

Efficacy and application of a novel topical anaesthetic wound formulation for treating cattle with Foot-and-Mouth disease: a field trial in Cameroon

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Abstract

Recently, a wound dressing formulation, (Tri-Solfen®), Medical Ethics Pty Ltd, Australia; TS) registered for use in ruminant husbandry in Australia, was registered for Foot-and-Mouth Disease (FMD) therapy in large ruminants in Laos, following clinical observations of improved welfare and healing following treatment of FMD lesions. In November 2019, an FMD outbreak in Cameroon provided an opportunity for a field trial, comparing clinical responses and recoveries to treatments on a sample of cattle (n = 36) comprising three equal groups of animals (n = 12), comparing responses to three treatments:(i) the application to lesions of TS, (ii) the administration of parenteral oxytetracycline commonly used for FMD in Cameroon; and (iii) an untreated control group (C). Appetite scores, lesion healing scores, and changes in dimensions of lesions, were recorded over a 15-day study period. Cattle treated with TS achieved both superior appetite and lesion healing scores with more rapid reduction in dimensions of lesions than other groups. Farmer observations indicated the TS treatment group had a more rapid return to eating with cessation of excessive salivation, and more rapid return of mobility (walking) with absence of overt lameness. The findings indicate that although mortality is usually low in FMD outbreaks, the disease is a debilitating and painful disorder with negative animal welfare impacts that should be addressed. All farmers expressed their desire that the product be made available for use in their region and modelling indicates that TS therapy imposes no additional financial burden on farmers, with the treatment likely to be provided at a similar or reduced cost to current treatment choices. As use of antibiotics for treatment of a viral disease potentially increases pressures for development of antimicrobial resistance and residues in the food chain, TS as an alternative non-antimicrobial therapy should be promoted for wider use in FMD outbreaks.

INTRODUCTION

Cameroon, with a population of almost 28 million people, is located in central Africa, bordering the Gulf of Guinea. It is a key transit link, sharing borders with six countries: Nigeria, Chad, Central African Republic, Republic of Congo and Equatorial Guinea. The gross domestic product (GDP) per capita of Cameroon is estimated at USD3,700 (CIA, 2017). The approximately 7.1 million cattle in Cameroon are susceptible to regular outbreaks from Foot-and-Mouth Disease (FMD), and endemic disease in much of Africa. With no preventive control programs in place and no access to commercial FMD vaccination, farmers are focused on treatment choices for affected cattle, with antibiotics and/or traditional therapies commonly used and prolonged periods for animals to recover.

FMD is a most important global viral pathogen of artiodactyl farmed and wildlife animals. The disease is characterised by lesions in and around the mouth and feet (Fakhrul-Islam et al., 2016). Globally, there

are seven pools of circulating FMD viruses recognised. Each pool represents independently circulating and evolving FMD virus (FMDV) genotypes (Russo, 2018). Within the pools, cycles of emergence and spread occur that usually affect multiple countries in the region. In the absence of specific and laboratory-confirmed reports, it should be assumed that the prevalent serotypes are continuously circulating in parts of the pool area and would be detected if sufficient surveillance was in place (Russo, 2018). Cameroon sits in the West/Central African region designated as Pool 5. FMD causes huge economic losses in Cameroon, with estimation of the total annual cost of FMD management at USD112 million (FAO, 2015).

Of the seven FMDV serotypes, four (O, A, SAT 1 & SAT2) occur in Cameroon (Ludi et al., 2016; Sevidzem et al., 2019b; Ehizibolo et al., 2019). There is no mass vaccination program for FMD and no commercial FMD vaccines are available in Cameroon (Bertram et al., 2018). A pilot trial using commercial trivalent vaccine (Aftovax®) was conducted in 2015 in Ngaoundere, with observations that clinical infection of FMD appeared to have been prevented, although persistent infection occurred. Cattle owners in Cameroon routinely manage the disease using a range of therapies, including anti-inflammatory preparations, antibiotics and traditional formulations (Sevidzem et al., 2019a).

In the absence of strategic preventive control programs, there are urgent needs for alternative FMD management options for endemic FMD-infected countries, including Cameroon. With affordable and efficacious vaccine candidates for managing FMD unlikely in the near future, the review and rationalizing of FMD therapies is advisable. Several topical treatments with ethno-veterinary and recognized veterinary pharmaceuticals have been examined in FMD endemic settings in Africa (Gakuya et al., 2011; Misk et al., 2015; Fakhrol-Islam et al., 2016; Al-Lethie et al., 2018). However, the widespread use of parenteral antibiotics for FMD globally, including numerous countries in Africa and Cameroon in particular, presents antimicrobial resistance (AMR) and potential food safety residue risks that need to be addressed.

Recently, an Australian wound dressing formulation, Tri-Solfen® (Medical Ethics Pty Ltd, Australia; TS) registered for use in cattle and small ruminant husbandry in Australia and New Zealand, was registered for FMD therapy in large ruminants in Laos in south east Asia (Windsor et al., 2020). The wound and lesion dressing formulation contains two local anaesthetics (lignocaine and bupivacaine), adrenalin and cetramide in a gel matrix that creates a barrier effect, numbing the pain of lesions, rapidly reducing their infectivity, and hastening healing, potentially reducing the weight loss in affected individuals (Windsor et al., 2020). If made available for purchase and administration by farmers, this product could provide a viable alternative approach for managing FMD in Cameroon, other African countries and developing countries globally. This formulation offers numerous advantages over current therapies as it provides efficacious pain relief and more rapid healing of wounds and lesions (Windsor et al., 2016; Roberts and Windsor, 2019; Windsor et al., 2020). Further, with a pH of ~2.7, and containing the antiseptic cetrimide, it potentially has viricidal impacts and anti-bacterial properties respectively, avoiding the need for other treatments, including antibiotics (Windsor et al., 2020).

In November 2019, TS was provided to the Cameroon research team for trials during FMD outbreaks in cattle. This report describes a field trial in Cameroon aimed at evaluating the efficacy of this therapy for FMD lesion management, particularly for enhancing recovery and wound healing, comparing the clinical responses to the most commonly used and available antimicrobial therapy currently in use. If TS is proven to be as efficacious for FMD in this current study as it appeared to be on first use in Laos as described (Windsor et al., 2020), it is likely that the product could offer an important innovation for improving FMD lesion treatment, potentially globally, with reduced animal welfare burdens, risks of AMR issues and possibly, increased transboundary disease reporting and surveillance.

MATERIALS AND METHODS

Trial location and design

This clinical trial was conducted between the 5th of November and the 5th of December 2019 in Ngaoundere II, Ngaoundere III and Martap subdivisions in the Vina Division of the Adamawa plateau. Around 40% of the cattle population of Cameroon is located in the Adamawa region (MINEPIA, 2013). Ngaoundere is the

capital of the Adamawa region, with geo-referenced coordinates between 6° 40' 0" and 7° 30' 0" north latitude and between 13° 20' 0" and 14° 10' 0" east longitude (Fig. 1). An FMD outbreak in the municipalities of Ngaoundere II, Ngaoundere III and Martap was advised to the lead author by a field technician in Ngaoundere on the 4th of November 2019. The following day, the outbreak areas were accessed, and the treatment trials commenced immediately. Two teams of two members each were formed to enable animal treatments and follow-up of the treated cases. Participating farms were selected following demonstration of owner willingness to participate, and included: Horé Mayanga, Borongo, Tchabal Baouro and Mbidjoro. The GPS data of all farms was recorded.

As Moore-Oxy® (manufactured in China; MO) was the reference antibiotic commonly used by farmers to manage clinical FMD in the study area, it was decided to compare the efficacy of this formulation on the healing of FMD lesions with that of TS. As MO is administered intra-muscularly and TS is administered topically, on each farm, three animals were matched by age and breed, with similar FMD clinical presentations; with one treated with MO, one with TS and the third left untreated. This created 12 sets of cattle for this comparative clinical trial on the different farms.

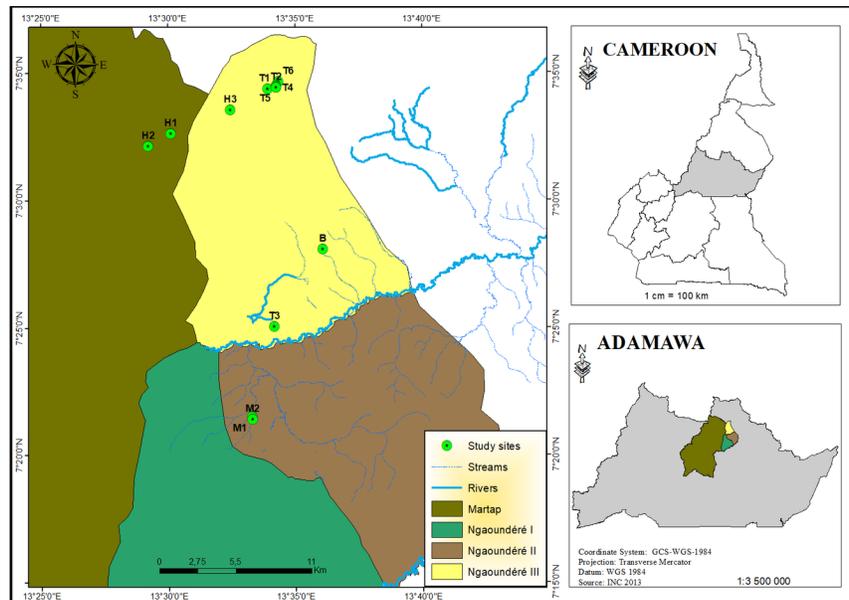


Figure 1 : Maps showing the study sites (Ngaoundere II, Ngaoundere III and Martap)

M1: Mbidjoro 1, M2: Mbidjoro 2, T1: Tchabal Baouro 1, T2: Tchabal Baouro 2, T3: Tchabal Baouro 3, T4: Tchabal 4, T5: Tchabal 5, T6: Tchabal 6, B: Borongo, H1: Horé Mayanga 1, H2: Horé Mayanga 2, H3: Horé Mayanga 3.

Treatment Applications

The clinical treatment trial was conducted on ‘fresh’ FMD lesions, containing intact vesicles or recently ruptured vesicles. Animals treated with TS had lesions liberally sprayed with 2ml of the product, as per label instructions. Animals treated with MO received an intra-muscular injection of the product, also at doses per label instructions. Clinical response observations were noted, including any improvements in capacity and ability of the animal to walk and eat. The following semi-quantitative clinical measurements were made:

Lesion healing score (LHS) on a scale of 1 to 4 (adapted from Al-Lethie et al., 2018)

Appetite score (AS) on a scale of 1 to 4 (from Al-Lethie et al., 2018)

Lesion size (cm): healing of lesions was measured quantitatively in the field using a 30-cm ruler, every 3 days from day zero (D0) through day 15 (D15), with lesions photographed using android mobile phones.

Farmer Observations

The farmers were requested to provide their observations for each animal for each treatment group, with the following information recorded:

Number of days until cattle mobility (walking) returned

Number of days cattle were not eating

Number of days cattle continued to salivate

Number of days displayed lameness

Number of days required for cattle to return to grazing

Treatment cost model

The costs of treatments were estimated and used to develop a cost-benefit model enabling comparison of therapies that could assist decisions for both individual farmers and public health policy makers on FMD outbreak management.

Statistical Analyses

Ordinal scale analysis

Both the lesion healing scores and the appetite scores were recorded on a four-point ordinal scale, not a quantitative scale, so appropriate ordinal categorical methods were required for this analysis (Agresti, 2002). In addition, walking was also modelled on a three-point ordinal scale: (1: immobile; 2: walking with difficulty; 3: walking normally). An ordinal logistic mixed model was fitted to each data set with fixed effects for Treatment, Day, Breed and Age (covariate), and a random effect for the individual Animal ID. A Treatment \times Day interaction was included in each of the models, to allow for a different shaped time course for each treatment. Note that the fitted model returned a set of model-based probabilities of obtaining each possible score (1 through 4, or 1 through 3), for the particular combination of terms in the model. The model was fitted using the `clmm` function in the ordinal package of R (Christensen, 2019), and probability estimates obtained using the `emmeans` (Lenth, 2020) and `RVAideMemoire` (Hervé, 2020) packages in R.

Binary data analysis

The status of cattle being on pasture and cattle salivating (Yes = 1, No = 0) are binary outcomes, and for this, binary logistic mixed models were used to analyse these data. As above, fixed effects for Treatment, Day, Treatment \times Day, Age, Breed and Sex, and a random effect for Animal ID were included in the model. The model was fitted using the `glmer` function in the `lme4` package of R (Bates et al., 2015), and model-based means obtained using the `emmeans` package.

Quantitative data analysis

Lesion size (cm) was analysed using a linear mixed model, with fixed effects for Treatment, Day, Treatment \times Day, Age, Breed and Sex, and a random effect for Animal ID. Model fitting was via the `lmer` function in the `lme4`, and model-based means obtained using the `emmeans` package. Event duration data (number of days that cattle are immobile, walk with difficulty, walk normally, on pasture, and salivating) were analysed using linear models with fixed effects for Treatment, Age, Breed and Sex. Due to the positive skew, a $\log_e(y + 1)$ transformation was applied. However, with the large number of ‘zero’ durations, hypothesis testing was conducted with permutation tests rather than F -tests, using the `aovperm` function in the `permuco` package (Frossard & Renaud, 2019) in R.

RESULTS

Of the 12 farmers enrolled into the 15-day treatment trial with three matched-cattle, each receiving a different treatment, the age and breed of the cattle recruited into each treatment group are presented (Table 1).

Table 1 . Age and breed of cattle recruited into each treatment group.

	Treatment group	Treatment group	Treatment group	
Variable	Moore-Oxy®	Tri-Solfen®	Control	Overall
Average age (yr)	3.7	4	3.3	3.7
Age range (yr)	2 to 5	2 to 8	2 to 5	2 to 8
Breed count				
Goudali	9	9	10	28
Holstein	1	1	1	3
White Fulani	1	2	1	4
Red Fulani	1	0	0	1
Total	12	12	12	36

Lesion healing scores

There was a highly significant Treatment \times Day interaction ($P = 3.4 \times 10^{-7}$), indicating differing lesion healing score time courses across the three treatment groups. Model-based lesion score probabilities are displayed (Figure 2). While control cattle maintained the presence of erosions/ulcers (a score of 1), recoveries were observed in the two treatment groups. From Day 9 onwards, cattle treated with TS had significantly higher lesion healing scores than those on MO (Day 9: $P = 0.025$; Day 12: $P = 0.016$; Day 15: $P = 0.0008$). None of the other terms had a significant association with lesion healing score: Age: $P = 0.464$; Breed: $P = 0.311$; Sex: $P = 0.376$.

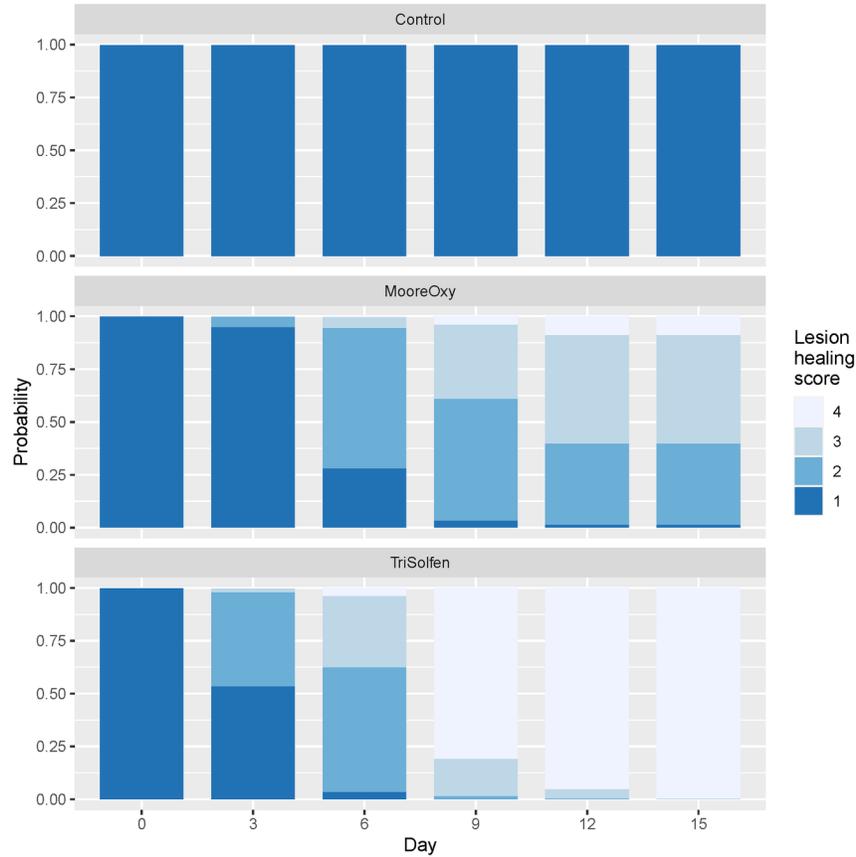


Figure 2 . Model-based probabilities of obtaining lesion healing scores 1 through 4 across the six study days, for the three treatment groups.

Appetite scores

Again, there was a highly significant Treatment \times Day interaction ($P = 2.0 \times 10^{-6}$) for appetite score, indicating treatment effects were changing over the study period. Model-based appetite score probabilities are displayed (Figure 3). Most control cattle had no appetite over the whole study period (score of 1). However, the two treatment groups had high appetite scores on Day 0, low on Day 3, then progressively increasing appetite scores after that. From Day 9 onwards, cattle treated with TS had significantly higher healing scores than those on MO (Day 9: $P = 0.025$; Day 12: $P = 0.016$; Day 15: $P = 0.0008$). After Day 0, there were no significant differences in appetite score between MO and TS treated cattle (all $P > 0.10$). Neither Age ($P = 0.810$), Breed ($P = 0.524$), nor Sex ($P = 0.324$) had a significant effect in appetite score.

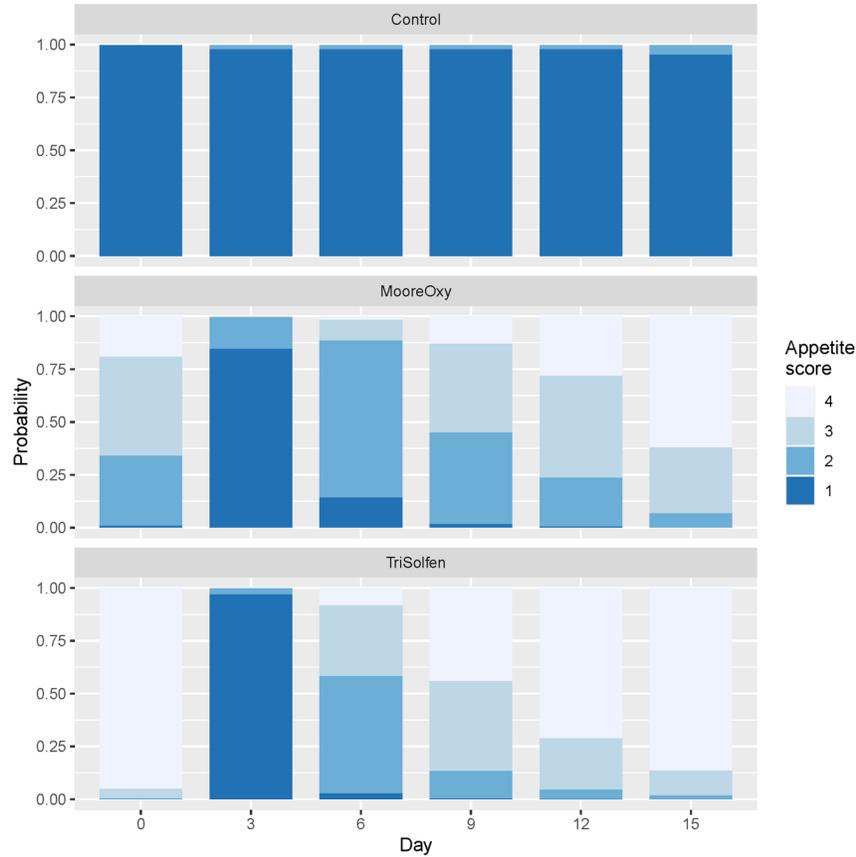


Figure 3 . Model-based probabilities of obtaining appetite scores 1 through 4 across the six study days, for the three treatment groups.

Lesion size

There was a highly significant Treatment \times Day interaction ($P < 2 \times 10^{-16}$) for lesion size (Figure 4), indicating different rates of healing across the three groups. Initially, there were no significant differences in mean wound sizes amongst the three groups (all $P > 0.25$). While mean wound size increases for Control cattle, they reduced for the two treatment groups, with those in the TS treatment reducing at a faster rate (differences were significant from Day 9, all $P < 0.01$). There was no significant effect of Age ($P = 0.454$), Breed ($P = 0.225$), nor Sex ($P = 0.374$) on lesion size.

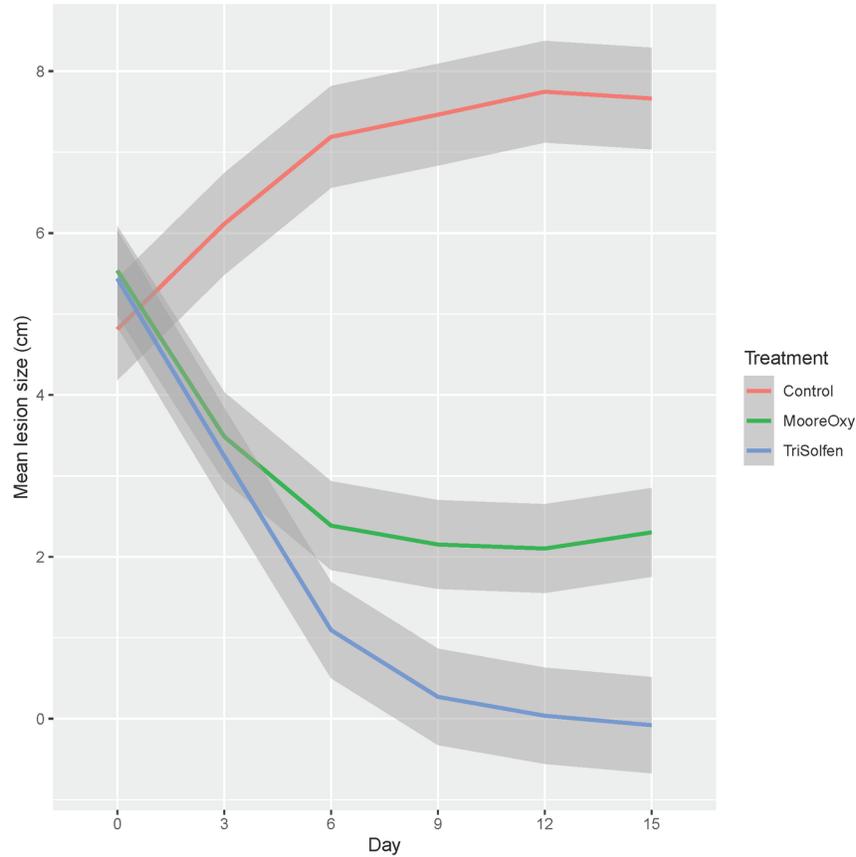


Figure 4 . Model-based mean lesion size (cm) across the six study days, for the three treatment groups. Shaded areas are ± 1 standard error of the mean.

Walking

When walking is considered as an ordinal score (Immobile < With difficulty < Normal), there was a highly significant Treatment \times Day interaction ($P = 1.8 \times 10^{-13}$). Initially (Day 0), there were no differences in walking scores between the three groups (all $P > 0.15$) and some cattle remained ‘Immobile’ or ‘With difficulty’ throughout the study period (Figure 5). However, walking improved for the two treatment groups, particularly for the TS treatment group. However, the differences between these two groups never reached threshold significance (all $P > 0.05$). There were no significant effects of other factors on walking (Age: $P = 0.583$; Breed: $P = 0.9012$; Sex: $P = 0.778$).

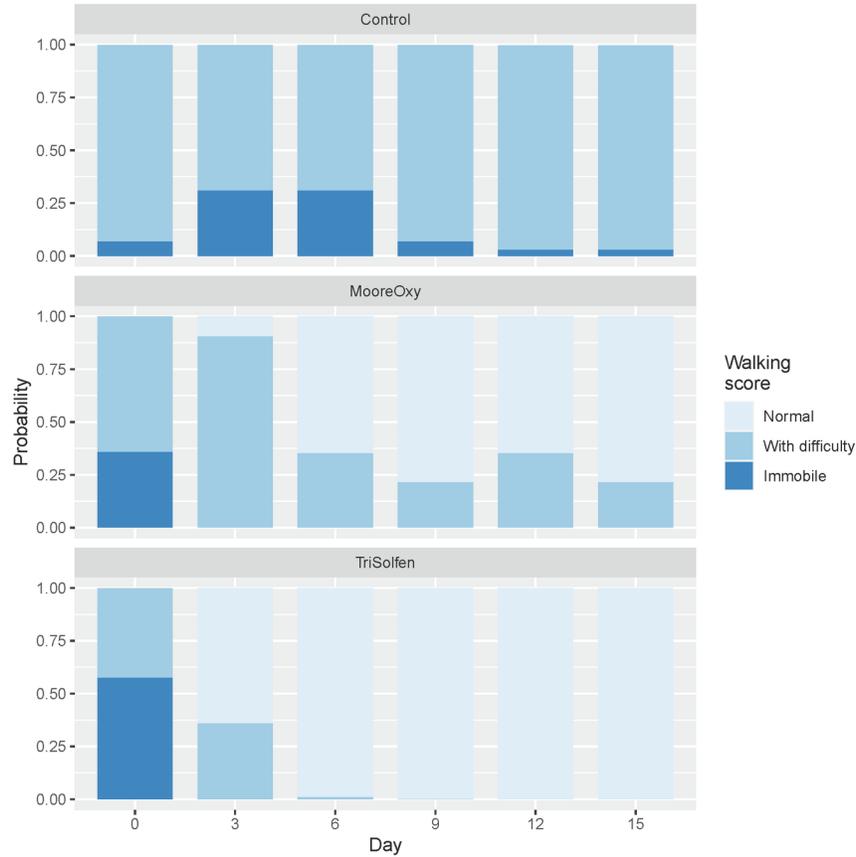


Figure 5. Model-based probabilities of obtaining walking scores of ‘Immobile’, ‘With difficulty’ and ‘Normal’ across the six study days, for the three treatment groups.

An additional analysis was conducted to compare the number of days that cattle are immobile, number of days they walk with difficulty, and number of days they walk normally (Table 2). In all cases, these durations differed significantly between the three treatment groups (all $P < 0.01$). Control cattle had a significantly longer period of immobility compared with the two treatment groups (both $P < 0.005$).

Table 2. Analysis of the number of days that cattle are immobile, number of days walking with difficulty, and number of days walking normally

Walking	Variable	<i>P</i> -value	Control	Moore-Oxy®	Tri-Solfen®
Immobile	Treatment	0.002	2.47 ^A ± 1.39	0.05 ^B ± 0.35	0.00 ^B ± 0.30
	Age	0.942			
	Breed	0.590			
	Sex	0.268			
With Difficulty	Treatment	0.009	8.27 ^A ± 4.95	3.49 ^{AB} ± 2.00	0.70 ^B ± 0.85
	Age	0.471			
	Breed	0.875			
	Sex	0.724			
Normal	Treatment	0.000	0.60 ^A ± 0.40	2.72 ^B ± 0.78	7.18 ^C ± 1.92
	Age	0.221			
	Breed	0.117			

Walking	Variable	<i>P</i> -value	Control	Moore-Oxy®	Tri-Solfen®
	Sex	0.033			

^{A, B, C}: Means sharing the same superscript are not significantly different ($P > 0.05$).

TS-treated cattle had a significantly shorter period of walking with difficulty compared with Control cattle ($P = 0.0022$), and also had significantly longer period walking normally compared with both Control and MO-treated cattle (both $P < 0.005$). Note that there were no effects of Age, Breed, not Sex on the three durations, with the exception of Sex on duration of normal walking ($P = 0.033$) with male cattle having mean duration 3.72 ± 1.14 days, compared with female duration of 1.82 days.

Time on Pasture

There was a highly significant Treatment \times Day interaction for presence of cattle on pasture ($P = 1.5 \times 10^{-6}$). It was apparent that Control cattle basically never returned onto pasture during the trial (Figure 6). However, for the two treatment groups, after initially none being on pasture, all were on pasture by Day 6. Although TS showed a faster return to pasture than MO, it was not possible to formally test this. There were no significant effects of Age ($P = 0.791$) nor Sex ($P = 0.661$) on the probability of being on pasture; there was some evidence of breed differences ($P = 0.023$), but breed estimates could not be relied upon because of small number in some breeds.

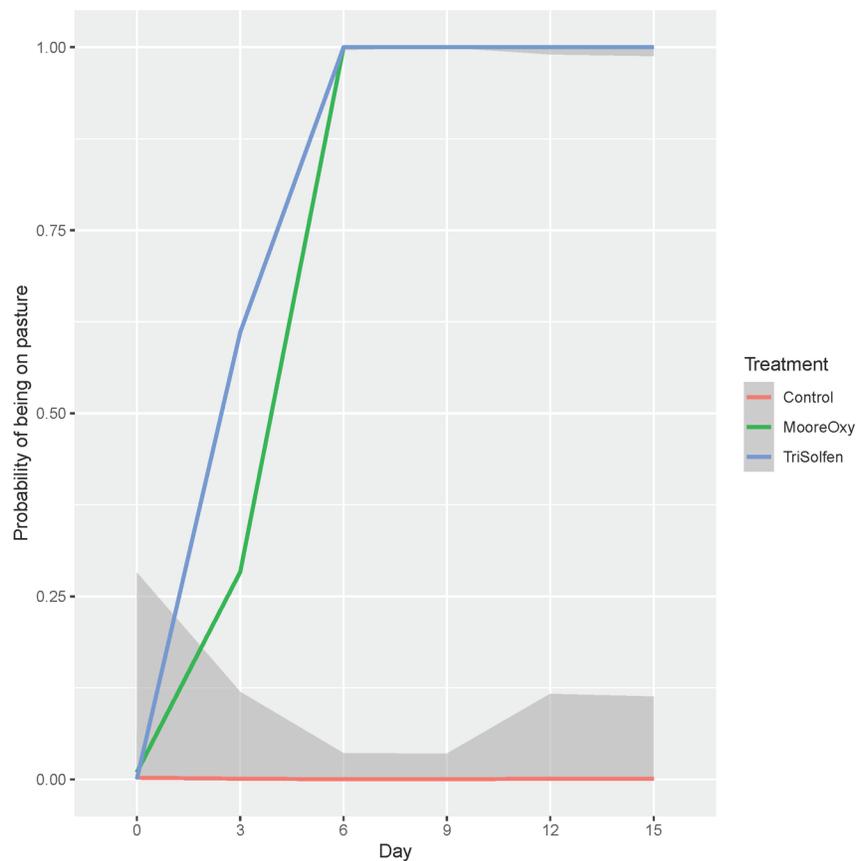


Figure 6. Model-based probabilities of cattle being on pasture across the six study days, for the three treatment groups. Shaded areas are ± 1 standard error of the mean (where available).

The results of an additional analysis to compare the number of days that cattle are on pasture is displayed (Table 3). As in the previous analysis, no significant effects of Treatment, nor Age, Breed or Sex were detected (all $P > 0.4$). However, consistent with the previous analysis, the two treatment groups spent more time on pasture.

Table 3 . Analysis of the number of days that cattle are salivating.

Variable	P -value	Control	Moore-Oxy®	Tri-Solfen®
Treatment	0.146	$1.59^A \pm 0.82$	$0.49^A \pm 0.39$	$0.56^A \pm 0.46$
Age	0.171			
Breed	0.537			
Sex	0.500			

^A: Means sharing the same superscript are not significantly different ($P > 0.05$).

Salivation

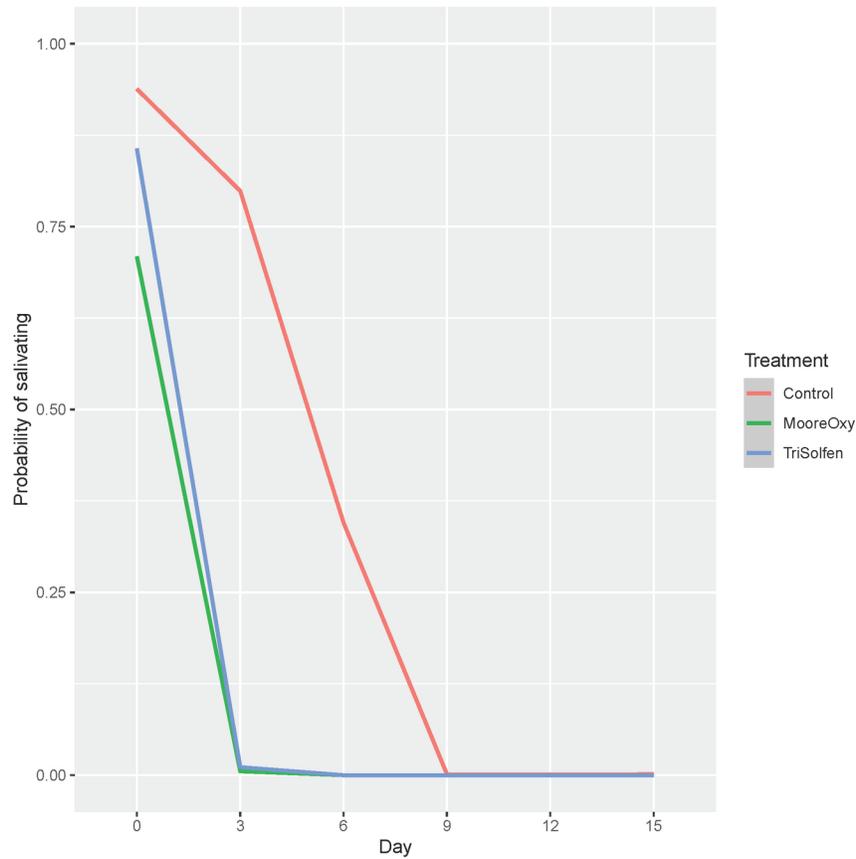


Figure 7 . Model-based probabilities of cattle salivating across the six study days, for the three treatment groups.

There was no significant Treatment \times Day interaction for occurrence of salivation ($P = 0.496$), nor was there an overall main effect of Treatment ($P = 0.401$). Model-based probabilities of salivating are shown (Figure 7). However, from initial high rates of salivating, there was a significant decline over the study period ($P =$

6.4×10^{-12}), with an apparent faster rate of decline for the two treatment groups compared with the control group. There were no significant effects of Age ($P = 0.619$), Breed ($P = 0.096$) nor Sex ($P = 1.000$) on instances of salivation.

The results of the analysis to compare the number of days that cattle are salivating is displayed (Table 4). As in the previous analysis, no significant effects of Treatment, nor Age, Breed or Sex were detected (all $P > 0.1$). However, consistent with the previous analysis, the two treatment groups spent less time salivating.

Table 4 . Analysis of the number of days that cattle are salivating.

Variable	<i>P</i> -value	Control	Moore-Oxy®	Tri-Solfen®
Treatment	0.146	1.59 ^A ± 0.82	0.49 ^A ± 0.39	0.56 ^A ± 0.46
Age	0.171			
Breed	0.537			
Sex	0.500			

^A: Means sharing the same superscript are not significantly different ($P > 0.05$).

Cost of therapy model

The treatment types and costs for FMD therapy in Cameroon were estimated, enabling a model to support therapy decisions for both individual farmers and public health policy makers (Table 5). With a single treatment of 1ml per lesion for TS, at USD0.50 per ml, the cost of treatment per animal is estimated between USD1.50-2.50.

Table 5. Treatment types used by farmers, application method, days of treatment and estimated daily and total costs.

Treatment choice	Application	Est. cost/day (USD)	Treatment days	Est. cost/animal (USD)
Moore-Oxy	Injection	0.85	3	2.55
Procaine penicillin	Injection	0.17	3	0.51
Oxytet 30%	Injection	0.85	3	2.55
Survidium	Injection	0.85	5	4.25
Insecticide & Petrol	Topical	0.42	7	2.94
Traditional drugs	Topical/oral	0.51	7	3.57
OXYDOZER 50	Injection	0.85	3	2.55
Tri-Solfen	Topical	/	1	2.50

Moore-Oxy® has a 7day milk WHP and 21 meat WHP.

Tri-Solfen® has a recommended 4day WHP for milk and meat in Lao PDR

DISCUSSION

This study reports the first field treatment trial using Tri-Solfen® as a therapy for the clinical management of FMD in Africa, comparing the clinical efficacy of this novel therapy with the commonly used parenteral antibiotic treatment Moore-Oxy® and animals remaining untreated. Despite necessary limitations due to resources availability on numbers of farmers and cattle recruited for the trial, the results and all participants, considered it was very successful, with high levels of appreciation by participants for their involvement and the clear results obtained.

Lesion healing scores across the three groups revealed superior results for the TS-treated cohort and although the MO-treated cohort also achieved a reasonable score, the untreated control animals had lesions persisting

for in excess of 2 weeks. Although a subjective measurement, these scores provide a useful indication of the healing rate of FMD lesions in field conditions in Cameroon.

Similarly, appetite scores were highest in the TS-treated cohort, indicating that the treatment supported the rapid return of appetite, even after 3 days. Superior appetite scores in this group were recorded at each data collection between day 3 and day 15, with the cohort receiving treatment with MO also having reasonable appetite scores between days 3 and 15. The control cohort cattle had poor appetite scores throughout, indicating appetite remains poor for in excess of 2 weeks when FMD remains untreated.

Lesion size was measured (in cm) at six data collection intervals, with the FMD infected cattle lesion size decreasing rapidly in the TS-treated cohort. By day 6 this cohort was performing well with average lesion size at 0.33 cm, despite commencing in the trial with higher average lesion size. By day 9, the lesions in the TS-treated cohort had almost entirely disappeared, remaining at 0 cm through day 12 and 15. Interestingly, the control group average lesion size increased as the trial progressed. In the MO-treated cohort, the average lesion size decreased until day 12, where it was recorded to increase from 0.17 cm to 0.75 cm at day 15. Stage of infection may have affected the study here, particularly as the lesion size in the control cohort was 0 cm at trial on day 0.

The farmer observations of clinical impacts also provided interesting results which could be used as animal welfare indicators as well as indicators of clinical response to the treatments. The mobility of all 12 cattle in the TS-treated cohort returned immediately, suggesting this is a very useful therapy to enable cattle to walk and gain access to water and feed. In the MO-treated cohort, 10 of the animals also immediately returned to walking. However, of the control group, only five had immediate mobility and four animals did not return to walking even by day 15. There was one animal in the MO-treated cohort that did not return to mobility until day 12. In total, 7/36 (19.4%) cattle had not returned to mobility by day 9, indicating that FMD is a severe disease as it renders animals immobile and that although FMD is considered a low mortality disease, the animal welfare impacts are considerable.

As oral vesicular lesions can have significant impacts on animal behaviours, recording the number of days the animals were anorexic was considered important. In total, 11/12 cattle in the TS-treated cohort were reported to be eating the same day as treatment (day 0). This compared to 9/12 cattle in the MO-treated cohort, with only 2/12 in the control cohort, with 9/12 of these untreated cattle remaining anorexic until day 15. Further, salivation is a common clinical sign with cattle with vesicular disease, considered an overt indicator of oral lesions and presumably oral pain. In all three cohorts the majority of cattle had ceased salivating at day 0, with the TS-treated cohort achieving 11/12, MO-treated cohort 10/12 and the controls 8/12.

Farmer reports of visible lameness of trial cattle indicated that 9/12 cattle in the TS-treated cohort ceased any lameness at day 0, compared to 5/12 in the MO-treated cohort and 3/12 in the controls. By day 6, no further cattle in the TS-treated cohort showed any lameness compared to 5/12 cattle in the MO-treated cohort still showing lameness by days 12 and 15, with lameness only ceasing by day 15 in 9/12 control cattle. For the question of how many days prior to cattle returning to grazing, mixed results were recorded. In the control cohort, 9/12 were reported grazing by day 0, with 8/12 in the TS-treated cohort returned to grazing by day 3 and all 12/12 in the MO-treated cohort returning to grazing by day 6.

When asked for a broad overview of treatments applied to FMD, six options were provided, including antibiotic formulations, traditional drugs, insecticides and petrol. Without speculating on the therapeutic potential of each treatment, it does indicate that farmers and paraveterinarians are inclined to treat affected animals with *something*. The data on costs of treatment suggest there are only minimal differences between treatments and that cost should not be an impediment to changing of treatment choice.

Field studies involving animal treatments with assessments of clinical impacts are challenging and subject to potential bias, reflected in the limited published literature on FMD therapy. However, the results obtained in this study demonstrate the superior clinical efficacy of a single application to FMD lesions of the TS topical anaesthetic wound formulation. The participating farmers reported a 100% appreciation for the product in

the treatment of FMD and expressed they were happy to have this product available for use in the region. These findings were consistent with those from a recently reported clinical investigation of TS therapy for FMD in Laos (Windsor et al, 2020) and reports of the use of this product for FMD in other countries in Africa, including Niger, Nigeria, and Kenya. This study in Cameroon provides the quantitative assessment that confirm that TS is efficacious in hastening clinical recoveries, immediately addressing pain and invoking more rapid healing of FMD lesions, as observed qualitatively in Laos (Windsor et al, 2020). As this innovation provides superior animal welfare outcomes for animals suffering from FMD, without the risks of AMR that arise from the more typical therapies in use for FMD globally, efforts to promote this new therapeutic approach should be supported.

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ETHICS STATEMENT

The authors confirm that the ethical policies of the journal as noted on the authors guidelines page, have been adhered to. In addition to following current procedures on animal and human ethics processes in Cameroon, the authors communicated with their Australian collaborators to ensure they complied with the National Health and Medical Research Council's (NHMRC) National Statement on Ethical Conduct in Human Research (2007) and the Universities Australia Australian Code for the Responsible Conduct of Research. This included ensuring that all participants provided verbal informed consent for the collection of animal blood samples, tissues, farmer interviews and participation in videos and images, where written consent was unavailable due to farmer illiteracy.

AUTHOR CONTRIBUTIONS

PW and SL designed the study and PW, JY and PT provided analytical and writing support. SL with support from JM, KB, KA and SD, performed the field trial activities. PT conducted the statistical analyses and all authors contributed to the final draft of the manuscript.

CONFLICT OF INTEREST

The authors report no conflicts of interest in this work. Studies evaluating Tri-Solfen® and other therapies for aversive animal husbandry interventions occurring prior to this study, were funded by an Australian Research Council Linkage Grant from the Australian government with financial contributions from Animal Ethics Pty Ltd Australia and Bayer Animal Health Australia. However, this current study did not receive funding from either of these companies, nor did they have a role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

DATA AVAILABILITY STATEMENT

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

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Table 1 . Age and breed of cattle recruited into each treatment group.

	Treatment group	Treatment group	Treatment group	
Variable	Moore-Oxy®	Tri-Solfen®	Control	Overall
Average age (yr)	3.7	4	3.3	3.7
Age range (yr)	2 to 5	2 to 8	2 to 5	2 to 8
Breed count				
Goudali	9	9	10	28
Holstein	1	1	1	3
White Fulani	1	2	1	4
Red Fulani	1	0	0	1
Total	12	12	12	36

Table 2. Analysis of the number of days that cattle are immobile, number of days walking with difficulty, and number of days walking normally

Walking	Variable	P-value	Control	MooreOxy®	Tri-Solfen®
Immobile	Treatment	0.002	2.47 ^A ± 1.39	0.05 ^B ± 0.35	0.00 ^B ± 0.30
	Age	0.942			
	Breed	0.590			
	Sex	0.268			
With Difficulty	Treatment	0.009	8.27 ^A ± 4.95	3.49 ^{AB} ± 2.00	0.70 ^B ± 0.85
	Age	0.471			
	Breed	0.875			
Normal	Treatment	0.000	0.60 ^A ± 0.40	2.72 ^B ± 0.78	7.18 ^C ± 1.92
	Age	0.221			
	Breed	0.117			
	Sex	0.033			

^A, ^B, ^C: Means sharing the same superscript are not significantly different ($P > 0.05$).

Table 3 . Analysis of the number of days that cattle are salivating.

Variable	<i>P</i> -value	Control	MooreOxy®	TriSolfen®
Treatment	0.146	1.59 ^A ± 0.82	0.49 ^A ± 0.39	0.56 ^A ± 0.46
Age	0.171			
Breed	0.537			
Sex	0.500			

^A: Means sharing the same superscript are not significantly different ($P > 0.05$).

Table 4 . Analysis of the number of days that cattle are salivating.

Variable	<i>P</i> -value	Control	MooreOxy®	TriSolfen®
Treatment	0.146	1.59 ^A ± 0.82	0.49 ^A ± 0.39	0.56 ^A ± 0.46
Age	0.171			
Breed	0.537			
Sex	0.500			

^A: Means sharing the same superscript are not significantly different ($P > 0.05$).

Table 5. Treatment types used by farmers, application method, days of treatment and estimated daily and total costs.

Treatment choice	Application	Est. cost/day (USD)	Treatment days	Est. cost/animal (USD)
Moore-Oxy	Injection	0.85	3	2.55
Procaine penicillin	Injection	0.17	3	0.51
Oxytet 30%	Injection	0.85	3	2.55
Survidium	Injection	0.85	5	4.25
Insecticide & Petrol	Topical	0.42	7	2.94
Traditional drugs	Topical/oral	0.51	7	3.57
OXYDOZER 50	Injection	0.85	3	2.55
Tri-Solfen	Topical	/	1	2.50

Moore-Oxy® has a 7day milk WHP and 21 meat WHP.

Tri-Solfen® has a recommended 4day WHP for milk and meat in Lao PDR

Figure Legends

Figure 1 : Maps showing the study sites (Ngaoundere II, Ngaoundere III and Martap)

M1: Mbidjoro 1, M2: Mbidjoro 2, T1: Tchabal Baouro 1, T2: Tchabal Baouro 2, T3: Tchabal Baouro 3, T4: Tchabal 4, T5: Tchabal 5, T6: Tchabal 6, B: Borongo, H1: Horé Mayanga 1, H2: Horé Mayanga 2, H3: Horé Mayanga 3.

Figure 2 . Model-based probabilities of obtaining lesion healing scores 1 through 4 across the six study days, for the three treatment groups.

Figure 3 . Model-based probabilities of obtaining appetite scores 1 through 4 across the six study days, for the three treatment groups.

Figure 4 . Model-based mean lesion size (cm) across the six study days, for the three treatment groups. Shaded areas are ± 1 standard error of the mean.

Figure 5. Model-based probabilities of obtaining walking scores of ‘Immobile’, ‘With difficulty’ and ‘Normal’ across the six study days, for the three treatment groups.

Figure 6. Model-based probabilities of cattle being on pasture across the six study days, for the three treatment groups. Shaded areas are ± 1 standard error of the mean (where available).

Figure 7 . Model-based probabilities of cattle salivating across the six study days, for the three treatment groups.

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