Veterinary Microbiology 292 (2024) 110037

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Use of a local anaesthetic and antiseptic wound formulation for the treatment of lambs naturally infected with Orf virus

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ARTICLE INFO

Keywords:
Orf
Multisolfen
Treatment
Lambs
Welfare

ABSTRACT

Contagious ecthyma (CE) is a worldwide highly contagious zoonotic viral skin disease of sheep and goats. Treatment for Orf virus (ORFV) infection usually involves topical and oral antibiotics. An anaesthetic and antiseptic topical gel (Multisolfen® or Tri-Solfen®; MS®, Medical Ethics, Australia) has been documented as an efficacious therapy for lesions from mucosal and epithelial viral infections in ruminants. The present study tested a new treatment protocol of MS® for CE therapy on-farm in 150 lambs naturally infected with ORFV. Lambs were divided into three cohorts of 50 lambs each (C, D and E). Cohort C was treated with MS® 3 times with an interval of 3 days between treatments, cohort D was treated daily with hypochlorous acid, whilst cohort E served as untreated controls. The lambs were examined clinically every two days, weight measured weekly, with whole blood and sterile swabs from ORFV lesions collected for haematological analysis and specific ORFV PCR. Cohort C presented fewer lambs displaying ORFV-associated lesions than other cohorts at different times of the experiment. Further, lesions treated with MS® were milder compared with other cohorts. However, following cessation of therapy, most of the lambs again developed ORFV-associated lesions. No differences between cohorts were observed in weight, haematological and PCR results. These findings suggest that topical treatment with MS® is effective for CE in field conditions, especially in the first stages of the clinical course, although treatment with MS® may need to be extended a minimum of 4 weeks.

1. Introduction

Contagious ecthyma (CE), also known as Orf, is a highly contagious global zoonotic viral skin disease affecting mainly sheep and goats (Bala et al., 2018). CE causes significant economic losses in the sheep and goat farming sector, from mortalities in young animals and reduced feed consumption and weight gain. Further, CE is considered to promote secondary bacterial infections in the skin and oral mucosa that increase the use of antibiotics (AMU), risking the generation of antimicrobial resistance (AMR). CE is a significant zoonotic disease in veterinarians and farmers (Lovatt et al., 2012; Windsor et al., 2017), commonly causing lesions in contact sites, primarily the hands. Lesions are characterised by erythema, papules, vesicles, and sometimes granulomatous dermatitis that usually can take weeks to months to heal (Nandi et al., 2011; Spyrou and Valiakos, 2015).

CE is caused by the Orf virus (ORFV) from the *Poxviridae* family, *Chordopoxvirinae* subfamily and *Parapoxvirus* genus (Bergqvist et al., 2017). ORFV is an epitheliotropic virus that replicates mainly into the cytoplasm of the stratum basale keratinocytes (Fleming et al., 2015). Although generally causing a self-limiting disease, ORFV encodes several immunomodulatory proteins permitting evasion of the immune system and inducing the reinfections of sheep and goats (Lloyd et al.,

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https://doi.org/10.1016/j.vetmic.2024.110037

Available online 3 March 2024

2000; Rohde et al., 2012; Bukar et al., 2021). Clinical presentation in lambs or kids is characterised by papules, vesicles and pustules that develop into scabby proliferative lesions, mainly affecting skin of the muzzle and lip mucosae although may extend to the oral mucosa and beyond, causing multifocal erosions and ulcers of the nostrils, ears, eyelids, feet, scrotum, vulva and udder (De La Concha-Bermejillo et al., 2003; McElroy and Bassett, 2007; Nandi et al., 2011; Spyrou and Valiakos, 2015; Windsor et al., 2017). ORFV lesions usually resolve in approximately 3–8 weeks (Nandi et al., 2011).

ORFV is mainly transmitted cutaneously (Spyrou and Valiakos, 2015), although other infection routes are possible (Allworth et al., 1987; Sargison et al., 2007). Morbidity can reach 100% and although mortality is usually less than 5%, there are reported outbreaks with a 90% of mortality rate in very young animals (Gumbrell and McGregor, 1997; Hosamani et al., 2009). CE outbreaks may cause both significant financial losses and animal welfare concerns in livestock production, particularly in association with intensive sheep and goat husbandry and compromising the international trade in small ruminants (Windsor et al., 2017).

Control of CE should be based on vaccination (Zhu et al., 2022), a routine procedure widely conducted in Australian sheep flocks using a live virus vaccine (Windsor et al., 2017). The further development of effective vaccines for ORFV infection is an important priority, particularly as there are no universally approved sheep or goat vaccines (Lacasta et al., 2015; Windsor et al., 2017). Registered vaccines are purified scab-based vaccines (Musser et al., 2008; Bukar et al., 2021) and cell culture-based live-attenuated vaccines (Buddle and Pulford, 1984; Pye, 1990; Bukar et al., 2021; Zhu et al., 2022). Purified scab-based vaccines can revert to virulence (Jorge and Dellagostin, 2017) and cell culture-based live-attenuated vaccines do not elicit complete protective immunity against ORFV (Tan et al., 2009) and also risk reversion to virulence (Friebe et al., 2004; Musser et al., 2008). For this reason, prototypes of DNA and subunit vaccines based on proteins ORFV B2L (ORFV011 gene) and ORFV F1L (ORFV059 gene) have been studied as vaccines against ORFV infections (Zhao et al., 2011; Yogisharadhya et al., 2017, 2018; Wassie et al., 2019; Zhu et al., 2022). Recently, a double gene-deleted recombinant vaccine, with deletions in CBP and GIF genes and a triple gene-deleted mutant of ORFV) with deletions in CBP, GIF and gene 121 have been studied in kids (Zhu et al., 2022; Shen et al., 2023). However, these promising prototype vaccines are yet to be approved and registered.

Despite the widespread distribution of ORFV amongst sheep and goat populations, there is no effective treatment against CE (Lacasta et al., 2023). Several topical antiseptics such as 10% Potassium permanganate solution (Van De Kerk, 1954), Stibophen (trivalent antimony compound) (Walder et al., 1979), 7% iodine, creosote dip and 3% phenol in vaseline (Beck and Taylor, 1974), Lotagen (metacresolsulfonic acid and formaldehyde 36% (Rapuntean et al., 1975), 6% aqueous suspension of lithium antimony thiomalate (Sanderson, 1976), sodium permanganate and salicylic acid (Tontis et al., 1981) and ointment (petrolatum and mineral oil) (Lansade, 1959; Larsson and Zahoory, 1983), have been suggested for ORFV treatment. In cases with secondary bacterial infections, parenteral antibiotics, such as penicillin (Mortelmans and Vercruysse, 1953) and chloramphenicol-ointment (Lansade, 1959), have shown lesions improvement. However, antimicrobial therapy is ineffective against ORFV infection (Greig et al., 1984) and may lead to increased resistance to antibiotics (AMR). Additionally, surgical treatment consisting of debridement and liquid nitrogen spray cryotherapy of the dermis has been proved in lambs, resulting in a rapid resolution and no recurrence, but an anaesthesia protocol was needed, and the time per lamb used was more than five minutes (Meynink et al., 1987, 1990). In humans, topical imiquimod (Lederman et al., 2007) or cidofovir have been successfully used for ORFV treatment (McCabe et al., 2003). Recently, other innovative treatments, such as (S)-HPMPA alkoxy alkyl esters (Dal Pozzo et al., 2007), systemic IFN-α injection and topical imiquimod (Ertekin et al., 2017) and genistein (isoflavone) (Lv et al., 2024) have been shown to inhibit the ORFV replication and infection.

Recently, MultiSolfen® (Dechra, UK), a local anaesthetic and antiseptic wound formulation, also marketed in some countries as TriSolfen (Medical Ethics, Australia; MS®) has been found to be an efficacious wound therapy formulation appropriate for the treatment of erosions and ulcers in oral mucosa caused by foot and mouth disease (FMD) (Windsor et al., 2020; Lendzele et al., 2021; Roughan and Windsor, 2022). In addition, MS® therapy was previously examined in 50 lambs infected experimentally with ORFV (Lacasta et al., 2023). Although MS® therapy had no effect on weight gain and clinical progression, this was attributed to early topical administration of animals infected by intra-dermal inoculation and lack of treatment of lesions in mid-latter stages of the disease; ORFV lesions having continued for more than 3 weeks (Lacasta et al., 2023). It was concluded that further studies in natural infections on-farm with a different treatment protocol were required to evaluate whether MS® could improve the clinical course of CE.

2. Materials and methods

In the present study, 150 Lacaune neonatal lambs 25–30 days old from a commercial sheep farm affected by a CE outbreak were selected for evaluation of MS® treatment. All the procedures were supervised and approved by the Ethics Advisory Commission for Animal Experimentation (nº PI33/21), the Biosafety Committee and the Occupational Risk Prevention Unit of the University of Zaragoza, in accordance with current regulations regarding these procedures. aspects (R.D. 53/2013, Law 31/1995, R.D. 664/1997, R.D. 1299/2006).

2.1. Studied lambs and weighing

The lambs were selected following presentation with a range of skin and oral lesions considered consistent with a diagnosis of CE. Confirmation of ORFV infection used a polymerase chain reaction (PCR) targeting ORFV 045 gene on swabs sampled from ORFV-associated skin and oral lesions.

Subsequently, lambs were randomly divided into 3 cohorts (C, D and E) of 50 lambs each. Lambs were registered with individual ear tags for identification and weights of all lambs were measured 4 (W1), 10 (W2), 18 (W3) and 22 (W4) days post-initial treatment (dpt) (Table 1).

2.2. Treatment application

Animals of cohort C were treated with MS® on 3 occasions, with an interval of 3 days between treatments (Table 1). Lambs were treated by spraying 1.5 mL of MS® (Dechra, UK) using a commercial dosing gun. The MS® was spread on the ORFV-associated lesions and into the mouth. Animals of cohort D were treated daily with hypochlorous acid (HA) (Brinasan, LEONVET, Spain), using the same technique as in group C. Animals of group E (control) were not treated. All lambs were examined and sampled over a 22-day period (Table 1).

2.3. Clinical examination

The lambs were examined at 2-day intervals (Table 1) with digital images of individual animal collected for detailed study of the type and severity of the lesions throughout the study. For each lamb, both frontal and lateral profiles were photographed, including the anterior mouth and the vicinity of the gums and palate, with photos grouped by lamb by the inclusion of ear tag numbers. For statistical study, the images were analysed individually, and lesions were classified according to the pathological nature of the lesion, directly correlated with the stage of CE, including erythema and/or papules in the first stages of CE; vesicles and/or pustules into mid-latter stages of the disease; and proliferative scabby lesions in the latest stages of CE. The severity of each lesion was graded from 0 to 4: 0 = absence; 1–3 = mild to moderate; and 4 =

Table 1
Treatment, clinical examination, weighing and sampling schedule.

	Days post-treatment										
	-1	0	2	4	6	8	10	12	15	18	22
MS® treatment		1 TS		2 TS		3 TS					
Clinical examination	CE 0	CE 1	CE 2	CE 3	CE 4	CE 5	CE 6	CE 7	CE 8	CE 9	CE 10
Weighing				W1			W2			W3	W4
Haematological analysis	He 0										He 1
PCR	P0						P1				P 2

Abbreviations: Days post-treatment: days post-first treatment of MS® and hypochlorous acid; MS®: Multisolfen; CE: clinical examination; W: Weighing; He: haematological analysis from whole blood samples; P: PCR targeting ORFV 045 gene from swabs of skin lesions.
Note: Animals from group D were treated daily with hypochlorous acid and animals from group E were not treated. In these groups, the clinical examination and sampling were performed as described in the table.

severe.

2.4. Haematological analysis

Whole blood samples were collected from the jugular vein into EDTA anticoagulant tubes for haematological analysis of all study animals. Samples were collected prior to treatment (He0) and 22 dpt (He1) (Table 1). Haematology was performed with an IDEXX ProcyteDx automatic haematology counter (IDEXX laboratories, Westbrook, ME, USA). Measured parameters included leukocytes (K/mL), erythrocytes (M/μL), haemoglobin (g/dL), haematocrit (%), platelets (K/μL), VCM (Mean Corpuscular Volume; fL), HCM (Corpuscular Hemoglobin Mean; pg), MCHC (Mean Corpuscular Hemoglobin Concentration; g/dL) and reticulocytes (K/μL). White series blood cells were also evaluated by counting neutrophils (K/μL), lymphocytes (K/μL), monocytes (K/μL), basophils (K/μL), and eosinophils (K/μL).

2.5. PCR and virus culture

For the detection of ORFV viral DNA in infected skin and mucous membranes, samples were collected from ORFV-associated lesions using sterile swabs and were preserved in Dulbecco's Modified Eagle Medium (DMEM) (Deltalab). Samples were obtained from all animals prior to treatment (P0) and on 10 (P1) and 22 (P2) dpt (Table 1). Nucleic acid extraction was performed manually (E.Z.N.A.® Blood DNA Kit, Omega Bio-tek). The extracted DNA samples were stored at −80 ∘C. For PCR, primers for ORFV 045 gene (forward primer: 5′-CCTACTTCTCG-GAGTTCAGC-3′; reverse primer: 5′- GCAGCACTTCTCCTCGTAG-3′) were used in amplification on a FAST 7500 cycler (Applied Biosystems).

Sterile swabs collected at P0, P1 and P2 were submitted to incubation with primary tissue cultures from ovine skin fibroblasts (OSF). Briefly, swabs were immersed in 2 mL of DMEM supplemented with 2% foetal bovine serum, 1% glutamine and 1% antibiotics (Sigma Aldrich, St. Louis, Missouri, USA) and then added to OSF. Cells were incubated at 37° C, 5% CO2 atmosphere for 7 days. DNA extraction was performed in cells using E.Z.N.A® Blood DNA Mini Kit (Omega, bio-tek). PCR was performed as described above.

2.6. Statistical analysis

All the data collected were integrated into a statistical matrix of the SPSS STATISTICS 26.0 program (IBM Corp., Armonk, NY, USA). The absence/ presence of lesions was codified as 0/1 and comparisons among treatment groups were carried out by Pearson Chi-square test. Grade of lesions (0−4) were considered as a quantitative variable, described by means and standard deviation (SD). Since distributions were not normal (as assessed by the Shapiro-Wilks test), comparisons among treatment groups were performed by the Kruskal-Wallis test (nonparametric test). For comparisons of initial weight among treatment groups, one-way Anova was applied. When considering weight at following assessments, Anova was conducted, with weights from previous assessments used as covariates. P-values <0.050 were considered statistically significant. The Bonferroni correction was applied in multiple pairwise comparisons.

3. Results

3.1. Weighting

At 4 dpt, the mean total weight was 6.616 ± 1.6397 (SD) kg. The comparison between the means of the cohorts revealed no significant differences at the commencement of the study: 6.904 ± 1.9083 (SD) kg in cohort C (treated with MS®); 6.436 ± 1.3717 (SD) kg in cohort D (treated with hypochlorous acid); and 6.491 ± 1.5751 (SD) kg in cohort E (control group). The progression of the average weight of the lambs per cohort during the study is displayed (Fig. 1). Significant differences were not found ($p > 0.05$) between cohorts. However, the average weight of cohort C treated with MS® was higher throughout the entire study, reaching its maximum difference with the other two groups on days 18 and 22 dpt, after the third treatment with MS®.

3.2. Clinical examination

At commencement of the study, all the animals displayed clinical signs consistent with CE, with variations in location, number and pathological nature of the lesions. Following treatment, the development of lesions in each cohort differed. Cohort C treated with MS® contained fewer lambs with ORFV-associated lesions than other cohorts at different periods of the study (Table 2). At 2 dpt, cohort C (MS® group) displayed a lower percentage of animals with erythema/papules and proliferative scabby lesions than cohort E (control group). At 6 dpt, cohort C displayed a lower percentage of animals with erythema/

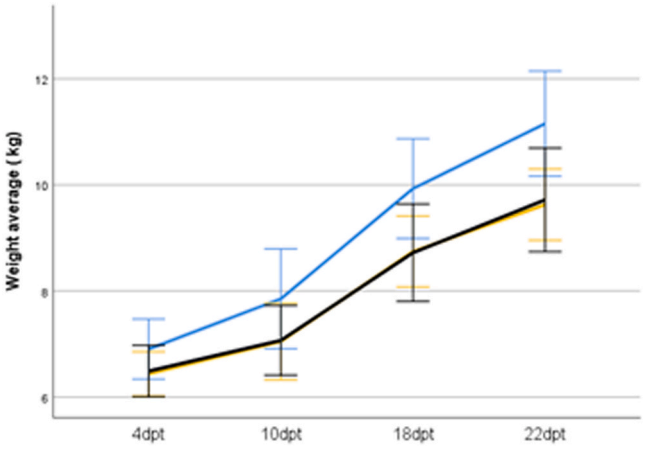

Fig. 1. Progression of the average weights of the three MS® (blue), HA (yellow) and control (black) cohorts throughout the study where all lambs were infected naturally by ORFV. Error bars: 95% CI for the mean.

Table 2
Presence of lesions (%) by type, days post-first treatment and cohort. Only results with statistically significant differences are shown.

Type of lesion	Dpt	Group			p-value
		Control	HA	MS®	
Erythema/papules	2	68.1%[a]	81.8%[a]	38.3%[b]	<0.001
	6	72.7%[a]	84.4%[a]	47.8%[b]	0.001
	12	88.1%[a]	76.2%[a,b]	61.9%[b]	0.020
	18	25.6%[b]	53.7%[a]	31.6%[a,b]	0.024
Vesicles/pustules	6	56.8%[a]	37.8%[a,b]	28.3%[b]	0.020
Proliferative scabby lesions	2	25.5%[b]	4.5%[a]	4.3%[a]	0.001

Abbreviations: Dpt: days post-first treatment of MS® and hypochlorous acid. Control: infected and not treated; HA: treated daily with hypochlorous acid; MS®: treated with Multisolfen 3 times with an interval of 3 days. a,b: Different letters in a row mean significant differences (p<0.05).

papules and vesicles/pustules than cohort E. At 12 dpt cohort C displayed a lower percentage of animals with erythema/papules than the control group. At 18 dpt, cohort E displayed the lowest percentage of individuals with erythema/papules, with cohort D (HA group) the highest, and cohort C (MS ® group) intermediate between the other two cohorts. All differences were statistically significant ($p < 0.005$).

Cohort C displayed a lower mean severity (0–4 scale) in all categories of ORFV-associated lesions than the other two cohorts at the different periods of the study (Table 3). At 2dpt, cohort C displayed a lower mean severity of erythema/papules than the other cohorts; whereas for proliferative scabby lesions, cohort C displayed a lower mean severity than cohort E (control). At 6 dpt, cohort C displayed a lower mean severity of erythema/papules than other cohorts, and for vesicles/pustules, cohort C displayed a lower mean severity than cohort E. At 12 dpt, cohort C displayed a lower mean severity of erythema/papules than cohort E. However, at 18 dpt, the lowest mean severity for erythema/papules was cohort E, the highest was in cohort D (HA group); with cohort C between the other cohorts. All differences were statistically significant ($p < 0.005$). At 15 dpt, cohort C presented a lower mean severity of proliferative scabby lesions than the other group, although multiple comparisons failed to find significant differences, with the global p-value under 0.05 (p=0.047).

3.3. Haematological analysis

Analysis of hemogram and leukogram parameters, including concentrations and percentages of total leukocytes, were found within the normal ranges in all lambs sampled. In addition, no significant differences ($p > 0.05$) were detected between the three cohorts at any of the sampling periods.

3.4. PCR and virus culture

Positive and negative PCR results of sampled swabs obtained at P0, P1 and P2 found no significant differences between cohorts ($P > 0.05$) (Table 4). Swabs submitted to incubation with primary tissue cultures from ovine skin fibroblasts (OSF) showed positive results in the three groups throughout P0, P1 and P2, with no significant statistical differences observed between groups ($p > 0.05$) (Table 4).

4. Discussion

Contagious ecthyma is a highly contagious zoonotic viral skin disease causing significant economic losses in global sheep and goat populations (Lovatt et al., 2012; Bala et al., 2018). Despite worldwide distribution and significant economic impact, there are few registered vaccines (Lacasta et al., 2015) to control this disease in some countries (Buddle and Pulford, 1984; Pye, 1990; Bukar et al., 2021; Zhu et al., 2022), with concerns that can revert to virulence or elicit incomplete immune protection (Friebe et al., 2004; Musser et al., 2008; Tan et al., 2009; Jorge and Dellagostin, 2017). Prototypes of DNA and subunit vaccines (Zhao et al., 2011; Yogisharadhya et al., 2017, 2018; Wassie et al., 2019; Zhu et al., 2022; Shen et al., 2023) have been experimentally studied with promising results, yet require approval. Further, some topical antiseptic formulations have been suggested for ORFV treatment (Van De Kerk P., 1954; Lansade, 1959; Beck and Taylor, 1974; Rapuntean et al., 1975; Sanderson, 1976; Walder et al., 1979; Larsson and Zahoory, 1983), although, generally, the results were not promising. Lotagen was effective in oral mucosa lesions but less effective in haired skin (Rapuntean et al., 1975). Only Stibophen reduced lesion severity in 8–10 days after application (Walder et al., 1979), although a detailed lesion typification was not performed to determine in which type of lesion this drug had the greatest effect. Additionally, antimony

Table 4
Results in percentage of PCR targeting ORFV 045 gene. Samples were collected using sterile swabs preserved in DMEM (Deltalab) from ORFV-associated lesions.

Sample	Result	-1 dpt Group			p-value
		Control	HA	MS®	
Swabs	Positive	65.00%	64.70%	91.70%	0.06
Virus culture	Positive	81.80%	73.30%	75,.00%	0.874
Sample	**Result**	**10 dpt Group**			**p-value**
		Control	**HA**	**MS®**	
Swabs	Positive	80,00%	87.50%	100.00%	0.345
Virus culture	Positive	27.30%	33.30%	45.80%	0.404
Sample	**Result**	**22 dpt Group**			**P-value**
		Control	**HA**	**MS®**	
Swabs	Positive	46.20%	46.70%	53.30%	0.911
Virus culture	Positive	66.70%	62.50%	53.80%	0.780

Abbreviations: Dpt: days post-first treatment of MS® and hypochlorous acid; Control: group infected and not treated; HA: group treated daily with hypochlorous acid; MS®: group treated with Multisolfen 3 times with an interval of 3 days.

Table 3
Severity of lesions (graded 0–4) for lesion-type, by days post-initial treatment and cohort.

Type of lesion	Dpt	Group						p-value
		Control		HA		MS®		
		Mean	SD	Mean	SD	Mean	SD	
Erythema/papules	2	0.74[a]	0.57	1.02[a]	0.66	0.47[b]	0.83	<0.001
	6	1.43[a]	0.70	1.38[a]	0.72	1.04[b]	0.87	0.014
	12	1.07[b]	0.56	0.88[a,b]	0.59	0.74[a]	0.67	0.040
	18	0.26[b]	0.44	0.66[a]	0.69	0.37[a,b]	0.63	0.012
Vesicles/pustules	6	0.64[b]	0.61	0.38[a,b]	0.49	0.30[a]	0.51	0.015
Proliferative scabby lesion	2	0.32[b]	0.63	0.05[a]	0.21	0.04[a]	0.20	0.001
	15	0.57	0.73	0.97	1.04	0.44	0.55	0.047

Abbreviations: Dpt: days post-initial treatment of MS® or hypochlorous acid; Control: infected and not treated; HA: treated daily with hypochlorous acid; MS®: treated with Multisolfen 3 times with an interval of 3 days; SD: standard deviation. a,b: Different letters in a row show significant differences (p<0.05).

compounds used as therapy can cause cardiotoxicity and pancreatitis (Sundar and Chakravarty, 2010). Therefore, no effective treatments for viral infections are available for use in farm conditions, with local antiseptics and antibiotics often used, assuming these may assist control of secondary bacterial infections. Recent studies have confirmed the efficacy of the wound therapy formulation Multisolfen® (MS®) for reducing pain and hastening the healing of skin and mucosal lesions in sheep and cattle (Windsor et al., 2020; Lendzele et al., 2021; Roughan and Windsor, 2022). Thus, prior to this study, MS® was examined as a therapeutic treatment in 50 lambs experimentally infected by intra-dermal inoculation with ORFV. Although MS® did not improve the clinical progression of CE in lambs, it was considered that this could have been due to the brief possibly inadequate treatment procedure (Lacasta et al., 2023).

In the present study, a new MS® treatment protocol was applied, using 150 Lacaune lambs from a commercial sheep farm affected by a natural CE outbreak. The protocol involved application of the MS® on 3 occasions with an interval of 3 days between treatments. The results indicated that the cohort treated with MS® presented with fewer lambs displaying ORFV-associated lesions than other cohorts at different times of the study (Table 2). The type of lesion that presented the most significant differences between groups was erythema/papules, a lesion observed in the initial phase of the clinical course of CE (Nandi et al., 2011; Spyrou and Valiakos, 2015). The cohort treated with MS® showed a lower number of animals with erythema/papules on 2, 6, 12 and 18 dpt than the other two cohorts, suggesting that MS® can reduce the erythema/papules if it is applied in an early stage of CE. However, vesicles/pustules and proliferative scabby lesions were observed in a significantly lower number of animals only in 6 and 2 dpt, respectively. These results may suggest that, although MS® appears to reduce the vesicles/pustules and proliferative scabby lesions after treatment, they proliferate again when treatment is discontinued. These findings reflect the prolonged clinical course of CE and suggest that multiple treatments with MS® could produce better results in controlling the presence of these types of lesions. Treatment with MS® could be extended for a minimum of 4 weeks, the average period necessary for resolution of ORFV lesions (Nandi et al., 2011).

Further, the severity of each lesion-type was evaluated, with grading from 0 to 4. The cohort treated with MS® displayed lower mean severity scores in all types of ORFV-associated lesions than the other cohorts with the same lesion-type and days as described above, with the exception that on 15 dpt, when the cohort treated with MS® displayed milder lesions categorised as proliferative scabby lesions, than in other cohorts (Table 3). The indications were that MS® likely reduced the severity of ORFV-associated lesions, especially of erythema/papules and proliferative scabby lesions. The findings concluded that in this, MS® therapy reduced both the number and severity of orf lesions, especially immediately after treatment. However, it appeared that after removal of the therapeutic gel solution, most of the lambs again developed ORFV-associated lesions in other locations. It is well-known that in a natural CE outbreak, ORFV lesions usually resolve within 3–8 weeks (Haig et al., 2002; Nandi et al., 2011) and during clinical progression of CE, ORFV replicates in the keratinocytes of stratum basale (Fleming et al., 2015; Windsor et al., 2017). Whilst topical application of MS® appears capable of improving the healing of erupted lesions, the virus continues to multiply in the stratum basale and new lesions will likely appear during the prolonged 4–6 weeks of CE disease (Bergqvist et al., 2017). For this reason, it is recommended that application of the product continues on repeated occasions during the pre-healing phase as it may assist controlling the development of the sub-acute and chronic-active ORFV lesions.

Therapy with a single dose of MS® has been showed to be efficacious for treating erosions and ulcers in oral mucosa and on the feet of animals affected by foot and mouth disease (FMD) (Windsor et al., 2020; Lendzele et al., 2021; Roughan and Windsor, 2022). Foot and mouth disease virus (FMDV) replicates principally in the stratum spinosum, comprising the most superficial layers of the epithelium and mucosa, with lesions commencing as vesicles then progressing to erosions and ulcers (Alexandersen et al., 2003). These characteristics enable topical application of MS® to readily contact the virus and resolve the clinical progression of FMD more quickly than other therapies, with previous observations suggesting a viricidal effect of MS® against FMDV due to the low pH of the product (2.7–2.9) or lidocaine concentration ranging from 0.5 mg/mL (0.05%) to 100 mg/mL (10%) (Windsor et al., 2020; Lendzele et al., 2021; Roughan and Windsor, 2022). In contrast, ORFV affects the stratum basale, the deepest layer of the epidermis and oral mucosa, and the principal lesions are papules, vesicles, pustules and proliferative lesions, with the latter encrustations potentially compromising the penetration of MS® into the basal epithelium and delivering the viricidal effect. In previous studies, MS® has not been effective in reduce ORFV viral load *in-vivo* (Lacasta et al., 2021, 2023). In the present study, 48 and 53.8% of swabs of the group treated with MS® from 10 and 22 dpt, respectively, were PCR positive on virus culture, suggesting that ORFV lesions may prevent MS® from inactivating the virus *in vivo* (Table 4). The results suggest that the clinical improvement in the lambs naturally infected with ORFV and treated with MS® are likely due prolonged pain-relieving and wound healing effects following blockage of local nociceptors during treatment of ORV lesions. In addition, fewer secondary infections occurred following the application of MS®, as recorded in previous studies (Lacasta et al., 2021, 2023), improving wound healing, an important attribute of MS® therapy (Windsor et al., 2020; Lendzele et al., 2021). Although it remains controversial whether provision of some forms of analgesia reduces acute inflammation, there are numerous studies demonstrating improved immune system function in different animal species (Yardeni et al., 2009; Cabral et al., 2015; Amodeo et al., 2018; DeMarco and Nunamaker, 2019).

The results obtained in the present study indicate that topical treatment with MS® is effective for CE in field conditions, especially in the early stages of the clinical course, and that it would likely to be beneficial to prolong the therapy for a minimum of 4 weeks to reduce the development of new ORFV lesions. Further, this study reinforces the hypothesis that whilst MS® may not penetrate to the stratum basale of proliferative lesions and inactivate ORFV, there is merit in the proposal that this multi-modal anaesthetic and antiseptic combination inhibits inflammation in viral skin diseases, improving welfare and assisting the control of secondary infections, promoting the healing of ORFV and viral lesions without promotion of AMR risks.

Author contributions

Conceived and designed the experiments (D.L., P.A.W and M.R.); performed the treatment and sample collection (H.R, A.G., D.L., M.R., P. Q., M.V., M.B., and J.J.R); did the laboratory examination (S.V., A.O., R. R., and T.N.); wrote the manuscript (A.G.); did the statistical analysis (M.T.T.); did the project management (D.L. and J.J.R.); reviewed the manuscript (D.L., P.A.W., S.V., M.T.T., A.O., M.B., M.V., H.R. and T.N.). All authors have read and agreed on the manuscript.

Funding

This research was supported by funding and product from the Australian company Animal Ethics Pty Ltd. The work was also supported by the Aragón Government and the European Social Fund (A15_17R, Construyendo Aragón 2016–20) and Project CONECTIM funded by Gobierno de Navarra (PC052-053).

CRediT authorship contribution statement

Peter Andrew Windsor: Writing – review & editing, Validation, Resources, Methodology, Funding acquisition, Conceptualization. **Teresa Navarro:** Investigation. **Pablo Quílez:** Investigation. **Marta Borobia:** Investigation. **Maite Verde:** Investigation. **Álex Gómez:** Writing –

original draft, Investigation, Formal analysis, Data curation. **Aurora Ortín:** Writing – review & editing, Methodology, Investigation. **Héctor Ruiz:** Writing – review & editing, Methodology, Investigation. **Ramsés Reina:** Investigation. **Sergio Villanueva-Saz:** Writing – review & editing, Methodology, Investigation. **María Teresa Tejedor:** Writing – review & editing, Validation, Formal analysis, Data curation. **Delia Lacasta:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Juan José Ramos:** Project administration, Methodology, Investigation. **Marta Ruiz de Arcaute:** Methodology, Investigation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that support the findings of this study are available from the corresponding author upon request.

Acknowledgements

We would like to thank Alberto Bordalde, the farmer who owns the farm where the study was carried out, for his help and support. Likewise, we would like to thank the laboratory technician Mariangeles Lostao and the intern students of the Ruminant Clinical Service (SCRUM) of the Faculty of Veterinary of the University of Zaragoza, especially Helena, Lucía and Marina.

Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of the University of Zaragoza (Project Licence PI 33/21, 2021) for studies involving animals.

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