

Full Length Research Paper

Seroprevalence of contagious caprine pleuropneumonia and field performance of inactivated vaccine in Borana pastoral area, southern Ethiopia

Matios Lakew¹, Tesfaye Sisay², Gelagay Ayelet³, Eyob Eshetu⁴, Gebremikael Dawit⁵ and Tadele Tolosa^{6,7*}

¹Goal Ethiopia, Afar Region, Gulina District, Ethiopia.

²Institute of Biotechnology, Addis Ababa University, Ethiopia.

³National Veterinary Institute (NVI), P.O. Box 19, Debre Zeit, Ethiopia.

⁴Yabello Regional Veterinary Laboratory, Oromia Regional State, Yabello, Ethiopia.

⁵Department of Animal Science and Ecotourism, Aksum University, Aksum, Ethiopia.

⁶Department of Veterinary Public Health and Veterinary Microbiology, College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia.

⁷M-team and Mastitis and Milk Quality Research Unit, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820, Merelbeke, Belgium.

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This study was conducted between September 2012 and May 2013 in three Districts of Borana pastoral area to determine seroprevalence of contagious caprine pleuropneumonia (CCPP) and assess field performance of inactivated commercial vaccine, produced by the National Veterinary Institute (NVI) in Ethiopia, against CCPP. Both pre and post vaccination sera samples were tested using competitive enzyme linked immunosorbent assay (cELISA). Out of 510 examined sera, 161 samples were positive for CCPP, giving an overall seroprevalence of 31.6% (95% CI = 27.57-35.64%) in the study area. Seroprevalence of 35.2, 35.1 and 25% were recorded in Arero, Dhas and Yabello districts, respectively. However, there was no significant difference ($\chi^2=5.56$, $P=0.062$) in seropositivity among the three districts and between male and female goats ($\chi^2=0.068$, $P=0.794$) examined in this study. On the other hand, the differences in seroprevalence among the age categories were statistically significant ($\chi^2=24.48$, $p<0.0001$). A rise in antibody (seroconversion) was observed after field vaccination of goats with inactivated CCPP vaccine and a total of 253 of the 414 examined sera samples were positive for *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*) specific antibodies, thus 61.1% of goats seroconverted following vaccination. Comparison of *Mccp* specific antibodies in the goat population before and after vaccination indicated that the number of positive reactors increased significantly ($P<0.0001$) following CCPP vaccination. Seropositivity following vaccination was analyzed among the three age groups and statistically significant differences ($\chi^2=45.48$, $P<0.0001$) were recorded. The change in the serum antibody after vaccination was found to be higher in younger and adult aged goats than old aged goats. In conclusion, the present study indicates that CCPP is one of the major goat health problems in Borana pastoral area which warrants appropriate measures to be in place towards the prevention and control of the disease. Moreover, field vaccination of goats by inactivated CCPP vaccine induced seropositivity in majority of the inoculated goats. Future controlled experimental studies with challenge infection after vaccination need to be conducted for further evaluation of the vaccine efficacy.

Key words: Borana pastoral area, contagious caprine pleuropneumonia (CCPP), goats, inactivated vaccine, seroconversion, seroprevalence.

INTRODUCTION

Ethiopia possesses an estimated 22.6 million heads of goats (CSA, 2012). Although goats represent a great national resource, their productivity is sub-optimal. Among the several factors that hamper the productivity of this animal, diseases take a lion share. Contagious caprine pleuropneumonia (CCPP) is one of the most important infectious diseases of goats that pose a significant threat to production capacities of this animal. It is a highly contagious and severe respiratory disease caused by *Mycoplasma capricolum* subspecies *capripneumoniae* (*Mccp*) (OIE, 2008).

The presence of CCPP in Ethiopia had been suspected since 1983 and was confirmed later in 1990 by isolation and identification of *Mccp* following an outbreak of CCPP in Ogaden, Eastern Ethiopia (Thiaucourt et al., 1992). Since then the disease has been known to be endemic in different regions of the country (Sharew et al., 2005). Outbreaks of CCPP have been reported from almost all regions of the country, especially from the lowland areas, which are known goat-rearing regions (APHRD, 2010). The frequently reported outbreaks of CCPP in Ethiopia almost certainly represent an underestimate as this disease is having a major socio-economic impact in the country (Nicholas and Churchward, 2012).

Borana rangeland area is among the known pastoral areas of Ethiopia and possesses huge livestock resource. Goats being an important component of livestock play a significant role in supporting the pastoralist's livelihood in the area (CARE-Ethiopia, 2009). Despite the high population density of goats estimated at 849,261 (CSA, 2012) in the Borana pastoral area, little attention has been given to the health problems of goats. To date there has not been sufficient study on CCPP in the area; hence there is dearth of well-documented information on the current status and precise distribution of the disease in the area.

CCPP is a highly contagious disease; hence control of the disease is one of the priority areas of the country. Like most African nations, vaccination remains the most cost effective strategy to control animal disease in Ethiopia. To realize success in vaccination campaigns, use of effective vaccine is crucial (AU-IBAR, 2013). From past pilot experiments conducted in Kenya, inactivated CCPP vaccines consisting of saponized organisms have been shown to be protective but the quality and efficacy may be variable (Rurangirwa et al., 1987a, b). Vaccine against CCPP is currently produced by the National Veterinary Institute (NVI) in Ethiopia, which is inactivated *Mccp* vaccine with saponin as an adjuvant. This vaccine is extensively used for the control of the disease in endemic areas of the country (APHRD, 2010).

Experimental trials conducted in the country have demonstrated that immunization of goats with inactivated *Mccp* (F-38 Kenyan strain) in adjuvant confers protection against a contact challenge (Ayelet et al., 2007). However, the efficacy of inactivated *Mccp* vaccine under field conditions has not been clearly documented.

The current study was conducted in Borana pastoral area, the largest pastoral area in Ethiopia, to determine the seroprevalence of CCPP and assess field performance of the currently available inactivated vaccine against the disease, regarding its seroconversion level following vaccination of goats in Borana pastoral area.

MATERIALS AND METHODS

Description of the study area

The study was conducted between September 2012 and May 2013 in three districts (Yabello, Arero and Dhas) of Borana pastoral area, Southern range lands of Ethiopia. Borana zone is under Oromia regional state and comprises of mainly pastoral areas and seldom agro-pastoral areas. Yabello, Arero and Dhas districts are among the pastoral areas and located at distance of 570 to 665 km from Addis Ababa, the capital city of Ethiopia.

Study animals

The study animals were local Borana goat breeds (Long-eared Somali breeds) managed under extensive pastoral production system by the Borana pastoralists (Gizaw, 2009). Goats with no history of vaccination for CCPP and above 6 months of age were used as source of sera samples for the study. Goats of both sex and various age groups were sampled. Age of the animals was determined based on owners information and dental eruption; accordingly, animals were categorized into three age groups: > 6 months and ≤ 2 years (young), >2 years and ≤5 years (adult) and >5 years (old) (Bekele et al., 2011).

Study design

A cross-sectional survey was carried out on goats in three districts of Borana Zone to determine seroprevalence of CCPP and assess post-vaccinal antibody response of inactivated CCPP vaccine at field level. Blood samples were collected twice before vaccination and three weeks post vaccination from jugular vein of individual goats. Sera samples were tested using competitive enzyme linked immunosorbent assay (cELISA) according to the standard test procedure at the National Veterinary Institute (NVI) of Ethiopia.

Sample size and sampling method

The three study districts (Yabello, Arero and Dhas) were selected purposively. Pastoral associations and villages (locally known as

*Corresponding author. E-mail: tadeletolosa@yahoo.com. Tel: +251 (917) 804070.

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Olla) under each district were considered as primary and secondary sampling units used for random sampling, respectively. Pastoral associations (PAs) were selected randomly from each district after obtaining the total number of PAs in the districts. Subsequently 3 PAs were selected from each district and a total of 9 PAs (one village from each PA) were considered for the present study. The total number of samples in each district and PAs were allocated proportionally.

Systematic random sampling method was employed for sampling of individual animals. Animals below six months of age were excluded from sampling. Animals were selected systematically the first animal being selected randomly from the flock then at an interval until the required number of sample size in that village was attained; this was done early in the morning before animals were released for grazing. During sampling, history of goats whether they were the member of the flock or not were asked and a newly recruited goats replaced by existing flocks to avoid the risk of including vaccinated animals.

The sample size was calculated according to the formula given by Thrusfield (2007) by considering 20% expected prevalence in the area (Gelagay et al., 2007), using 95% confidence interval at 5% absolute precision. Thus, the calculated sample size was 246. In order to increase precision, the sample size was doubled to get a total of 510 pre-vaccination blood samples. For the field assessment of inactivated CCPV vaccine, it was possible to collect post-vaccination blood samples from 414 previously sampled (pre-vaccinal blood sample) and vaccinated goats. However, from previously sampled animals, 96 goats were missed during the second round sample collection (post-vaccinal sample collection).

Study methodology

Blood sample collection

Blood samples were collected twice from individual goats, that is before vaccination and three weeks post vaccination. The separated sera were stored in deep freezer at -20°C at Yabello Regional Veterinary Laboratory until processed at the NVI serology laboratory. Transportation to the National Veterinary Institute (NVI) laboratory was done by placing packed samples in an ice box containing preformed blocks of ice.

Vaccination

Inactivated CCPV vaccine, commercially produced by NVI (Ethiopia) from F-38 Kenyan strain of *Mccp*, was used to vaccinate goats in Borana pastoral area. After shaking gently, 1 ml/goat was injected subcutaneously under the loose skin at the neck region using automatic vaccination syringe. To follow rise in antibody (seroconversion level), 5-7 ml of blood sample was collected 3 weeks post-vaccination (at 21th day). Due to difficult working conditions in the pastoral area, it was not possible to follow up any post vaccination reactions and repetitive collection of blood samples from individual goats after vaccination was not possible too.

Serological test

Collected sera samples were examined for the presence of specific antibodies against *Mccp* by using cELISA in serology laboratory of the National Veterinary Institute (Ethiopia). The cELISA test was employed using *Mccp* antibody test kit and it was obtained from CIRAD-Montpellier, France. Similar studies have used cELISA kit to detect antibodies that appear after an infection or after an immunization with a relevant CCPV vaccine (Peyraud et al., 2014).

Thus, the same kit was used to determine the disease prevalence and to measure antibodies following vaccination.

The 924 test sera (both pre and post vaccination sera samples) were examined in twelve 96-well flat bottom microplates, according to the test protocol supplied with the kit. Test samples and controls were pre-diluted on the preplate (uncoated). Samples to be tested were premixed with a specific monoclonal anti-*Mccp* antibody (Mab 4.52) in a preplate (uncoated) and homogenized contents of the preplate were transferred into the *Mccp* antigen coated microplate. The contents were incubated for 1 h at 37°C with a gentle agitation, washed two times, dried and then an anti-mouse IgG enzyme conjugate was dispensed and incubated for 30 min. Then, after three times washing enzyme substrate was added to be incubated for 20 min. Finally, stop solution was added and color development was observed and read at 450 nm by ELISA reader to determine the optical density and percentage of inhibition was calculated. For the assay to be valid, results of internal quality control sera were first checked to make sure they are within the acceptable ranges. Those samples with percentage of inhibition greater than or equal to 50% are considered positive for presence of *Mccp* antibodies.

Data analysis

All collected data were entered into Microsoft Office Excel 2007 computer program and then summarized first by using a descriptive statistics. All statistical analyses were performed using Statistical Package for Social Science (SPSS)-Version 20. Seroprevalence of CCPV and post-vaccination seroconversion percent was calculated as the proportion of the number of cELISA positive animals to the total number of tested animals expressed in percent. Chi-square test was used to assess association of the disease with districts, age group and sex. Paired-sample t-test was used to analyse if there is difference between pre and post vaccination antibody titer (paired scores). A P-value less than 0.05 at 95% confidence interval was considered for significance.

RESULTS

Seroprevalence

Out of 510 examined sera, 161 samples were positive for *Mccp* specific antibodies using cELISA. An overall seroprevalence of 31.6% (95% CI= 27.57-35.64%) was observed in the study area (Table 1). The seroprevalence was highest in Arero district (35.2%) followed by Dhas (35.1%) and the lowest seroprevalence was recorded in Yabello (25%). There was no significant difference ($\chi^2=5.56$, $P=0.06$) in CCPV seroprevalence among the three districts. Analysis of the seroprevalence of CCPV with respect to sex showed no significant difference ($\chi^2=0.068$, $P=0.79$) between male and female goats (Table 2).

The result of age groups were compared for seropositivity of CCPV as indicated in Table 3. Seroprevalence of 26.4, 25.8 and 50.4% were recorded in young, adult age and old age categories, respectively.

The differences in seroprevalence among the age categories were significant ($\chi^2=24.48$, $p<0.0001$). Old age category has showed significantly higher seroprevalence as compared to the young and adult age category. The odds of the disease in old age category was 2.83 (95%

Table 1. Seroprevalence of CCPP in the three districts of Borana pastoral area, Ethiopia.

District	Sample tested	Sample positive	Seroprevalence (%)	95% Confidence interval
Yabello	180	45	25	18.67-31.33
Dhas	168	59	35.1	27.88-42.32
Arero	162	57	35.2	27.85-42.55
Total	510	161	31.6	27.57-35.64

Table 2. Seroprevalence of CCPP with respect to sex in Borana pastoral area, Ethiopia.

Sex	Sample tested	Sample positive	Seroprevalence (%)	95% Confidence Interval	OR (95% CI)
Male	172	53	30.8	23.9-37.7	1
Female	338	108	32	27.03-36.97	1.05 (0.71-1.57)
Total	510	161	31.6	27.57-35.64	

Table 3. Seroprevalence of CCPP in different age groups in Borana pastoral area, Ethiopia.

Age	Sample tested	Sample positive	Seroprevalence (%) (95% CI)	P-value	OR (95% CI)
Adult	221	57	25.8 (20.03-31.57)		1
Young	174	46	26.4 (19.85-32.95)	0.885	1.03 (0.658-1.625)
Old	115	58	50.4 (41.26-59.54)	0.000	2.93 (1.82-4.70)
Total	510	161	31.6 (27.57-35.64)		

CI=1.72-4.65) and 2.93 (95% CI=1.82-4.70) times to occur as compared to the young and adult age categories, respectively.

Post vaccination seroconversion

A rise in antibody (seroconversion level) was assessed after vaccination of goats with inactivated CCPP vaccine. Paired sera samples (pre- and post-vaccination) were collected from 414 vaccinated goats. The serum antibody level was monitored three weeks (at 21th day) post vaccination; thus, out of 414 examined serum samples, 253 were positive for *Mccp* specific antibodies after vaccination using cELISA.

Thus seroconversion was observed in 61.1% of the vaccinated goats of various age groups and both sexes in the three districts of Borena zone (Table 4). Comparison of *Mccp* specific antibody levels in the goat population before and after vaccination indicated that the number of positive reactors increased significantly ($P < 0.0001$) following CCPP vaccination. Moreover, paired sample t-test analysis indicated that there was statistically significant difference between pre and post vaccination antibody level based on the mean percentage of inhibition ($t = 9.82$, $P < 0.0001$) (Table 5).

When considering only those goats which were negative for *Mccp* specific antibodies in the pre-vaccination sera, a total of 132 (48.4%) of the 273 examined sera samples were positive after vaccination. However, sera samples collected from 141 (51.6%) vaccinated goats remained seronegative for *Mccp* specific antibodies. Seropositivity following vaccination was analyzed among the three age groups and statistically significant differences ($\chi^2 = 45.48$, $P < 0.0001$) were recorded (Table 6). The change in the serum antibody after vaccination was found to be higher in younger goats (61.45%) followed by adult age goats (56.6%) and the lowest seropositivity following vaccination were recorded among old age goats (8.8%). Analysis of serological response following vaccination among goats in the three districts as well as between sexes showed no significant differences.

DISCUSSION

In Ethiopia, goat rearing carries tremendous importance in house hold economy, especially in pastoral areas where goats are raised in large number (Hirpa and Abebe, 2008). CCPP is one of the most important infectious diseases of goats that pose a significant threat

Table 4. *Mccp* specific antibody levels in the goat population before and after vaccination.

Seroconversion level by	Category	No. of goats from which paired sera samples collected	No. of seropositive goats pre-vaccination (%)	No. of seropositive goats post-vaccination (%)
District	Yabello	163	43 (26.4)	107 (65.6)
	Dhas	120	43 (35.8)	71 (59.2)
	Arero	131	55 (42)	75 (57.3)
Sex	Female	264	94 (35.6)	166 (62.9)
	Male	150	47 (31.3)	87 (58)
Age	Young	139	43 (30.9)	94 (67.6)
	Adult	167	47 (28.1)	115 (68.9)
	Old	108	51 (47.2)	44 (40.7)
Overall		414	141 (34.1)	253 (61.1)

Table 5. Comparison of pre and post vaccination antibody level based on mean percentage of inhibition.

Antibody level during	Sample tested	Mean percentage of inhibition	Paired differences	t-score	P-value
Pre vaccination	414	46.54			
Post vaccination	414	57.2	10.67	9.82	0.000

Table 6. Seroconversion following field vaccination among goats that were negative for *Mccp* specific antibodies before vaccination.

Seroconversion level among age groups	No. of seronegative goats for <i>Mccp</i> specific antibodies pre-vaccination	No. of seropositive goats post vaccination	Seroconversion (%)	P-value
Young	96	59	61.45	0.000
Adult	120	68	56.66	0.000
Old	57	5	8.8	
Overall	273	132	48.4	

to production capacities of this animal. The first objective of the present study was to determine the prevalence of contagious caprine pleuropneumonia in Borana pastoral area.

The overall seroprevalence of CCPP in the study areas was 31.6% (95% CI= 27.57-35.64%). The finding in the present study was in line with reports of Hadush et al. (2009) 32.68% in Tigray and Afar, Sharew et al. (2005) 29% in Wollo and Eshetu et al. (2007) 31% in an export abattoir from goats that had been collected from Borana, Afar, Bale and Jinka. Similarly, Sherif et al. (2012), Mohammed (2008) and Ingle et al. (2008) had reported seroprevalence of 32.63% in selected districts of Jijiga Zone, 32% in Eastern Ethiopia and 33.67% in Nagpur District of Vidarbha region of India, respectively.

The overall prevalence of CCPP in the present study was higher than some of previous studies which were conducted in Southern part of Ethiopia. A relatively lower seroprevalence of 18.61% in South Omo and Arbaminch

areas (Mekuria and Asmare, 2010), 15.5% in Hammer and Benna-Tsema (Mekuria et al., 2008), 20.12% in Borana pastoral area (Gelagay et al., 2007) and 13.2% in Borana and Guji lowlands (Bekele et al., 2011) had been reported. Moreover, Eshete (2006) had also reported seroprevalence of 19.19% using cELISA in Afar pastoral area. However, the prevalence recorded in this study was lower than that of Sharew et al. (2005) who reported prevalence rates ranging from 52 to 100% using CFT and B-ELISA in outbreak samples from the lowland districts of the country.

The variation in the seroprevalence of CCPP reported from different studies may be as a result of the temporal and spatial factors associated with sampling, the situation of the disease during the time of sampling and the variation in the specificity and sensitivity of the different serological tests employed.

The difference in seroprevalence between the three districts was not statistically significant (P=0.062). This

may be associated with the non restricted animal movement between this neighboring districts and the highly contagious nature of *Mccp* infection, as well as similar climatic factors in the districts. Since pastoralism is the mainstay of livelihood in the study districts, there has been regular mixing of flocks at watering points and communal grazing areas, which is likely to spread the infection between flocks. This may be explained by the fact that the infection needs proximity to source of infection (Thiaucourt and Bolske, 1996).

Sex of the animal was not associated with seropositivity in this observation. This result was agreeable with the observations made by Eshete (2006), Mekuria and Asmare (2010), Bekele et al. (2011), Yousuf et al. (2012) and Sherif et al. (2012) in studies conducted in different parts of Ethiopia. It has also been reported that CCPP is highly contagious and fatal disease affecting susceptible goats of both sexes (OIE, 2008).

Old age category has showed significantly higher seroprevalence as compared to the young and adult age category ($p < 0.0001$). Similarly, significant variation among age groups was also reported by Mekuria and Asmare (2010), Regassa et al. (2010), Yousuf et al. (2012) and Sherif et al. (2012). In all these studies, it was reported that the prevalence of the disease was significantly higher in old age than young animals. The higher prevalence of the disease in old age goats as compared to the young and adult ages might be explained by the fact that as age increases, the goats are often repeatedly exposed to different stress conditions (due to malnutrition, movement over long distances, adverse weather conditions and the likes) which can predispose the animal to the disease. Advancing age in goats, as with other species, is eventually associated with a decline in body condition as well as an increase in susceptibility to infections. Moreover, they also tend to be infected repeatedly. Therefore, the probability to be seropositive in older ages for CCPP would be high as compared to young and adult goats. However, there was also a report that suggested absence of age factor in CCPP epidemiology (Eshetu et al., 2007; Hadush et al., 2009). Perhaps this assumption needs further investigation.

With the next objective of the present study, some observations were done on field performance of the inactivated CCPP vaccine after vaccination of goats in Borana pastoral area. A rise in antibody was assessed and seroconversion was observed in 61.1% of the vaccinated goats. Comparison of *Mccp* specific antibodies in the goat population before and after vaccination indicated that the number of positive reactors increased significantly following CCPP vaccination. Thus, the inactivated *Mccp* vaccine induced seropositivity in majority of the inoculated goats. The finding in the present study was in agreement with that of Tarekegn et al. (2012) who reported seroconversion in 68.4% of vaccinated goats using inactivated CCPP vaccine produced by NVI (Ethiopia). Seroconversion indicates that sufficient

Mccp antigen and adjuvant were present in the vaccine to induce the proper response. Similar finding was reported by Peyraud et al. (2014) where goats vaccinated at CIRAD with a batch of the reference vaccine displayed rapid marked seroconversion, that was detectable after as little as one week.

While considering only those goats which were negative for *Mccp* specific antibodies in the pre-vaccination sera 48.8% goats seroconverted following vaccination. However, sera samples collected from 51.6% vaccinated goats remained seronegative for *Mccp* specific antibodies 3 weeks post-vaccination. In the present study, blood samples were collected only once after vaccination hence those goats that possibly became seropositive after the third week of vaccination may have been missed from the result. On the other hand, Peyraud et al. (2014) have reported that vaccine quality directly affected the intensity and duration of seroconversion. From their study, lower antigen content and the use of smaller amounts of adjuvant resulted in weaker responses. Thus, quality control is particularly important for vaccines, as the industrial product used is generated on a large scale and may behave differently from the original laboratory product for which efficacy was demonstrated (Rurangirwa et al., 1987a, b).

In addition, the management situation at the time of vaccination (before and after vaccination) is important for a very good immune response (antibody production). Vaccines should be administered at times of low stress and several weeks prior to expected changes in management that may increase stress or exposure to infectious agents. Good nutrition, both in protein and energy as well as trace minerals and vitamins is required for an adequate immune response (Rashid et al., 2009). With the present field trial, study animals were owned and managed by several pastoralists in the area; hence, vaccinated goats were not managed under uniform and controlled field environment. Thus, several factors like poor nutrition, movement over long distances, high environmental temperatures and immunosuppressive concurrent diseases may have interfered with ability of the animal to mount a good immune response following field vaccination.

The differences in post-vaccinal seroconversion among the age categories were statistically significant. Significantly lower number of positive reactors was recorded among old age goats when compared with young and adult age goats. This observed variation in vaccine responses of the old aged goats might be due to individual genetic variations, health status and other external factors. In addition, several studies have indicated that age-related changes in the immune system may hamper successful vaccination and currently development of vaccination strategies that are effective in all age groups is an important area of research (Weinberger et al., 2008).

Vaccination programs are designed to protect an animal

from infection; however, depending upon the age and health of the animal, vaccination may not stimulate a protective humoral response. It is possible that, as in the human being and other animals, old aged goats may be less responsive than their younger counterparts to current vaccination protocols. In human being, several studies have reported that ageing of the immune system (immunosenescence) contributes to the increased susceptibility of the elderly to infectious disease and to the poor outcome of vaccination (Pawelec, 2007). Vaccination could protect them against several infectious diseases, but it can be effective only if cells that are capable of responding are still present in the repertoire. Recent vaccination strategies in the elderly might achieve low effectiveness due to age-related immune impairment. Immunosenescence affects both the innate and adaptive immunity (Ongradi and Kovessdi, 2010).

It is now becoming apparent that the immune system undergoes age-associated alterations, which accumulate to produce a progressive deterioration in the ability to respond to infections and to develop immunity after vaccination (Weinberger et al., 2008). While these changes have been extensively documented in humans and mouse models, little is known regarding the effect of ageing on the immune response to vaccination in the goat population. Vaccination remains to be the most cost-effective measure for preventing and reducing the severity of infectious diseases like CCPP in the goat population. Past trials have demonstrated that immunization of goats with inactivated CCPP vaccine confers protective immunity; however, all of those trials used approximately equal aged goats for their studies (Rurangirwa et al., 1991; Ayelet et al., 2007; Tarekegn et al., 2012). Hence, further investigation is needed regarding the effectiveness of currently used CCPP vaccine in all (various) age groups of goat population.

In conclusion, serological findings indicated that CCPP is one of the major goat health problems in Borana pastoral area. Therefore, appropriate disease prevention and control strategies should be designed and implemented to mitigate the disease impact.

Field vaccination of goats by inactivated *Mccp* vaccine induced seropositivity (seroconversion) in majority of the inoculated goats. However, it is difficult to establish a correlation between this level and protection. Hence, it would be valuable to conduct CCPP challenge experiment, first under controlled laboratory condition, and then under field level.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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