Determination of Antibody Response against Inactivated Agalactia Vaccine in Small Ruminants

Short Communication

Sotoodehnia, A*[1]., Moazeni Jula, G[1]., Madani, R[2]., Golchinfar, F[2]., Naserirad, A[1].

1. Aerobic Bacterial Vaccines Dept., Razi Vaccine & Serum Research Institute, karaj, Iran

2. Biotechnology Dept. Razi Vaccine & Serum Research Institute, karaj, Iran

Received 11 Dec 2004; accepted 15 Sep 2005

Summary

An inactivated agalactia vaccine was prepared with *Mycoplasma agalactiae* in fermenter. The culture was inactivated and subsequently adjuvanted with aluminum hydroxide gel. Four sheep and four goats were immunized with the experimental vaccine. Two doses of 1ml of vaccine were administered three weeks interval. Monthly, blood samples were taken from the vaccinated animals up to the fifth month along with hyperimmune and negative controls. Elisa was set up and sera were assayed against Lorestan live antigen. Results indicated that antibodies were determined in vaccinated sheep and goats with the maximum overall mean titres of 1.25 and 1.028, at one month post-vaccination and minimum mean titres of 0.674 and 0.538 respectively.

Key words**:** *Mycoplasma Agalactiae*, Inactivated vaccine, Small ruminants

Introduction

Contagious agalactia (CA) of sheep and goats has been known for about two centuries. *Mycoplasma agalactiae* is the primary agent of contagious agalactia in small ruminants. The disease is clinically manifested by mastitis, arthritis, and kerato conjunctivitis (Cottew 1979, DaMassa 1983). Similar clinical and pathological feature can be produced in small ruminants by other *Mycoplasmas* namely, *Mycoplasma mycoides* subspecies mycoides LC, *Mycoplasma capricolum*

* Author for correspondence. E-mail: sotoodeh347@yahoo.com

subspecies. *Capricolum* and *Mycoplasma putrifaciens* (DaMassa *et al* 1992, Nicholas 1996, Bergonier *et al* 1997). CA is frequently occurred in Mediterranean countries, the north of Africa and Near and Middle East countries (Al-Zeftawi 1979; Erdag 1989, Sarris 1996, Kusikula *et al*, 2000) in which it has caused remarkable losses.

CA is a highly infectious disease of small ruminants in Iran. The first isolation of *Mycoplasma agalactiae*, the causative agent of CA was reported from ewe milk (Bory & Entessar1959). Later an inactivated vaccine was prepared and used in limited area(Baharsefat *et al* 1971). *Mycoplasma mycoides* subspecies mycoides LC, another agent of CA was reported from the same country (Aarabi & Sotoodehnia 1984). In a report, status of the disease in the country has been described (Sotoodehnia & Aarabi 1986).

Vaccination strategies against CA are based on both live and inactivated vaccines (Leon-Vizcaino *et al* 1995, Tola *et al* 1999). In Europe, where live vaccines for *Mycoplasma agalactiae* are not acceptable, attention has focused on the use of killed organisms, mostly using formalin as inactivating agent of choice and aluminium hydroxide or mineral oil as adjuvant (Leon-Vizcaino *et al* 1995, Sarris *et al* 1989). Formalised vaccine has given some protection against experimental infection but it could not prevent clinical disease following the introduction of naturally infected animals. Recently vaccines inactivated with phenol or with saponin have given superior protection against experimental infections compared with formalin, sodium hypochlorite or heat-inactivated vaccines (Tola *et al* 1999). Live attenuated vaccines against *Mycoplasma agalactiae* have been applied in Turkey since 1972 and have been reported to provide better protection in ewes and lambs than inactivated vaccines (Turkaslan 1990). However they can produce a transient infection with shedding of mycoplasma. The purpose of this study was to determine the antibody induced against agalactia vaccine in vaccinated sheep and goats by Elisa.

Materials and Methods

Vaccine preparation. *Mycoplasma (M.) agalactiae* strain *Lorestan* was used for seed culture. This strain had already been isolated from sheep milk and identified as *M. agalactiae* (Aarabi and Sotoodehnia 1984). Seed culture was prepared with PPLO broth medium and supplemented with equine normal serum, incubated at 37°C for 24 hours. The seed was inoculated into fermenter containing the above medium (sterilize at 110°C for15 minutes, pH 7.2). Viable mycoplasma number was determined according to standard procedure (Rodwell *et al* 1983). The culture was inactivated with 0.4 %(v/v) formaldehyde and subsequently, the vaccine was adjuvanted with 10 %(v/v) aluminium hydroxide gel. Vaccine safety was performed on guinea pigs with administration of 2 ml (2doses) of vaccine then animals observed for a week.

Animals. Two groups of four sheep and four goats about 1-year-old without history of contagious agalactia were selected from a clean area and transferred to animal house of Razi Vaccine & Serum Research Institute (RVSRI). 0ne healthy sheep and one healthy goat were also used as negative controls.

Vaccination and sera collection. All animals were bled before vaccination. Two doses of 1 ml vaccine (three weeks interval) were subcutaneously administered in each sheep and goat. Blood samples were then taken from all animals at 1, 2, 3, 4, and 5 months after the second vaccination. Sera were separately collected by centrifugation and kept at –20°C until use. Sera from sheep and goat controls were also prepared by the same way**.**

Preparation of live antigen and hyperimmune sera. One litre pure culture of *M. agalactiae* strain Lorestan was prepared in PPLO broth medium and live cells were then centrifuged at 12000 rpm. Cells were washed twice with sterile PBS as described by (Tola *et al* 1999) and harvested in 60ml PBS adjusted to No.6 *Mc ferlan* opacity tube. Antigen was stocked at –20°C before injection to animals. One sheep and one goat were immunized against live antigen. Injections of 3ml

antigen were intraveneously administered in jugular vein, five days apart. After six injections, animals were bled and hyperimmune sera were collected by centrifugation.

Elisa. Polystyrene plates were coated with 100 µl of live *M. agalactiae* strain, Lorestan, (10µg/ml) and incubated at 4°C for an overnight. Half Plates were incubated for 1 hour at 37°C with vaccinated sheep sera and half with vaccinated goats sera (diluted 1:200). After several washings with PBS and Tween 20, plates were incubated for another hour with 100 µl/well of peroxidase - conjugated rabbit anti - sheep or anti - goat. After washing, the ready use substrate (BM blue POD) was used (100 µl /well). The reaction was stopped with N sulphuric acid (50µl/well) and monitored at 450 nm with an Elisa reader. Simultaneously, sheep and goat hyperimmune sera as well as negative sheep and goat sera were used in the test.

Results and Discussion

Before vaccination, none of the animals showed antibody titre against live antigen except sheep No.3 and goat No.6. The moderate titres in sera of these animals were probably due to their exposition before this experiment. During vaccination a progressive increase in antibody production against mycoplasma immunogenic proteins was determined with one peak in the first month. The results of immune response are given in tables.1, 2 and Figures 1 and 2. In table 3, the mean titre and standard deviation (SD) of vaccinated sheep and goat sera are shown. The overall mean titres, in the first bleeding after one month, were 1.25 and 1.028 respectively. These maximum overall mean titres of antibody were gradually decreased to the minimum of 0.674 for sheep and 0.538 for goats. In this study, the duration of antibody was determined 3 months in groups of sheep and 2 months in groups of goats while they have received two doses of inactivated vaccine. The difference in antibody lasting between groups of sheep and goat was probably due to the origin of used strain, Lorestan, which was belonged to sheep origin.

Tola *et al* (1999) have used Elisa test for detection of antibody in vaccinated ewes with different inactivated vaccines. They reported that saponin and phenol-inactivated vaccines maintain high antibody levels with two peaks one in the third month and the other in the eight month after vaccination. Animals vaccinated with phenol and saponin inactivated mycoplasmas resisted experimental challenge.

Table 1. *Antibody titre of vaccinated and control groups of sheep.*

No	Before vaccination	Months after vaccination				
		1	2	3	4	5
1	0.308	1.179	1.187	0.725	0.262	0.321
2	0.526	1.29	1.327	0.955	0.394	0.418
3	0.698	1.306	0.914	0.474	0.368	0.330
4	0.366	1.225	0.815	0.542	0.199	0.292
Hyperimmune	0.259	0.450	0.353	1.529	1.066	1.390
Control	0.293	0.298	0.315	0.352	0.306	0.420

Table 2. *Antibody titre of vaccinated and control groups of goats.*

No	Before vaccination	Months after vaccination				
		1	2	3	4	5
5	0.196	1.254	0.722	0.515	0.313	0.349
6	0.802	1.153	0.711	0.309	0.389	0.352
7	0.179	0.565	0.249	0.183	0.190	0.361
8	0.151	1.141	0.473	0.256	0.251	0.361
Hyperimmune	0.163	0.194	0.162	1.069	0.891	0.529
Control	0.206	0.226	0.171	0.176	0.168	0.155

Table 3. Mean titre and standard deviation (SD) of vaccinated sheep and goat sera.

Post-vaccination bleeding(month)	***Mean sheep sera***	***S.D sheep sera***	***Mean Goat sera***	***S.D Goat sera***
First	*1.25*	*0.058*	*1.028*	*0.312*
Second	*1.060*	*0.237*	*0.538*	*0.224*
Third	*0.674*	*0.215*	*0.315*	*0.142*
Forth	*0.305*	*0.091*	*0.285*	*0.085*
Fifth	*0.340*	*0.054*	*0.355*	*0.006*

During the past years, efficacy of inactivated vaccines against CA has been repeatedly evaluated. Immunization of killed agalactia vaccine was carried out in Turkey by complement fixation and growth inhibition tests (Erdag 1989). The complement fixing and growth inhibiting antibodies appeared 2 weeks after vaccination and reached its peak 4 weeks later and disappeared 10 weeks after vaccination

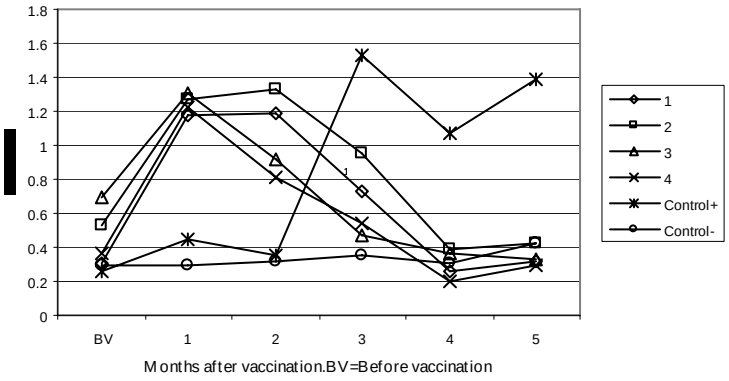

Figure 1. *Antibody titre of vaccinated and control groups of sheep*

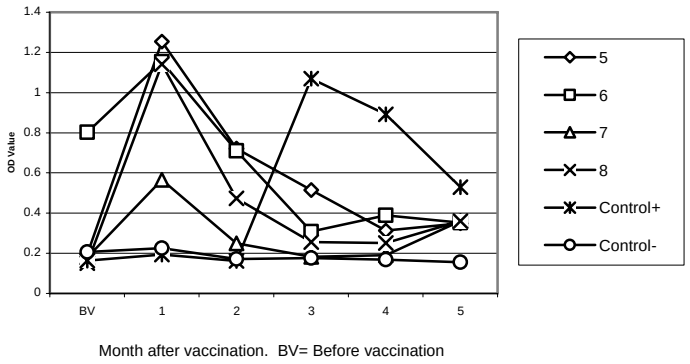

Figure 1. *Antibody titre of vaccinated and control groups of sheep*

The result obtained after challenging proved that formal inactivated oil-falba treated vaccine gave full protection in sheep and goats.

Perez *et al* (1990) stated that inactivated vaccines provide inadequate immune protection against a clinical outbreak of contagious agalactia. Regalla (1987) recommended immunization three times a year although he considered that the efficacy of vaccination was controversial when the infection pressure was very high. Leon Vizcaino *et al* (1995) believed that systematic immunization can reduce the incidence of disease. They recommended that three doses of vaccine should be administered before, and one dose after parturition, and that the herd should be kept isolated in order to control the disease.

Owing to the antigenic heterogeneity of *M. agalactiae*, the efficacy of vaccination has so far been relatively poor (Sarris & Papadopoulos 1987), and it should always be used a wider policy of hygienic prophylaxis. It is possible that in some instances, he apparent lack of protection given by inactivated vaccines could be result of animals being infected with one of the other three mycoplasmas involved with the syndrome of contagious agalactia. However, no single vaccine has been universally adopted, and no standard methods of preparation and evaluation would apply to all vaccines that have been used through the world (Manual of standards for diagnostic tests and vaccines, OIE 2004). Despite the widely disputes concerning The use and efficacy of conventional agalactia vaccines (Food and Agriculture Organization 1992), vaccination is recommended in affected regions particularly in areas with a low social and economic standard where implementation of more radical measures will be difficult.

References

Aarabi, I. and Sotoodehnia, A. (1984). Isolation and carbohydrate fermentation tests of Mycoplasma agalactiae and mycoplasma mycoides subsp.mycoids strains in Iran. *Archives of Razi Institute* 34:67-70.

Al-Zeftawi, N.M.N. (1979). The role of *Mycoplasma tales* in diseases of sheep and goats in Egypt. *PhD Thesis.* Pp:188. University of Cairo, Egypt.

Baharsefat, M., Yamini, M. and Ahourai, P. (1971). Comparison of three different

contagious agalactia vaccines. *Archives of Razi Institute* 23:113-118.

Bergonier, D., Bertholet, X. and Poumarat, F. (1997). Contagious agalactia of small ruminants: Current knowledge concerning epidemiology, diagnosis and control. *Revue Scientifique et Technique OIE* 16: 848-873.

Bory, G. and Entessar, F.(1959). Etude sur l' agalaxie contagieuse des chevres et des moutons. *Archives of Razi Institute* 14:109-127.

Cottew, G. S. (1979). The Mycoplasma II. In: *Caprine - Ovine Mycoplasma.* Pp:103-32. Academic Press, New York.

Da Massa, A.J. (1983). Recovery of *Mycoplasma agalactiae* from mastitic goat milk. *Journal of American Veterinary Medicine Association* 183: 548-54.

Da Massa, A.J., Wakenell, P.S. and Brooks, D.L. (1992). Mycoplasmas of goats and sheep. *Journal of Veterinary Diagnosis and Investigation* 4: 101-103.

Erdag, O. (1989). Investigation on the preparation and application of vaccine against contagious Mycoplasma agalactiae for sheep and goats in Turkey. *Proceeding of International Symposium of Mycoplasma.* Pp: 20-22.

Food and Agriculture Organisation (1992). Animal health year book. *FAO/Office International des Epizooties (OIE) / World Health Organization (WHO).* Pp: 271. Rome.

Kusikula, L.J.M., Ojeniyi, B., Friis, N.F., Kazwala, R.R. and Kokotovic, B. (2000). Mycoplasmas isolated from the respiratory tract of cattle and goats in Tanzania. *Acta Veterinaria Scandinavica* 41: 299-309.

Leon - Vizcaino, I., Garrido- Abellan, F., Cubero- Pablo, M.J. and Perales, A. (1995). Immunoprophylaxis of caprine contagious agalactia due to Mycoplasma agalactiae with an inactivated vaccine. *Veterinary Record* 137: 266-9.

Manual of Standards for Diagnostic Tests and Vaccines, OIE (2004). (5^{th} edn.). Pp: 1-14.

Nicholas, R.A.J. (1996). Contagious agalactia an update. In: *Mycoplasmas of ruminants:* pathogenicity, diagnostics, epidemiology and molecular genetics. EUR 16934, COST, *European Communities Official Publications Office.* Pp:

60-62. Luxembourg.

Perez, I., Solanes, M., Alvarez, J., Algar, J., Marco, J. and Feced, J. (1990). *Proceedings of the 15th jomadas de la sociedad espanola de ovinotecnia y caprinotecnia cordeba.* In: A. Vera. M. Herrera Zaragoza (Eds.), *Advances en Alimentacion y Mejora Animal.* Pp : 151.

Rigalla, J. (1987). Contagious agalactia and other mycoplasmal diseases of small ruminants. M. Lambert, G. Jones (Ed.), *Commission of the European Community*. Pp: 7. Luxembourg.

Rodwell, A.W. and Whitcomb, R.F. (1983). Methods for direct and indirect measurement of Mycoplasma growth In: *Razin S and Tully J. editors.* Methods in Mycoplasmology I**.** Pp: 185-196. Academic Press, New York.

Sarris, K. (1996). *Contagious agalactia*. In: Frey and Sarris, K.: Mycoplasma of ruminants: pathogenicity, diagnostics, epidemiology and molecular genetics. EUR16934, *Cost, European Commission, European Communities Official Publications Office.* Pp: 12-15. Luxembourg.

Sarris, K.Z., drakas, A., Papasteriadis. A., and Papadopoules, O. (1989). Experimental contagious agalactia vaccine. *Bulletin of Helenic Veterinary Medicine Society* 40: 71-4.

Sotoodehnia, A., Aerabi, I. (1986). Agalactia disease and its geographical distribution in sheep and goats in Iran. *Archives of Razi Institute* 37: 75-78.

Tola, S. (1999). Experimental vaccination against Mycoplasma agalactiae using different inactivated vaccine. Veterinary Microbiology 17:2764-2768.

Tukaslan J. (1990). Control of important mycoplasma diseases in Turkey with special emphasis on CCPP and contagious agalactia. *IOM Letter* 1: 184-185.