African Journal of Microbiology Research Vol. 6(12) pp. 3085-3087, 30 March, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.232
ISSN 1996-0808 ©2012 Academic Journals

Short Communication

An experimental live vaccine trial against contagious caprine pleuropneumonia

Solomon Tarekegn[1], Wudu Temesgen[2], Sefinew Alemu[2]* and Gelagay Ayelet[3]

[1]Ministry of Agriculture, Oromiya National Regional State, Ethiopia.
[2]Department of Clinical Medicine and Epidemiology, Faculty of Veterinary Medicine, University of Gondar, P. O. Box, 196, Gondar, Ethiopia.
[3]National Veterinary Institute, P.O. Box 19, Debre Zeit, Ethiopia.

Accepted 29 February, 2012

Live attenuated *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) vaccine prepared from local isolate at the National Veterinary Institute (NVI), Ethiopia, was injected to 56 contagious caprine pleuropneumonia unexposed goats with the aim of determining level of seroconversion and safety compared to a killed commercial vaccine, produced by the NVI. The 56 goats were randomly allocated into three groups vaccinated with: candidate live vaccine (n=19) and Mccp killed vaccine (positive control) (n=19), and negative control (n=18). Antibody was detected by compliment fixation test (CFT). The candidate live vaccine induced seropositivity in higher proportion of inoculated goats than that of the killed vaccine in use. Moreover, the seroconversion occurred slightly earlier and for slightly prolonged period compared to the killed vaccine. Increase in body temperature was recorded in 4 of 19 (21.1%) goats inoculated by the killed vaccine. Back to seronegativity was rapid in both types of vaccines. In conclusion, absence of any post vaccination reaction, early appearance and longer persistence of antibodies made the live candidate Mccp vaccine better than the killed vaccine. However, further experimental and field trial of the vaccine is suggested and reversion to the attenuated virus to virulent form and carrier state should be considered.

Key words: Contagious caprine pleuropneumonia (CCPP), goats, seroconversion, vaccine.

INTRODUCTION

Contagious caprine pleuropneumonia (CCPP) is a severe contagious disease of goats caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) (OIE, 2004). CCPP is economically important disease of worldwide distribution (Radostits et al., 2007) and a major cause of loss of goats in many countries of Africa and Asia (OIE, 1996). CCPP has been reported from almost all regions of Ethiopia and is a special problem of goats in pastoral areas of the country including Afar, Borana, Omo Valley, and in the lowlands of Tigray where there is large population of goats (Yigezu et al., 2004; Ayelet et al., 2006, Hadush et al., 2009). Mccp was isolated from the lungs of necropsied goats in an outbreak of respiratory disease complicated with *Mannhemia haemolytica* in goats and sheep in Afar (Shiferaw et al., 2006).

* Corresponding author. E-mail: sefiale@yahoo.com.

At field level, CCPP can be controlled by application of treatment, controlling animal movement, slaughtering infected animals and vaccination (Mare, 2004). Generally, CCPP is refractory to commonly used antibiotics and therapy is unavailable or too expensive for many poor farmers which make this option increasingly less successful. Eradication of CCPP can be best achieved by the slaughter of affected and in contact animals, but is not always practical, especially in developing countries like Ethiopia. Both live attenuated and inactivated F_{38} vaccines have been tested with varying degrees of success. However, inactivated vaccine is both difficult and expensive to implement on a large scale, and immunity is generally short-lived (Nicholas, 2002). In Ethiopia, killed vaccine prepared from Mccp F_{38} strain by Ethiopian National Veterinary Institute (NVI) is extensively used; however, this vaccine causes fever and local reaction at the site of inoculation. Therefore, new vaccines which can generate

better immunity, safe and can provide effective prophylactic measures and cheap for farmers is necessary. Therefore, this work was carried out to test a live CCPP vaccine obtained from local isolate of Mccp and study its ability of seroconversion and safety in comparison to the available killed vaccine produced by the NVI.

MATERIALS AND METHODS

Experimental animals

Experimental study was conducted from October, 2008 to May, 2009 at the NVI, Ethiopia. Fifty six goats older than six months and of approximately equal age which had no previous exposure to CCPP and negative for Mccp specific antibodies were used for the experiment. The goats, purchased from central highland of Ethiopia, were treated by Albendazole and oxytetracycline and left for three weeks for adaptation.

Preparation of test and control vaccines

The live candidate vaccine was prepared from local isolate of Mccp obtained after ten passages *in vitro* culture of the bacteria in Hyaflik medium. Killed vaccine, commercially produced by NVI which was prepared from the Kenyan isolate of Mccp was used as a positive control. Eighty hours old culture of Mccp in Hyaflik medium were collected by centrifugation at 15,000 rpm for 20 min, inactivated by saponin and washed three times with sterile phosphate buffer saline (PBS). Protein determination was done by Buretic and Bradford methods using bovine serum albumin as a standard according to the description of OIE (2000).

Experimental protocol

The fifty six goats were randomly assigned in to three groups: first group (n=19) inoculated with a dose of 10^5 Mccp candidate live vaccine, second group (n=19) vaccinated with a dose of 0.15 mg of *Mycoplasma* protein of Kenyan isolate of Mccp killed vaccine and the last group (n=18) was left for control. Except the negative control group, all goats were shaved at sites of inoculation, right thoracic wall, to appreciate post inoculation reactions and both groups inoculated subcutaneously. Finally, all groups of the goats were strictly monitored for any post-vaccination reactions and seroconversion.

Assessment of post vaccination reaction was made by measuring rectal temperature, examination of typical signs of CCPP and localized skin reactions. Rectal temperature were taken twice daily for 9 consecutive days of post vaccination by clinical thermometer. Occurrence of localized skin reactions (swellings) at the sites of inoculation and typical signs of CCPP like nasal discharge, coughing and other respiratory signs were monitored throughout the study period.

About 5-7ml of blood samples were collected from each of the fifty six goats once per week at days 1, 7, 14, 21, 28, 35, 42 and 49 of post vaccination. Collected blood samples were allowed to stand for 3-6 h at room temperature to enhance clotting and sera was further separated by centrifugation at 3000 rpm for 3 min to extract clear serum (OIE, 1992; Thiaucourt et al., 1994). The sera were then transferred into sterile tubes and stored in deep freezer at -20°C until processed in the NVI serology laboratory. Standard CFT was used to determine the amount of antibodies against Mccp according to the technique described by OIE (1992) . More than 50% haemolysis and antibodies detected at 1:20 titration were considered positive (OIE, 2000).

RESULTS

Post vaccination side reactions

Four of the 19 goats (21.1%) vaccinated by the killed vaccine showed slight raise in rectal temperature readings beyond 40°C which were measured at day one, day three, day five and day six of post inoculation. Rectal temperature readings were in normal ranges in all other goats in the experiment. There was no other post vaccination reactions observed in any of the experimental goats of all the three groups in this study.

Seroconversion

Sixteen of 19 (84.2%) goats were seropositive for Mccp antibodies after inoculated by the live vaccine; whereas 13 of 19 (68.4%) goats were seropositive from the positive control. But all goats in the negative control group remained seronegative for Mccp antibodies (Table 1).

Higher level of seropositivity was observed in the live vaccine inoculated group of goats. The higher proportion of seropositive goats in the live vaccine inoculated group (84.2%) was not statistically significant difference (*P>0.05*) compared to that of the killed vaccine inoculated group (68.4%). In both groups, higher level of seropositivity was observed at 14, 21, 28 and 35th days of post vaccination. The number of seropositive goats declined after 28th days of post vaccination and only one goat from each of the inoculated groups remained seropositive at the last day of post vaccination (Table 1).

DISCUSSION

Fever was observed in 4 of 19 (21.1%) goats inoculated by the killed vaccine; whereas no fever was observed in goats injected with live vaccine and in goats kept for negative control. The result in this study was in agreement with the work of Ayelet et al. (2007) who reported the presence of fever and other post vaccination side reactions following vaccination of goats with killed Mccp vaccine. The slight increase in rectal temperature in this and the previous studies can be partly associated with the adjuvant used in the vaccine. According to Hirsh et al. (2004) and OIE (2004), one of the main problems of killed vaccine is the effect of adjuvant used in the vaccine. Absence of any post vaccination reaction in goats inoculated by the live vaccine might be associated with the level of attenuation; which was enough to reduce virulence of the Mccp organisms. Rise in rectal body temperature observed as post vaccination side reactions in killed vaccine inoculated group of goats.

Relatively higher number of goats, 16 of 19 (84.2%), seroconvert from the live vaccine inoculated group than the number of goats, 13 of 19 (68.4%), in the killed

Table 1. Number of seropositive goats and pattern of seroconversion within 7 weeks of post inoculation by live and killed vaccines.

Group of goats	No. of goats	No. of seropositive goats in the seven weeks of post vaccination							Seropositive goats	Seropositive samples
		I	II	III	IV	V	VI	VII		
Live	19	4	6	6	8	7	2	1	16 (84.2)*	34 (25.6)
Killed	19	2	4	7	9	3	0	1	13 (68.4)	26 (19.5)
Control	18	0	0	0	0	0	0	0	-	-
Total	56	6	10	13	17	10	2	2	29 (51.8)	60 (15.3)

*numbers in parenthesis indicate percentages of the total.

vaccine inoculated group; however, the difference between the reactors was not statistically significant. The difference in the number of goats that seroconverted in the two types of vaccines can be associated with of amount antigenic protein in the vaccine which might be lower in the case of killed vaccine. High level of seroconversion was detected between 14 and 35th days of post vaccination; which is in agreement with the work of Ayelet et al. (2007) who reported high level of sero-conversion percent inhibition in between 20 and 40 days after vaccination of goats by killed Mccp vaccine.

The number of seropositive goats declined after 28th day of post vaccination; sero-converting back to negative for Mccp antibody in both inoculated groups was observed. However, antibodies were detected slightly earlier and took a bit longer to decline in case of the live vaccine, and hence gave protection for longer time compared to the killed vaccine. However, it is a fact that the antibody level of a certain antigens will decline in the absence of further challenge (Thrusfield 2005); therefore, it cannot warrant to leaving out the need for booster vaccination.

In conclusion, absence of any post vaccination reaction, early appearance and longer persistence of antibodies made the live candidate Mccp vaccine better than the killed vaccine. However, further experimental and field trial of the vaccine is suggested and reversion of the attenuated virus to virulent form and carrier state should be considered.

ACKNOWLEDGEMENT

We appreciate The National Veterinary Institute (NVI), Ethiopia, for allowing us to work this experimental study and the staff of NVI who directly or indirectly contributed in this work.

REFERENCES

Ayelet G, Laekemariam Y, Aschalew Z, Esayas G, Kassahun A (2007). Validation of immunity induced by inactivated CCPP vaccine with different adjuvants. Small Ruminant Res., 73: 200-205.

Ayelet G, Teshale S, Amsalu W, Esayas G (2006). Prevalence of contagious caprine pleuropneumonia in the Borena pastoral areas of Ethiopia. Small Ruminant Res., 97: 131-135.

Hadush B, Eshetu L, Mengistu W, Hailesilassie M (2009). Seroprevalence of contagious caprine pleuropneumonia in Kefta Humera, Alamata (Tigray) and Aba-'ala (Afar), Northern Ethiopia. Trop. Animal Health Prod., 41:803-806

Hirsh DC, Maclachlan NJ, Walker RL (2004). Veterinary Microbiology. 2nd ed. India: Black well, pp. 48-53, 240-249.

Mare' CJ (2004). Contagious caprine pleuropneumonia. In: Foreign animals disease veterinary Science/ Microbiology. University of Arizone, Tueson, AZ 85721: http: /WWW. Phsoulce. US/ph/ palm/ PDA/ com. Available at <file://A:/CCPP. htm accessed date 14/06/04. p. 1-7 Nicholas RAJ (2002). Improvements in the diagnosis and control of diseases of small ruminants caused by mycoplasmas. Small Ruminant Research. 45: 145–149.

Radostitis OM, Gay CC, Hinchclff KW, Constablle PD (2007). Veterinary Medicine: A Text book of the disease of Cattle, Horses, Sheep, Pig and Goats. 10th ed. Spain: Saunders Elsevier, pp. 1123-1156.

Shiferaw G, Tariku S, Ayelet G, Abebe Z (2006). Contagious caprine pleuropneumonia and Mannheimia haemolytica-associated acute respiratory disease of goats and sheep in Afar Region, Ethiopia. Rev. Sci. tech. Off. intl. Epiz., 25: 1153-1163.

Thiaucourt F, Bolske G, Liball G, Legoft C, Lefever PC (1994). The use of Monoclonal Antibodies in the Diagnosis of CCPP. Vet. microbiol., 44: 191-203.

Thrusfield M (1995). Veterinary epidemiology. 2nd ed. WB. Saunders., philaadelphia, p. 219.

World Organisation for Animal Health (OIE) (1992). *Contagious Caprine pleuro Pneumonia and Pestedes Petitis Rruminants*: In diagnostic manual. 1st ed. Reudeproney, Paris, France, pp.128-130.

World Organisation for Animal Health (OIE) (1996). World Organization for animal health: manual of standards for diagnostic tests and Vaccine. 3rd ed. Pairs, France, pp. 374-383.

World Organisation for Animal Health (OIE) (2000). *Manual of Standards for Diagnostic Tests and Vaccines. Contagious Caprine Pleuropneumonia*, 4th ed. Pairs France, pp. 503–514.

World Organisation for Animal Health (OIE) (2004). Contagious caprine pleuropneumonia. Chapter 2.4.6. *In* Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 5th Ed. OIE, Paris, pp. 623-635.

Yigezu L, Tariku S, Ayelet G, Roger F (2004). Respiratory mycoplasmosis of small ruminants in Ethiopia. Ethiop. vet. J., 8: 67-74.