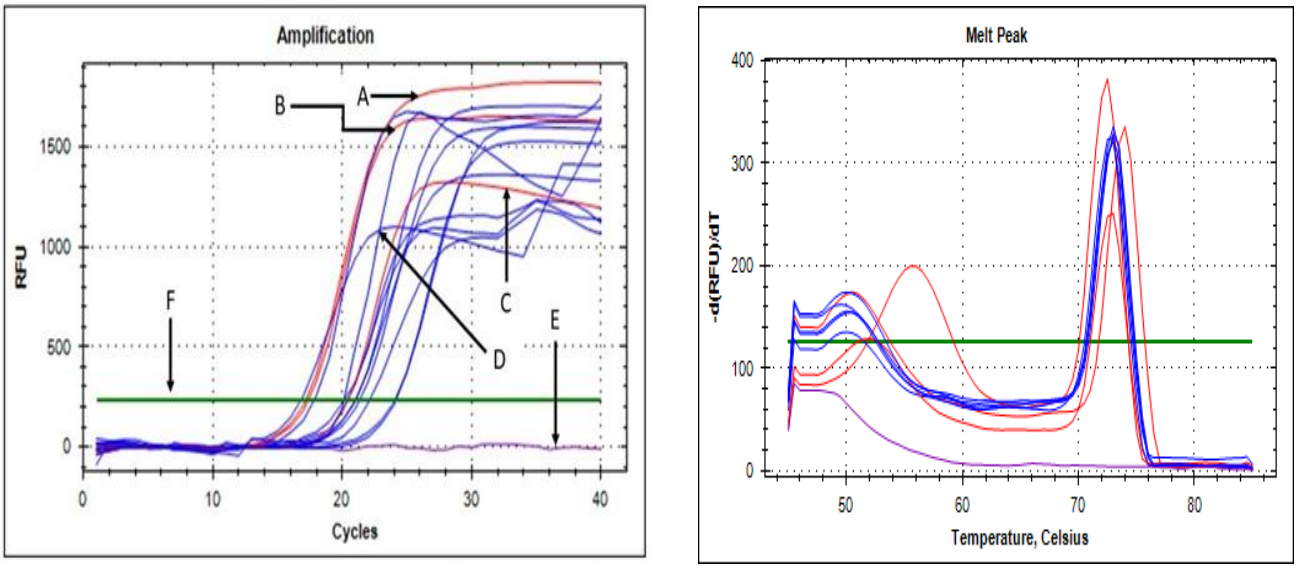

Figure 3. Detection of LSDV from nasal swab and skin nodule samples (crossbred calves [mean age ~ 9 months], five females, and one male). Real time polymerase chain reaction amplification result (left) and melting curve analysis (right). **A**: Known lumpy skin disease virus, **B**: Known goat pox virus, **C**: Known sheep pox virus, **D**: Field samples (blue colours), **E**: Negative control, **F**: Threshold value.

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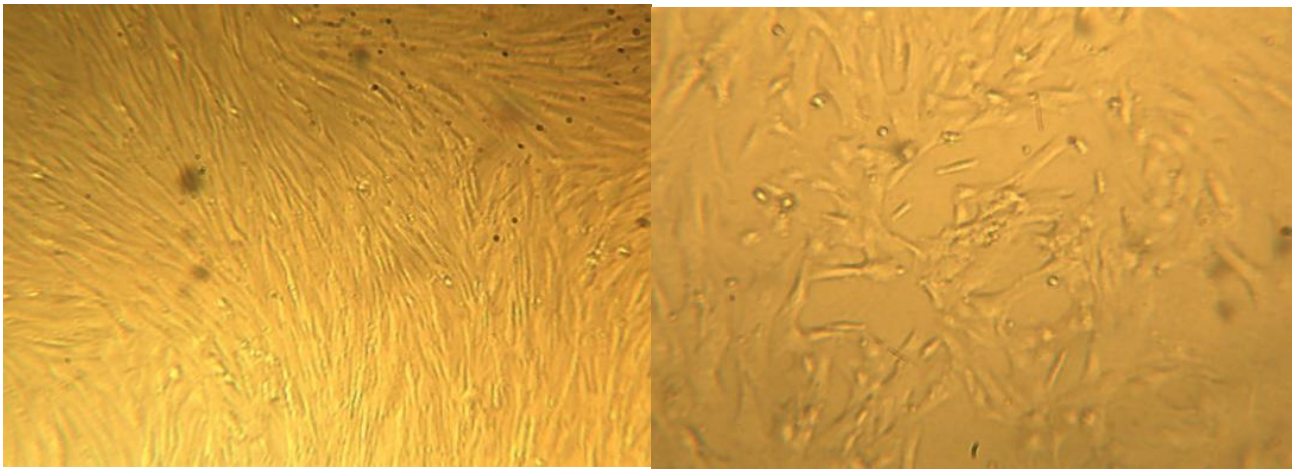

Figure 4. Isolation of Lumpy skin disease virus (LSDV) on Embryonic Sheep Skin cell line (ESH-L, left) and cytopathic effect provoked by LSDV (Crossbred dairy cattle, heifer, right).

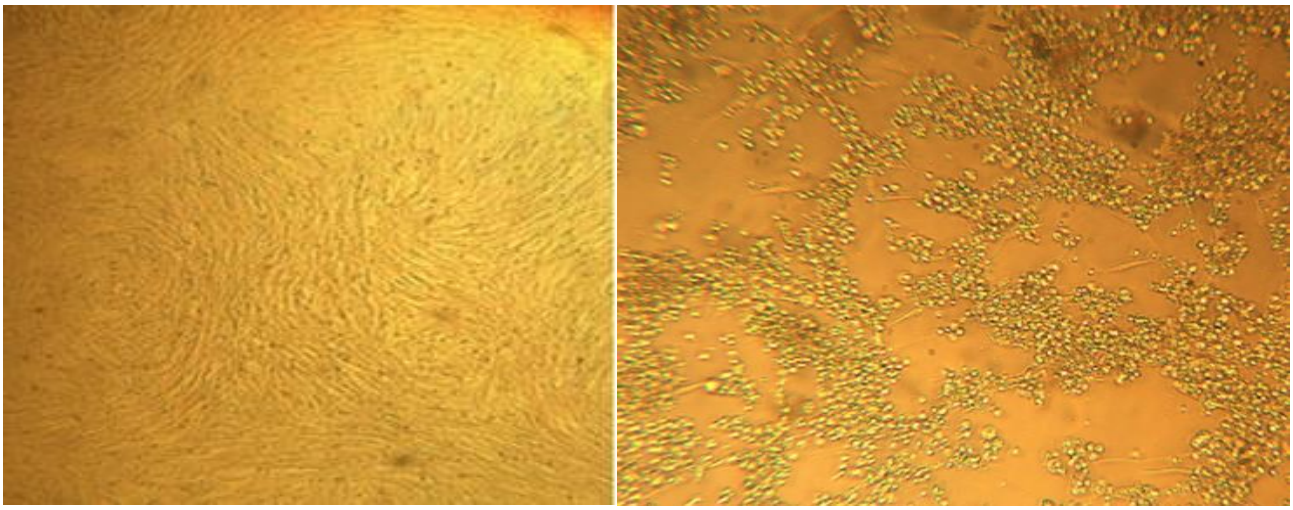

Figure 5. Noninfected Baby Hamster Kedney (BHK-21) cell line (left) and the cytopathic effect of FMDV on the cell line crossbred dairy cattle, 2-year-old heifer (right).

Figure 5. Calves recovered from Lumpy skin disease virus infection at two months of the follow-up period

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DISCUSSION

The LSDV and FMDV lack effective treatments, posing a substantial threat to the dairy and beef farming industries with potential for significant economic losses upon occurrence. Therefore, implementing routine vaccinations against both FMDV and LSDV emerges as the most efficacious strategy to curb the spread of these viruses (Sevik, 2017). Lumpy skin disease is a serious infectious viral disease in cattle. It occurs in an acute or chronic form and causes serious economic losses (Al-Salihi and Hassan, 2015). In this case study, the clinical infection of LSD was examined in crossbreed calves under one year old on a dairy farm. Likewise, LSD was found in four-month-old calves with nodular growth on different parts of the body, reduced appetite, emaciation, nasal discharge, and lameness in India (Halder and Seikh, 2022). Previously, a clinical case report of LSD was reported in Ethiopia in a bull with fever and the presence of large-sized circumscribed nodules on different body parts, particularly in the neck area, by Feyisa (2018). In this case study, varying-sized skin nodules, typical ring-like ulcerative lesions from the external nose, salivation and oculo-nasal discharge, and swelling of prescapular and prefemoral lymph nodes were observed on clinical examinations of the affected calves. Besides, conjunctivitis, corneal cloudiness, oedematous swelling of the limb, large areas of necrotic lesions over the oedematous limb with skin peeling off, and lameness were observed. Fever, depression, and reduced feed intake were also distinguished. As a result, the clinical findings in the current case report were in agreement with the LSD signs reported and described in previous studies (Feyisa, 2018; Mulatu and Feyisa, 2018; Tuppurainen et al., 2021).

In the present case, typical ring-like ulcerative lesions were seen on the muzzle and external nose. This is due to the sloughing of the necrotic lesions from the surrounding healthy epithelium (Al-Salihi, 2014). In this case report, conjunctivitis and corneal opacity were investigated in a nine-month-old calf. A similar sign was reported by Sudhakar et al. (2020) from outbreaks of LSD in cattle in India. The observed clinical signs, such as ulcerative lesions, conjunctivitis, corneal opacity, and lameness, might occur as severe clinical symptoms with complications of secondary bacterial infections (Al-Salihi and Hassan, 2015; Ratyotha et al., 2022; Whittle et al., 2023). The oedematous swelling of the limb in this case study was observed in one calf, which might be due to lymphangitis and vasculitis (Ratyotha et al., 2022). The presence of LSD-like nodules after vaccination ranges from 0.38% to 12% (Bamouh et al., 2021). In the present case study, two of the affected calves had a five-month vaccination history against LSDV. This is probably due to the incomplete effectiveness of the vaccine, the incorrect dosage of the vaccine at the time of vaccination, the low response level of the calves to the vaccine (Namazi and Khodakaram, 2021), or the fact that the calves might be missed during vaccination.

In the current case study, the calves were treated using short-acting oxytetracycline, multivitamins, and diclofenac sodium with the aim of avoiding secondary bacterial infection with antibiotics and reducing losses due to the disease and pain to the animal for welfare reasons. Similarly, the treatment protocol was in agreement with the management of the LSD case by Salib and Osman (2011). The most recent assessment by Dubey et al. (2023) revealed that while the mortality rate of LSD is typically 10%, it can occasionally rise to 40%. During the follow-up period, three calves were recovered and the others died. The case of death in the present instance might be due to lesions in the ocular and respiratory tract that probably prolong the period of anorexia and develop secondary pneumonia, which is a common sequel to LSD (Al-Salihi, 2014) that arises from the late detection and report of the cases by the owner.

In the present case report, a heifer was examined with clinical manifestations indicating both FMD and LSD in the study area. The presence of a cytopathic effect on BHK-21 and molecular examination of a tissue sample from an infected heifer revealed both FMD and LSD viruses, respectively. Thus, the results indicated that the clinical symptoms were co-infections caused by both LSDV and FMDV. The diagnosis of the case is also supported by the fact that FMD was present in the area at the time the current case occurred. Similar FMD-LSD co-infection was previously reported by Sevik (2017) in calves with clinical signs of fever (40°C), limping, nasal secretions, and lesions on the skin and vesicles on mucous membranes of the mouth.

CONCLUSION

Lumpy skin disease and foot and mouth disease are noticeable viral diseases that cause significant economic loss in the cattle industry. The current case report has shown the possibility of unusual LSD and FMD co-infections. Therefore, prevention by vaccination of animals against LSDV and FMDV and promotion of bio-security are more helpful than treatment, and early case reporting is also warranted to avoid losses due to death.

DECLARATIONS

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Authors' contribution

Abdi Feyisa Fufa and Asmamaw Bihonegn have generated the proposal. Both participated in designing the treatment and laboratory test protocol. Asmamaw Bihonegn did the follow-up and the write-up of the draft manuscript. Abdi Fufa reviewed and edited the documents. Both authors have read and approved the final version of the manuscript for publication in the World`s Veterinary Journal.

Competing interest

The authors declare that they have no competing interests.

Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked and compiled by the authors.

Availability of data and material

All clinical findings and laboratory results were included in the manuscript.

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