A live vaccine for contagious agalactia is protective but does not provoke an ELISA response

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Abstract

This study aimed to assess the efficacy of two *Mycoplasma agalactiae* vaccines, a live attenuated vaccine (strain AIK) which has been used in the field for over 50 years in Turkey, and an experimental vaccine which was inactivated and adjuvanted with saponin containing the same strain of *Mycoplasma agalactiae*. A total of 30 Angora goats were assigned to 3 groups, each with 10 animals. Group 1 was inoculated with the attenuated vaccine, group 2 with the inactivated vaccine and group 3 comprised the control group receiving phosphate buffered saline only. The three groups were challenged with 100 ID_{50} of *Mycoplasma agalactiae* strain Aşkale by the subcutaneous route one month after vaccination. Clinical signs were recorded after challenge and all goats were bled at 14, 21, 28, 42 and 64 days post-vaccination; serological responses to *Mycoplasma agalactiae* were measured using a commercial ELISA kit. All animals were killed at one month after challenge and internal organs were subjected to bacteriological examination to assess the invasion of the challenge strain. The inactivated vaccine stimulated a strong antibody response as detected by ELISA, whereas the attenuated vaccine did not induce any detectable antibody titres. The challenge strain was re-isolated from internal organs of all the controls and two goats in each vaccinated group. The vaccination-challenge results showed that both vaccines have high efficacy against *Mycoplasma agalactiae* and that they could be used to control the spread of *Mycoplasma agalactiae* infection.

Introduction

Contagious agalactia (CA), an important disease of small ruminants, presents mainly as mastitis in lactating animals; it can also cause arthritis, keratoconjunctivitis pneumonia, septicaemia and abscesses in all age groups and abortions in pregnant females [1,2]. The disease leads to significant economic losses because of the reduction in milk yield, death of young animals, abortion and treatment costs [3]. Although the major aetiological agent is *Mycoplasma agalactiae* (Ma), *Mycoplasma capricolum* subsp. *capricolum*, *Mycoplasma mycoides* subsp. *capri* and *Mycoplasma putrefaciens* are also responsible sporadically for a clinically similar syndrome [2]. The disease occurs worldwide, in particular in countries surrounding the Mediterranean and in the Middle East where traditional husbandry is often carried out [4].

CA is difficult to control as antibiotics are rarely completely effective [5]. Commercial vaccines are available and used widely and are mostly formalin, heat-inactivated or aluminum hydroxide- or oil-adjuvanted preparations [2,6]. In Turkey both live attenuated and inactivated vaccines have been used many years [7]. In a recent trial of commercial and experimental CA vaccines, the live vaccine made in Turkey was shown to be the most effective [8]. The aim of this study was to compare the efficacies of the live attenuated vaccine with a vaccine inactivated with saponin in a larger trial.

Material and method

Vaccines

Live attenuated vaccine: a vaccine was prepared with the *M. agalactiae* strain AIK isolated from a CA outbreak in Turkey in 1968 and attenuated by 40 serial passages *in vitro* [9].

Inactivated vaccine: a vaccine was prepared with the second passage of *M. agalactiae* strain AIK by inactivating with 2mg/ml of saponin as described by [10] and adjuvanted with oil and Falba [9]. Viability tests showed vaccine to be completely inactivated.

Both vaccines are manufactured and marketed by the Pendik Veterinary Control Institute, Istanbul, Turkey.

Challenge strain

The strain used for challenging the sheep had been isolated in 1982 from a severe outbreak of CA and designated Aşkale. Unlike the live vaccine strain, the challenge strain produced film and spots in mycoplasma medium and haemolysed medium containing 10% red blood cells.

Animals

A total of 30 Angora goats were used. The goats were first bled and tested by a commercial ELISA (Institut Pourquier Version: P00400/05) to confirm the absence of *M. agalactiae* antibodies prior to the experiment.

The goats were assigned to 3 groups consisting of 10 animals each. While group 1 was inoculated with 1ml of the live attenuated vaccine, group 2 was inoculated with the inactivated vaccine by the subcutaneous route. Group 3 was the control group and received only phosphate buffered saline.

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The goats in the experimental and control groups were bled at 14, 21, 28, 42 and 64 days post-vaccination and serological responses to *M. agalactiae* were measured by ELISA following the manufacturer's protocol.

The three groups were challenged with 1ml of inoculum containing 100 ID_{50} (10^4 microorganisms) of *M. agalactiae* strain Aşkale by the subcutaneous route at one month post-vaccination; clinical signs suggestive CA were recorded daily until autopsy.

All animals were killed at one month post-challenge and various organs: kidney, spleen, liver, lung, hearth, intestine, udder, trachea, joints (carpal, tarsal, knee) and lymph nodes (prescapular, inguinal, popliteal, supramammary, mesenterial, hepatic, mediastinal, bronchial, mandibular) were subjected to bacteriological analysis to assess the invasion of the challenge strain [9]). The mycoplasma were reisolated and identified by established methods [11].

The clinical and cultural findings recorded were scored according to the scheme developed in house at Pendik (Table 1)

Result

Serological results

Mean ELISA tests results (S/P%) of the goats in the groups are shown in Figure 1. The inactivated vaccine (group 2) stimulated a strong antibody response reaching a peak on day 21. The titres ranged between 57% and 161% on the same day. However, the attenuated vaccine did not induce any detectable antibody titers.

Clinical and culture findings

While no clinical signs were seen in the vaccinated groups, lameness (two animals), keratoconjunctivitis (two animals) and mild fever (one animal) were seen in the control group. Clinical monitoring and re-isolation study results are shown in Table 2.

The challenge strain, recognised by its ability to produce film and spots, was re-isolated and identified from a liver of one goat and lymph nodes of one goat in group 1. In group 2, the strain was reisolated and identified from lungs of one goat and lymph nodes of another. In the control group, *M. agalactiae* was reisolated and identified from the various samples of all animals.

Table 1. The scoring system to calculate immunity

| Findings	Score
Isolation of mycoplasma from blood culture, (for each animal)	2
Isolation of mycoplasma from eyes, noses, milk, feces, (for each isolation)	1
Isolation for each day	1
Death following severe disease	8
Death following moderate disease	4
Isolation of mycoplasma from internal organs, (for each isolation)	1
Isolation of mycoplasma from joints, (for each isolation)	2
Isolation of mycoplasma from lymph nodes, (for each isolation)	1

Calculation:
Arithmetic means of the scores of vaccinated and control animals are calculated. Per cent immunity is calculated using the formula below. A vaccine batch which has per cent immunity of 50 or above is considered satisfactory.

Mean score of controls   C

Mean score of vaccinated animals   V

$$\text{Per cent immunity} = \frac{C-V}{C} \times 100$$

Figure 1. Mean serological responses of the groups as detected by ELISA
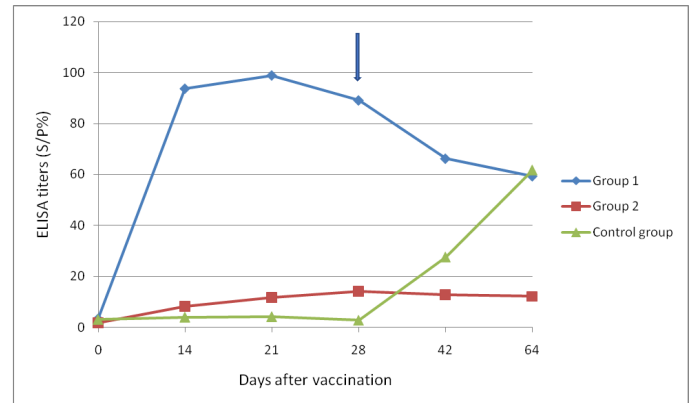
*According to the manufacturer's instructions, 60 S/P% and above should be interpreted as positive.
Arrow signifies time of challenge

Table 2. Clinical and culture findings after the challenge

Group	Goat ID	Clinical signs	Reisolation of *M agalactiae*
Group 1	361	Not seen	-
	366	Not seen	-
	434	Not seen	Liver
	435	Not seen	-
	792	Not seen	-
	244	Not seen	-
	253	Not seen	-
	786	Not seen	-
	793	Not seen	-
	784	Not seen	Lymph node
Group 2	228	Not seen	Lung
	315	Not seen	-
	795	Not seen	-
	831	Not seen	-
	832	Not seen	Lymph node
	801	Not seen	-
	60	Not seen	-
	310	Not seen	-
	451	Not seen	-
	255	Not seen	-
Group 3 (Control group)	312	Not seen	Lymph node
	785	Mild lameness	Lymph node, liver, lung, hearth
	836	keratoconjunctivitis	Lymph node, liver
	63	Mild fever	Lymph node
	228	Not seen	Lymph node
	321	keratoconjunctivitis	Lymph node, lung
	101	Not seen	Lymph node
	779	Mild lameness	Lymph node, liver, lung
	452	Not seen	Lymph node
	229	Not seen	Lymph node

Based on the scoring system the live and inactivated vaccines studied confer 85% immunity.

Discussion

Live Ma vaccines are not permitted in the EU but an attenuated product has been used successfully for many years in Turkey [2,9]. It was produced from the 40th passage of a local Ma strain grown in

selective media [9]; it retains some pathogenicity for lactating animals which can be further attenuated by another 30 passages but this reduced protection [12]. In the present study, in spite of the lack of a detectable ELISA immune response, animals inoculated with the live attenuated vaccine were largely protected although it must be acknowledged that this was not a severe challenge. Despite this *M. agalactiae* was re-isolated from all unvaccinated sheep.

This failure to generate an antibody response to this vaccine has been reported before [8] and may be due to the random loss of virulence genes during attenuation such as the NIF locus identified by [13]. Mutants lacking this locus also do not provoke an IgG response in experimentally infected ewes though whether the mutants were protective against challenge was not tested.

Alternatively the strains used in the vaccine and ELISA kit may be antigenically disimilar [14] showed extensive molecular diversity amongst *M. agalactiae* strains from several European countries using a range of molecular typing tools. Strains have also shown to be highly variable antigenically which is compounded by their variable surface proteins [15]. This clearly requires further study but this observation can be used to differentiate naturally infected from vaccinated animals in the field [16].

Live vaccines are inexpensive and easy to apply and could have a major impact in areas where disease is endemic. However, they may sometimes cause subclinical infection in lactating females [9] and thus are contra-indicated during this susceptible period. Anecdotal evidence from the field where the live vaccine has been used for many years suggest that they are highly protective with a long duration of immunity (unpublished results). The live vaccine has also been shown to be effective in the face of an outbreak with rapid reductions of clinical signs seen in affected animals similar to that reported recently by [3]. The use of live vaccines in disease-free areas would not be necessary as effective inactivated vaccines described here would be used here.

In a vaccine trial two widely available formalised commercial products were shown to be ineffective against experimental challenge raising concerns for their use in the field [8]. Clearly better vaccines are necessary. [10] compared a range of experimental vaccines. Sheep immunized with saponin- and phenol-inactivated vaccines performed better than those inactivated with formalin, heat and sodium hypochlorite. Our study has confirmed the efficacy of the saponinized inactivated vaccine against *M. agalactiae* producing high levels of immunoglobulin and is protective.

In conclusion this study shows hat both vaccines are highly effective against *M. agalactiae* infection and could be used to control CA by the use of a live vaccine in region where disease is widespread. Ideally, the live vaccines should be part of a regional plan in which all animals likely to come into contact are vaccinated around the same time; an inactivated product would be used in areas of lower disease prevalence and those surrounding infected zones.

Conflict of interest statement

None of the authors have any conflicting interests.

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