



Development of a live monovalent vaccine candidate for bovine trichophytosis

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Abstract

The purpose of this study was to isolate *Trichophyton verrucosum* from naturally infected calves, characterize it phenotypically, and evaluate a live monovalent vaccine candidate against bovine trichophytosis. The study was conducted in the Mycology laboratory of Vetinvest Innovative Enterprise LLP. A total of 100 calves from farms located in different regions of Kazakhstan were clinically examined for dermatophytosis. Skin and hair samples were collected from affected areas for isolation and characterization of *T. verrucosum*. In addition, haematological changes in calves, comparison of protective efficacy between field isolates and reference strain in rabbits, vaccine protective efficacy, therapeutic efficacy, and safety assessment in rabbits, and field evaluation of developed vaccine in naturally infected calves were also performed. Clinical examination revealed dermatophyte lesions in 45 calves, whereas laboratory confirmation revealed only 40 calves as dermatophyte positive, with the majority displaying mild localized lesions. The infected calves showed a significant increase in erythrocyte count, haemoglobin concentration, and haematocrit value, with reduced lymphocyte percentages. The vaccine formulation developed from field isolates of *T. verrucosum* showed 70% protective efficacy after experimental challenge at an immunizing dose of 20 million microconidia/cm³ with respect to the reference strain *T. verrucosum* F-02. Since the reference strain demonstrated 100% protective efficacy in rabbit model, it was selected for vaccine preparation. It provided complete protection in experimentally infected rabbits at a vaccine dose of 20.0 million microconidia/cm³, with detectable anti-*Trichophyton* IgG levels 10 days post vaccination, suggesting humoral immune response induction. Post-vaccination mild and short-lived local inflammatory reactions were observed in rabbits for three days. Field evaluation of the vaccine revealed clinical resolution of visible lesions in all vaccinated calves with mild local reactogenicity during the observation period. In conclusion, results demonstrated significantly favourable immune and clinical responses to *T. verrucosum* F-02-based live monovalent vaccine candidate with short-term tolerable safety profile under field conditions of the present study. However, the results should be cautiously interpreted and extrapolated because of small sample size and lack of confirmation of isolates at the molecular level.

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1. Introduction

Bovine trichophytosis is a contagious dermatophytosis caused predominantly by *Trichophyton verrucosum*, a zoophilic fungus that affects keratinised tissues, including the epidermis and hair follicles. The disease is clinically characterised by circumscribed alopecic, scaly, and crusted skin lesions, which typically appear on the muzzle and subsequently spread to the neck, back, and other body regions. In addition to its effects on animal health, bovine trichophytosis damages hides, thereby causing economic losses to cattle producers and enterprises involved in leather processing (Abastabar et al. 2019; Begum et al. 2020; Brasch et al. 2019; Elwart et al. 2021; Gosink 2022). The prevalence of bovine trichophytosis varies among herds and regions and is influenced by animal age, housing conditions, hygiene practices, and climatic factors. Infected animals, including those with mild or unapparent clinical signs, may act as reservoirs of infection. Transmission occurs mainly through direct contact with infected animals or indirectly through contaminated equipment, bedding, and housing surfaces. Young cattle are particularly susceptible. Additional risk factors include high humidity, inadequate hygiene, high stocking density, poor nutrition, and reduced immune status. The host immune response to dermatophyte infection involves the activation of cellular immune mechanisms, including neutrophils, eosinophils, and

macrophages, together with the release of reactive oxygen mediators and antimicrobial peptides. The association between immune status and susceptibility to dermatophytosis supports the use of vaccination as a preventive and therapeutic measure. Live fungal vaccines may stimulate cellular and humoral immune responses, although their safety, reactogenicity, and effectiveness must be evaluated for each vaccine strain, formulation, and target animal population (Husseini et al. 2022; Kandel et al. 2022; Nami et al. 2019). Th1- and Th17-associated cellular immune responses are primarily responsible for the removal of dermatophyte infections, while antibodies are believed to have just a supporting function.

In Kazakhstan, *T. verrucosum* remains an important dermatophyte pathogen of cattle, while the national cattle population has continued to increase in recent years (Kassenbayev et al. 2024). Previous work in Kazakhstan has included the evaluation of polyvalent inactivated vaccines against animal dermatomycoses (Umitzhanov et al. 2020). Live vaccines against bovine trichophytosis are also available internationally, but many are supplied in lyophilised form and require reconstitution before administration. Therefore, the development of a locally evaluated, ready-to-use liquid monovalent vaccine could simplify administration under field conditions. The aim of the present study was to develop and evaluate a live monovalent vaccine against bovine

trichophytosis based on *T. verrucosum*. The objectives were to: (i) assess the clinical and haematological characteristics of affected calves; (ii) isolate and identify *T. verrucosum* from clinical samples; (iii) compare the protective efficacy of field isolates with that of the reference *T. verrucosum* F-02 strain; (iv) prepare and evaluate vaccine formulations containing different concentrations of microconidia in rabbits; and (v) assess clinical therapeutic efficacy and local reactogenicity in naturally infected calves under field conditions.

2. Materials and Methods

2.1. Study Area and Experimental Design

The empirical study was conducted in the mycology laboratory of Vetinvest Innovative Enterprise LLP, Kazakhstan. Sampling were collected from 1 February to 30 November 2023 on small farms in the Almaty, Kyzylorda, and Turkestan regions. Farms were selected based on the presence of calves with clinically visible dermatological lesions compatible with dermatophytosis and the availability of the animal history records. The study was conducted in four stages: (i) clinical screening and laboratory diagnosis of bovine trichophytosis, (ii) isolation and characterization of field strains of *T. verrucosum*, (iii) development and laboratory evaluation of a live monovalent vaccine, and (iv) field evaluation of vaccine efficacy in naturally infected calves. Following convenience sampling method, animals were allocated to experimental groups according to the study design and their availability at the time of enrolment. Clinical examination, microscopy, and culture assessment were performed using the same procedures for all groups. Detailed information on allocation concealment and assessor blinding was not recorded.

2.2. Clinical examination and sample collection

Before inclusion, information on breed, sex, housing conditions, previous antifungal treatment, and previous vaccination against dermatophytosis was recorded when available. Animals with documented recent antifungal treatment or vaccination against dermatophytosis were excluded from the vaccine-response assessment. Calves were examined for alopecia, scaling, crust formation, and the anatomical distribution of lesions. Before sample collection, affected skin areas were disinfected with 70% ethanol. Hair and epidermal scales were removed from the edges of the lesions using sterile disposable scalpels and tweezers. Samples were placed into sterile paper bags and transported to the laboratory. Fluorescent lamps PRK-2, PRK-4, and L-60 equipped with a Wood filter were used only for preliminary screening and differential diagnosis of microsporia. Because *T. verrucosum* does not produce characteristic emerald-green fluorescence, the final laboratory diagnosis was based on microscopy and culture.

2.3. Hematological evaluation

A general haematological examination was performed in 23 calves. Sixteen calves had clinical signs compatible with bovine trichophytosis, while seven calves without clinical signs served as the control group. Blood was collected from the jugular vein and analyzed using a Mindray BC-2800 haematology analyzer. The evaluated parameters included erythrocyte count, haemoglobin concentration, haematocrit, leukocyte count, lymphocyte percentage, monocyte percentage, and granulocyte percentage.

2.4. Microscopic identification of dermatophytes

Clinical samples were transferred to sterile Petri dishes in accordance with State Standard GOST 1770-74 (1976). Dense hair fragments in contact with affected skin, together with white coating and desquamated epithelium, were selected using a dissecting needle. Fragments measuring approximately 1–2 mm were used for examination. Eight to ten particles of desquamated epithelium were placed on a microscope slide prepared according to State Standard GOST 9284-75 (1978). The material was treated with 10–15% potassium hydroxide or sodium hydroxide prepared according to State Standard GOST 4328-77 (1977), with the addition of glycerol. A coverslip was applied, and the samples were examined at $\times 10$ and $\times 40$ magnification. The preliminary diagnosis of trichophytosis was based on the presence of arthrospores surrounding the hair roots and fungal elements in the desquamated epithelium.

2.5. Isolation and cultivation of *T. verrucosum*

Following microscopic examination, the material was inoculated onto M188 and M1026 nutrient media containing rice extract (HiMedia, India). Cultures were incubated at 25–30 °C for up to 21 days. Culture growth and the presence of contamination were recorded throughout the incubation period. Morphological and temporal culture characteristics were evaluated macroscopically and microscopically. The concentration of dermatophyte microconidia in 1 cm³ was determined using a Goryaev counting chamber. Species identification was based on the clinical findings, microscopic appearance, duration of culture growth, and macroscopic and microscopic colony morphology. Molecular confirmation using PCR, sequencing, MALDI-TOF mass spectrometry, or internal transcribed spacer typing was not performed.

2.6. Selection of vaccine strain

Field isolates obtained from calves and the reference *T. verrucosum* F-02 strain were compared according to their protective properties in rabbits. The evaluated immunizing concentrations were 10.0, 15.0, and 20.0 million microconidia/cm³. Each experimental group included five rabbits. The challenge suspension was administered in a volume of 0.5 cm³ and contained 2.0 million microconidia/cm³. Uninfected and unvaccinated rabbits were used as negative controls. Unvaccinated rabbits challenged with *T. verrucosum* F-02 were used as positive infection controls. The strain producing the highest protective efficacy was selected for subsequent vaccine preparation.

2.7. Preparation of the live monovalent vaccine

An 18-day culture of the *T. verrucosum* F-02 strain grown on wort agar was used to prepare the laboratory vaccine series. Cultivation was performed at pH 7.2–7.4 and 28 °C within a cultivation period of up to 21 days. Under aseptic conditions, the fungal biomass was harvested from the culture surface using a sterile glass scraper and transferred to sterile containers. Sterile saline solution in a volume of 300.0 cm³ was added, and the suspension was mixed using a BioSan vortex mixer. Three 1.0 cm³ samples were collected for control examination. The suspension was processed using a MiniSpin microcentrifuge at 6,000 rpm for 20 minutes. Sucrose and gelatin were added as stabilizing components, after which the preparation was homogenized. The resulting suspension was dispensed into containers with volumes ranging from 10.0 to 200.0 cm³, sealed with rubber stoppers and aluminium caps, and labelled. The vaccine was stored in a dry, dark location at 6–10 °C. Three vaccine formulations were prepared containing 10.0, 15.0, or 20.0 million microconidia/cm³.

2.8. Protective efficacy and safety evaluation

Thirty-five rabbits were divided into seven groups of five animals. Rabbits in the three vaccine groups received vaccine formulations containing 10.0, 15.0, or 20.0 million microconidia/cm³. Vaccination was performed twice at an interval of 14 days. Each administration consisted of 2.0 cm³, corresponding to a total administered volume of 4.0 cm³. Three control groups were included to confirm the absence of spontaneous lesions and the infectivity of the challenge culture. A seventh group received an increased intramuscular dose of 5.0 cm³ to evaluate vaccine safety and local reactogenicity. Twenty-one days after the final vaccination, rabbits were challenged experimentally. A 5 × 5 cm² skin area on the back was shaved, treated with 70% ethanol, and subjected to shallow, bloodless superficial scarification using a sterile blade. A 0.5 cm³ suspension containing 2.0 million *T. verrucosum* microconidia/cm³ was applied to the prepared area. Animals were observed by a veterinarian for 15–30 days, with examinations performed every 3–5 days. protective efficacy was defined as the absence of clinical and microscopic signs of trichophytic infection after experimental challenge. Clinical protective efficacy was defined as the disappearance of visible lesions and the absence of new lesions during the observation period following vaccination. The humoral immune response was assessed using an enzyme-linked immunosorbent assay. Anti-dermatophyte-specific IgG was measured 10 days after vaccine administration in rabbits receiving the formulation containing 20.0 million microconidia/cm³.

2.9. Therapeutic evaluation in calves

Field evaluation was conducted in 60 naturally infected calves aged 6–12 months from private farms in the Almaty region. Each calf received 4.0 cm³ of the selected live monovalent vaccine. Clinical therapeutic efficacy was defined as the complete disappearance of visible lesions and the absence of new lesions during the observation period. Microbiological efficacy was not evaluated as a separate field endpoint. No untreated or placebo-treated calf group was included.

2.10. Statistical analysis

Quantitative haematological variables are presented as the mean ± standard error of the mean. Differences between calves with clinical signs and control calves were evaluated using Student’s *t*-test after assessment of the distribution of the quantitative variables. Differences were considered statistically significant at *p*<0.05. Categorical vaccine-response outcomes were analyzed descriptively because of the small

number of animals in each group. When comparison with an unvaccinated control group was possible, Fisher’s exact test was used, and exact *p*-values were reported. Confidence intervals were not calculated for the vaccine-response outcomes because of the small group sizes.

2.11. Ethical statement

All procedures involving animals were reviewed and approved by the Scientific Committee of Ethics of the Kazakh National Agrarian Research University, with authorization No. 32-16. Animal handling, vaccination, sample collection, and experimental challenge were performed by trained personnel. Measures were taken to minimize animal stress and discomfort. Skin scarification was limited to shallow, bloodless disruption of the superficial skin layer, and the animals were regularly monitored for pain, distress, and adverse reactions.

3. Results

3.1. Clinical findings

Of the 100 calves examined, 45 showed clinical lesions compatible with dermatophytosis. Laboratory examination presumptively identified dermatophyte infection in 40 calves, representing 40% of the examined calves. Among the 45 calves with clinically compatible lesions, 27 animals (60%) had a mild form characterized by small areas of alopecia located predominantly on the head and neck. The remaining affected calves had lesions involving the back, and the lesions were not exclusively circular. No significant differences in lesion occurrence were observed according to sex or breed. Infection by another fungal species could not be excluded in animals with atypical lesion patterns.

3.2. Haematological changes

The haematological findings for calves with and without clinical signs of bovine trichophytosis are presented in Table 1. Calves with clinical signs of trichophytosis were associated with significantly higher erythrocyte counts than control calves (9.82±0.28 versus 8.31±0.21 ×10⁶/mm³; *p* < 0.001). Haemoglobin concentration and haematocrit were also higher in affected calves (*p* = 0.03 and *p* = 0.04, respectively), whereas the lymphocyte percentage was lower (*p* = 0.04). Differences in total leukocyte count and monocyte percentage were not statistically significant (*p* = 0.09 and *p* = 0.98, respectively). The granulocyte percentage was higher in affected calves, with a value at the statistical threshold of *p* = 0.05.

Table 1. General blood test of calves with and without clinical signs of bovine trichophytosis			
Indicator	Control (n = 7)	Calves with clinical signs (n = 16)	P-value
Red blood cells (×10 ⁶ /mm ³)	8.31±0.21	9.82±0.28	p<0.001
Haemoglobin (g/dl)	9.67±0.34	10.89±0.31	0.03
Haematocrit (%)	28.90±1.02	32.23±0.88	0.04
White blood cells (×10 ³ /mm ³)	7.00±0.47	9.12±0.74	0.09
Lymphocytes (%)	49.59±2.66	37.82±3.51	0.04
Monocytes (%)	12.69±0.62	12.66±0.87	0.98
Granulocytes (%)	37.73±2.63	49.40±3.55	0.05
Values are presented as mean ± standard error of the mean. p-values were calculated using Student's t-test; p < 0.05 was considered statistically significant. Reference ranges were interpreted according to generally accepted veterinary haematology reference values reported in the scientific literature			

Table 2. Protective efficacy of field isolates in calves of Almaty region tested on laboratory animals (rabbits)

Source	Number of animals	Immunization dose (million microconidia / cm ³)	Infecting dose (2 million/cm ³)	Protective efficacy (%)
Private farms in the Almaty region	5	10	0.5	40
	5	15	0.5	60
	5	20	0.5	70
Reference strain of <i>Trichophyton verrucosum</i> F-02	5	10	0.5	20
	5	15	0.5	60
	5	20	0.5	100
Control group (uninfected rabbits)	5	-	-	100
	5	-	-	100
	5	-	-	100
Control group (strain <i>Tr. verrucosum</i> F-02)	5	-	0.5	-
	5	-	0.5	-
	5	-	0.5	-

The negative control group consisted of uninfected and unvaccinated rabbits. The positive infection control group consisted of unvaccinated rabbits challenged with *T. verrucosum* F-02. The control groups were used to verify the absence of spontaneous lesions and the infectivity of the challenge culture, respectively

3.3. Microscopic and cultural identification of *T. verrucosum*

Microscopic examination revealed arthrospores surrounding the hair roots, fungal filaments in desquamated epithelium, and spores occurring individually or in elongated chains. The fungal filaments were thin, measured approximately 2–4 μm , and showed variable branching and distribution. No visible colony growth was detected during the first seven days after inoculation. During the following two to three weeks, colonies reached approximately 8–12 mm in diameter. The colony margins were dense, velvety, and light-beige in appearance, while the central areas were brown and convex. Chains of rounded chlamydospores together with small club-shaped microconidia were observed microscopically. Identification of *T. verrucosum* was based on approximately 21-day growth period together with the microscopic and macroscopic morphology of the colonies. Haemolysis was not used as a diagnostic criterion.

3.4. Protective efficacy of field isolates vs reference vaccine

The protective efficacy of the field isolates and the *Trichophyton verrucosum* F-02 reference strain at the evaluated microconidia concentrations is presented in Table 2. Field isolates produced protective efficacy of 40%, 60%, and 70% at concentrations of 10.0, 15.0, and 20.0 million microconidia/cm³, respectively. The *T. verrucosum* F-02 reference strain produced corresponding protective efficacy of 20%, 60%, and 100%. Because the F-02 strain achieved the highest efficacy at

20.0 million microconidia/cm³, it was selected for preparation of the live monovalent vaccine.

3.5. Protective efficacy in rabbits

All unvaccinated rabbits challenged with *T. verrucosum* developed clinical and microscopic signs of trichophytic infection, including alopecia, scaling, and chains of spores surrounding the hair roots. Vaccinated rabbits showed different levels of protection depending on the concentration of microconidia in the vaccine formulation (Table 3). Protective efficacy increased with the concentration of microconidia in the vaccine. Vaccine formulations containing 10.0, 15.0, and 20.0 million microconidia/cm³ produced protective efficacy values of 20%, 60%, and 100%, respectively. All five unvaccinated challenged rabbits developed clinical signs of trichophytosis infection. Comparison with the unvaccinated infected control group using Fisher's exact test showed a statistically significant difference only for the formulation containing 20.0 million microconidia/cm³ ($p = 0.008$).

3.6. Protective efficacy in rabbits

Clinical protective efficacy was evaluated according to the disappearance of visible lesions and the absence of new lesions in infected rabbits after vaccination. The results are presented in Table 4. Clinical protective efficacy increased with the concentration of microconidia in the vaccine formulation. The formulations containing 10.0 and 15.0 million microconidia/cm³ produced protective efficacy

Table 3. Protective efficacy of monovalent live vaccine against bovine trichophytosis tested on laboratory animals (rabbits, n = 5)

Vaccine options	Vaccine dose (cm ³)	Results		Protective efficacy (%)
		Animal with clinical signs	Animal without clinical signs	
10.0 million/cm ³	4	4	1	20
15.0 million/cm ³	4	2	3	60
20.0 million/cm ³	4	0	5	100
Control	0	5	0	0

Because each group included five rabbits, the results should be interpreted cautiously. Comparison with the unvaccinated infected control group using Fisher's exact test showed significance difference only for the 20.0 million/cm³ vaccine option ($p = 0.008$)

Table 4. Clinical protective efficacy of monovalent live vaccine against bovine trichophytosis tested on infected laboratory animals (rabbits; n = 5)

Vaccine options	Vaccine dose (cm ³)	Results		Protective efficacy (%)
		Animal with clinical signs	Animal without clinical signs	
10.0 million/cm ³	4	4	1	20
15.0 million/cm ³	4	2	3	60
20.0 million/cm ³	4	0	5	100

Because each group included five rabbits, the protective efficacy results should be interpreted descriptively. The small group size limits statistical power and reproducibility

values of 20% and 60%, respectively. The formulation containing 20.0 million microconidia/cm³ produced 100% clinical protective efficacy, as none of the five rabbits showed signs of trichophytic infection after treatment. Because each group contained only five animals, these findings were interpreted descriptively.

3.7. Humoral immune response in rabbits

In rabbits receiving 4.0 cm³ of the vaccine formulation containing 20.0 million microconidia/cm³, the geometric mean titre of anti-*T. verrucosum*-specific IgG reached 1,510 ELISA EIA/mL at 10 days post vaccination.

3.8. Vaccine safety and reactogenicity

The safety and local reactogenicity observed in rabbits following administration of the increased vaccine dose of 5.0 cm³ are presented in Table 5. Rabbits receiving the increased vaccine dose of 5.0 cm³ developed mild local hyperaemia, soreness, and swelling at the injection site. The local reaction resolved within three days. No systemic clinical abnormalities were observed during the 15-day observation period. The animals remained active and maintained normal feed and water consumption.

or placebo-treated control group, this outcome was interpreted descriptively.

4. Discussion

The laboratory-confirmed prevalence of dermatophyte infection was 40% among the examined calves, while 60% of the clinically affected animals had a mild form of the disease. The predominance of localized lesions on the head and neck corresponds to previously described clinical manifestations of bovine dermatophytosis, in which circumscribed alopecic and scaly lesions frequently begin on the muzzle and subsequently spread to other body regions (Elwart et al. 2021; Gosink 2022; Monod and Lanternier 2022). The high proportion of mild cases is epidemiologically relevant because animals with limited or unapparent lesions may continue to serve as sources of infection within a herd. The haematological findings revealed that significant differences in erythrocyte count, haemoglobin concentration, haematocrit, and lymphocyte percentage were associated with infection status. The reduction in lymphocyte percentage and the higher granulocyte percentage may potentially reflect an inflammatory response to dermatophyte infection. However, total leukocyte count did

Table 5. Safety and local reactogenicity of monovalent live vaccine against bovine trichophytosis (20.0 million/cm³) tested on laboratory animals (rabbits; n = 5)

Vaccine dose (cm ³)	Results		
	Day 1 post vaccination	Day 3 post vaccination	Day 15 post vaccination
5	Mild inflammation was observed at the injection site. The general health status was satisfactory, and the animals remained active and continued to consume feed and water	No inflammatory reaction was observed at the injection site. The general health status, activity, feed intake, and water consumption remained normal	The general health status remained satisfactory, and activity, feed intake, and water consumption were normal

Table 5 presents descriptive safety observations only. No formal severity grading score was applied in this experiment

3.9. Therapeutic evaluation of vaccine efficacy in calves

The clinical therapeutic efficacy of the live monovalent vaccine in calves from private farms in the Almaty region is presented in Table 6. Following administration of 4.0 cm³ of the live monovalent vaccine, all 60 calves showed complete disappearance of visible clinical lesions and no development of new lesions during the observation period. Therefore, all vaccinated calves exhibited clinical recovery during the observation period. Because the field trial did not include an untreated

not differ significantly, and the granulocyte result was at the statistical threshold. However, these haematological changes should not be interpreted as indicating a cause-and-effect relationship but possible associations can be explored in further investigations. Studies of bovine immune responses indicate that cellular immunity and the functional activity of leukocyte populations are important in resistance to infectious agents, but such responses may also be affected by age, physiological status, stress, and other concurrent conditions (Husseini et al. 2022; Kandel et al. 2022).

Table 6. Clinical therapeutic efficacy of a live monovalent vaccine in field trials against bovine trichophytosis conducted on calves aged 6-12 months (n = 60)

Dose (cm ³)	Results		Clinical therapeutic efficacy (%)
	Signs of infection	No signs of infection	
4	0	60	100

Clinical therapeutic efficacy was defined as the complete disappearance of visible clinical signs and absence of new lesions during the observation period. Microbiological efficacy was not assessed as a separate endpoint in the field trial. Because no untreated or placebo calf group was included, the field efficacy result should be interpreted descriptively

Microscopy and fungal culture supported the identification of *T. verrucosum*. The slow development of colonies over approximately 21 days was consistent with the recognized cultural characteristics of this dermatophyte. Microscopy enabled the detection of arthrospores and fungal filaments, while culture allowed evaluation of colony morphology. These findings support the combined use of direct microscopy and culture in routine diagnosis, as recommended in previous studies of dermatophytosis diagnostics (Begum et al. 2020; Hassan et al. 2020). Nevertheless, the absence of PCR, sequencing, MALDI-TOF mass spectrometry, or internal transcribed spacer typing limited the certainty of species-level identification. The field isolates demonstrated a maximum protective efficacy of 70%, whereas the F-02 reference strain produced 100% efficacy at the same concentration of 20.0 million microconidia/cm³. This difference formed the experimental basis for selecting F-02 for vaccine preparation. Efficacy increased with the concentration of microconidia, suggesting a concentration-dependent response. However, the groups included only five rabbits, and statistical significance in comparison with the unvaccinated infected control was demonstrated only for the formulation containing 20.0 million microconidia/cm³.

Live vaccination against bovine trichophytosis has been investigated earlier, because TF-130- and LTF-130-based preparations have previously been developed and used. The contribution of the present study is the preparation and evaluation of a ready-to-use liquid monovalent vaccine based on the F-02 strain under field conditions in Kazakhstan. Previous work in Kazakhstan evaluated a polyvalent inactivated vaccine against animal dermatomycoses (Umitzhanov et al. 2020), whereas the current preparation contained a single live strain intended specifically for bovine trichophytosis. The 20.0 million microconidia/cm³ formulation produced 100% protective efficacy and 100% clinical therapeutic efficacy in rabbits. The anti-*T. verrucosum*-specific IgG titre measured after vaccination also indicated induction of a humoral immune response. Nevertheless, the IgG result was obtained from the group showing the highest vaccine response, and no baseline titre or corresponding unvaccinated control titre was reported. It therefore confirms antibody production but does not establish a quantitative correlate of protection. The increased vaccine dose induced only mild local hyperaemia, soreness, and swelling, which resolved within three days without systemic changes. This result is consistent with the expected possibility of limited local reactogenicity following administration of live fungal preparations (Nami et al. 2019; Monod and Lanternier 2022). The absence of an added adjuvant may have contributed to the limited local response, although this mechanism was not directly tested.

The field trial showed complete clinical resolution in all 60 treated calves. This finding indicates potential therapeutic usefulness under practical farm conditions. However, bovine ringworm may be self-limiting, and the trial did not include untreated or placebo-treated calves. It is therefore not possible to determine how much of the observed clinical resolution resulted specifically from vaccination rather than spontaneous recovery or changes in management conditions. In addition, microbiological clearance was not assessed after clinical recovery. The ready-to-use liquid formulation may simplify vaccine administration because it does not require reconstitution, unlike lyophilised live vaccines such as Bovilis Ringvac LTF-130. However, no direct comparison with a commercially available vaccine was performed. Therefore, the present findings do not demonstrate

superiority in efficacy, cost, safety, or convenience. The potential practical advantage of the liquid formulation must also be considered in light of its unknown storage stability and shelf life.

The principal limitations of the study were the small rabbit-group sizes, the absence of an untreated or placebo-treated calf comparator group, the absence of molecular confirmation of the isolated dermatophytes, and the lack of evaluation of microbiological clearance in the field trial. Allocation concealment and assessor blinding were not documented. The withdrawal period, long-term storage stability, shelf life, and environmental persistence of the live vaccine strain were also not examined. Future studies should use larger animal groups and randomised comparator-controlled field designs. Molecular methods should be used to confirm the identity and genetic stability of the vaccine strain and clinical isolates. Additional studies should evaluate duration of immunity, microbiological clearance, optimal vaccination schedules, withdrawal periods, environmental persistence, storage stability, and shelf life. Direct comparison with existing live and inactivated vaccines would also be necessary to determine the relative advantages of the F-02 liquid formulation.

5. Conclusions

Bovine trichophytosis was laboratory-confirmed in 40 of the 100 examined calves, with mild lesions being the predominant clinical presentation. Microscopy and culture supported the identification of *T. verrucosum*. Field isolates achieved a maximum efficacy of 70%, whereas the reference F-02 strain achieved 100% efficacy at a concentration of 20.0 million microconidia/cm³ and was therefore selected for vaccine preparation. The resulting ready-to-use liquid monovalent vaccine provided complete protection in rabbit challenge model and clinical therapeutic efficacy in the infected calves receiving the selected formulation, while an increased dose caused only mild local reactogenicity that resolved within three days. Complete clinical resolution was observed in all naturally infected calves under the uncontrolled field-trial conditions. These findings indicate that the F-02-based formulation is a promising candidate for the treatment and prevention of bovine trichophytosis, although larger controlled studies are required before its efficacy and practical advantages can be confirmed.

Declarations

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