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Short Communication

Preparation of *agalactia* vaccine in fermentor

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ABSTRACT

Mycoplasma agalactia was cultured in PPLO broth medium supplemented with sterilized normal horse serum in fermentor. Cultivation process continued at 37 ºC under aerobic conditions and growth was terminated after 26 hours cultured period with 2.2×10^9 *Mycoplasma* per ml. Samples were taken at different stages of growth. Culture was inactivated with 0.04 %*v/v* formaldehyde and the vaccine was adjuvanted with saponin with final concentration of 1/1000 *w/v*. Results indicated that the yield of *Mycoplasma* was higher than the vaccine produced in large flasks using the same culture media. The produced vaccine in the fermentor was safe in guinea pig, sheep and goat according to OIE instructions. Based on the present findings, *Mycoplasma agalatia* can easily grow in fermentor for the purpose of vaccine production instead of its conventional production in large flasks.

***Keywords*:** *Mycoplasma agalactia*, Vaccine, Ferementor

INTRODUCTION

Contagious *agalactia* is a highly infectious disease of sheep and goats. The major causal agent in both sheep and goats is Mycoplasma *agalactia*. The disease can also be caused by Mycoplasma *mycoides subsp. Mycoides LC*, Mycoplasma *capriculum subsp. Capriculum* and Mycoplasma *putrifaciens* (Nicholas 1996, Bergonier *et al* 1997). The disease is usually manifested by mastitis, arthritis and keratoconjunctivitis (Damassa 1983). Contagious *agalactia* occurs in most of the countries with intensive production of sheep and goats, i.e. in the Mmediterranean regions, Africa and Western Asia (Erdag 1989, Belaid *et al* 1990, Sarris 1996, Bergonier 1997).

The vaccination strategy against contagious *agalactia* is based on the use of live and inactivated vaccines. For prevention of contagious *agalactia*, no single vaccine has been universally adapted, and no standard methods of preparation and evaluation of the vaccines has been applied (OIE 2004, Erdag, 1989).

Contagious *agalactia* has been known for a long time in small ruminants in Iran. The first isolation of Mycoplasma *agalactia* in Iran has been reported by Entessar in 1959. Later on, an inactivated vaccine composed of three local isolates was prepared in

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Razi Vaccine and Serum Research Institute (RVSRI) and used in limited area of Iran with successfully (Baharsefat *et al* 1971). Status of the disease in the country has been described by Sotoodehnia & Aarabi 1986. In the present study, agalactia vaccine was prepared in fermentor and the prepared vaccine was evaluated in guinea pig, sheep Mediterranean regions, Africa and Western Asia (Erdag 1989, Belaid *et al* 1990, Sarris 1996, Bergonier 1997). The vaccination strategy against contagious *agalactia* is based on the use of live and inactivated vaccines. For prevention of contagious *agalactia*, no single vaccine has been universally adapted, and no standard methods of preparation and evaluation of the vaccines have been applied (OIE 2004, Erdag 1989).

Contagious *agalactia* has been known for a long time in small ruminants in Iran. The first isolation of Mycoplasma *agalactia* in Iran has been reported by Entessar in 1959. Later on, an inactivated vaccine composed of three local isolates was prepared in RVSRI and used in limited area of Iran with successfully (Baharsefat *et al* 1971). Status of the disease in the country has been described by Sotoodehnia & Aarabi 1986. In the present study, agalactia vaccine was prepared in fermentor and the prepared vaccine was evaluated in guinea pig, sheep and goats according to OIE (2004). At the present time this vaccine is prepared using PPLO broth medium in 20 lit glass flasks.

Due to difficulties in handling of the flasks and also dangers of breakage of flasks for persons, limitation of production of vaccine in flasks, and yearly increasing demands for this vaccine we decided to design program to produce it in fermentor in large volumes.

MATERIALS AND METHODS

Lyophilised Mycoplasma *agalactia* in skim milk was used for seed culture. This strain had already been isolated from sheep milk and identified as Mycoplasma *agalactia* (Aarabi & Sotoodehnia, 1984), and was stored in lyophylised form in skim milk in specialized ampoules. A nine liter seed culture was prepared with PPLO broth medium (21 g/lit) and supplemented with 10% (*v/v*) sterilized normal horse serum and 10 g/lit yeast extract. Culture was incubated at 37 °C for 24 hours with constant agitation. After checking for purity, the seed was inoculated into fermentor containing 380 liters of the similar proportion of above mentioned media which was sterilized at 110 °C for 15 min, the pH was set at 7.2 then 10% (*v/v*) sterilized normal horse serum was added to the sterilized media in the fermentor. The culture was run in fermentor at 37 °C with constant agitation (100 rpm) and pH changes were recorded during the culture process. During growth of the bacteria in fermentor the pH of the culture was adjusted by adding 20% NaOH automatically. Samples were collected within the period of culture growth at zero time (1/2 hour after adding seed culture), 10, 18, 22, 24 and 26 hours after starting the growth in fermentor. Viable mycoplasma number was determined according to standard procedure (Rodwell *et al* 1983). Subsequently, the culture of mycoplasma was inactivated with 0.4% (*v/v*) formaldehyde and stored at 4°C. The vaccine was eventually adjuvanted with 1/1000 (*w/v*) saponin. Sterility test was carried out by conventional methods according to OIE (2004). Safety test was performed on guinea pig, sheep and goat. For this purpose according to standard protocol 2 doses of the vaccine inoculated subcutaneously into each of two animals and the animals observed for two weeks for any abnormal reactions or death.

RESULTS AND DISCUSSION

The growth curve of Mycoplasma in fermentor is shown in Figure 1. During the first ten hours of culture in fermentor, growth was slowly progressive, and then log phase along with turbidity of the culture medium was started and continued up to hour 26,

i.e., the highest point of culture curve. Then as the growth rate was become slower, the culture curve was gradually declined (hour 30). The maximum viable organisms was obtained 2.2×10^9/ml. Aerobic conditions were provided by sterile air flow (10 m^3/ hour) in fermentor and pH changes of the culture media were little between 0.2-0.3 during growth process. Viable mycoplasma cells in seed culture were 500×10^6/ml when added into the culture media in fermentor.

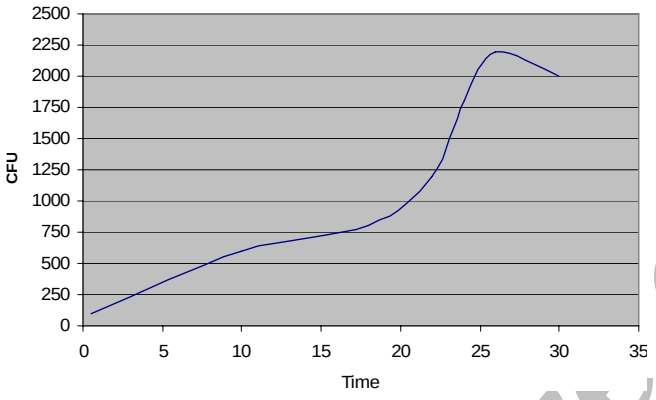

Figure 1. Growth culture of Mycoplasma *agalactia* in fermentor.

Because suitable conditions such as pH and air flow can not be provided for growth of mycoplasma in flasks, using the same culture media as in fermentor, so the growth of bacteria is very low and variable at different time and usually is between as low as 100×10^6 up to 1.2×10^9 bacteria/ml after 48 hours. While the rate of growth of the bacteria in fermentor in shorter time is too much higher.

According to the requirements for inactivated agalactia vaccine, formaldehyde as an inactivator, adjuvant such as saponin, and a high titer of mycoplasma preparation (10^8-10^{10} CFU/ml) are generally recommended for sheep and goats (OIE 2004).

In the present study, *agalactia* vaccine large production was scaled up and standardize in fermentor. Our conclusions indicated that fermentor system would provide a high yield of mycoplasma under the scheduled conditions. The results of safety test were good in guinea pigs, sheep and goats without any abnormal reactions. Study on induced antibodies against this vaccine has already been reported (Sotoodehnia *et al* 2005). Results of ELISA showed a maximum titer of antibodies in the stage of one month post vaccination. In this study the duration of antibody were determined up to five month in groups of sheep and up to four month in groups of goats while they have received two doses of inactivated vaccine one month apart. Vaccination against contagious agalactia is being done in sheep and goats in Iran since 1971. During the past years, annually demands of inactivated vaccine have been gradually increased up to 15 million doses at present time. This inactivated vaccine made in RVSRI has been routinely produced by conventional method in large glass flasks. Due to limited capacity for production and other disadvantages, this technique can be easily substituted by fermentor system which allows us for large production.

References

Baharsefat, M., Yamini, M. and Ahurai, 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. *Scientific and Technical Review* 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.

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 agalactia for sheep and goats in Turkey, *proceeding of international symposium on Mycoplasma*, Pp: 20-22. Theiler.

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

Nicolas, R.A.J. (1996). Contagious agalactia: an update, In: *Mycoplasma of ruminants: Pathogenicity, Diagnostics, Epidemiology and Molecular genetics.* Pp: 60-62. European Communities Official Publications Office, EUR 16934, COST, Luxembourg,

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

Sarris, K. (1996). Contagious *agalactia*. In: Frey and K. Sarris (Eds.), *Mycoplasma of ruminants: Pathgenicity, Diagnostics, Epidemiology and Molecular Genetics.* Pp: 12-15. European Communities Official Publications Office, EUR 16934, COST, Luxembourg.

Sotoodehnia, A. and Aarabi, I. (1986). Agalactia disease and its geographical distribution in sheep and goats in Iran. *Archives of Razi Institute* 36/37: 75-78.

Sotoodehnia, A., Moazeni-Jula, G. Madani, R., Golchinfar, F. and Naserirad, A. (2005). Determination of antibody responses against inactivated agalactia vaccine in small ruminants. *Archives of Razi Institute* 60: 67-75.