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ORIGINAL RESEARCH

Seroprevalence of contagious bovine pleuropneumonia at export quarantine centers in and around Adama, Ethiopia

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Abstract A cross sectional study was undertaken from October 2010 to March 2011 to determine the seroprevalence of contagious bovine pleuropneumonia (CBPP) and its related risk factors in export quarantine centers. A total of 3,111 cattle sera were collected from different export quarantine farms located in and around Adama, namely, Bekero, Jogo, Kedir, and Dera farms, and tested for the presence of *Mycoplasma mycoides* subsp. *mycoides* small colonies antibody using competitive enzyme-linked immunosorbant assay. Of the total 3,111 cattle sera examined, 124 (4 %) were found positive for CBPP. Among the potential predisposing factors assessed, origin, transportation condition, confinement level, and stay time of the animals in quarantine center were not found significantly ($P>0.05$) associated with the occurrence of the disease. Whereas age was found significantly ($P<0.05$) associated with the occurrence of the disease in which a high seroprevalence was recorded in aged (9.5 %) animals than young (3 %). Generally, this study showed that CBPP is a threat for Ethiopian livestock export market and a well established disease in Borana and Bale areas, where the animals originated.

Keywords Adama · c-ELISA · CBPP · *Mycoplasma mycoides* subsp. *mycoides* small colonies · Seroprevalence · Quarantine centers

Introduction

Contagious bovine pleuropneumonia (CBPP) is a disease of cattle caused by *Mycoplasma mycoides* subsp. *mycoides* small colonies (MmmSC) (OIE 2008). The disease affects the respiratory tract of cattle and characterized by fever, anorexia, dyspnea, polypnea, cough and nasal discharge (Schnee et al. 2011; Schubert et al. 2011). Macropathological examinations show gross lesions in the lung including marbling, severe fibrinous exudative pleuropneumonia, and thickened interlobular septa and sequestra (Schnee et al. 2011; Sacchini et al. 2012). However, clinical and pathological signs are not always evident, and chronically infected animals might act as carriers and sources of infections (Schnee et al. 2011). CBPP is an OIE-notifiable disease and was included among the former List "A" diseases (Tambi et al. 2006; Schubert et al. 2011). It is a prominent cattle disease in Africa, where outbreaks of the disease reported from 20 countries in 2006, with the highest number of cases in Ethiopia, Angola and Cameroon (Nicholas et al. 2008). CBPP has been eradicated in Australia, Europe, Asia and America through the application of restrictions to the movement of cattle, as well as test and slaughter policies combined with compensation for livestock keepers. Such policies are difficult to apply in most African countries because of pastoralism, lack of economical resources, and fragmented veterinary services (Neiman et al. 2009; Sacchini et al. 2012). As a result, the disease remains endemic in Africa particularly in tropical and subtropical regions (West, Central, East, and parts of Southern Africa) of the continent (Amanfu 2009; Neiman et al. 2009). The disease has serious implications for food security and peoples' livelihoods in affected countries (Amanfu 2009).

Contagious bovine pleuropneumonia transmission occurs from direct and repeated contacts between sick and healthy animals. The principal route of infection is by the inhalation of infective droplets from active or carrier cases of the disease. Outbreaks tend to be more extensive in housed animals and in those in transit by train and on foot (Radostits et al. 2007). Factors such as extremes of age, stress, and concurrent infections may predispose to tissue invasion (Thomson 2005).

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Contagious bovine pleuropneumonia is considered to be a disease of economic importance because of its high mortality rate, production loss, increased production cost due to cost of disease control, loss of weight and working ability, delayed marketing, reduced fertility, loss due to quarantine, loss of cattle trade, and reduced investment in livestock production (Tambi et al. 2006; Radostits et al. 2007). In addition to these, it leads to in imposition of rigorous limitation to international trade on CBPP-affected countries in accordance with World Organization of Animal Health (OIE) regulation (Muuka et al. 2011; Sacchini et al. 2012). The financial and economic loss caused by the disease in Africa is significant. Otte et al. (2004) reported that the continent has lost approximately 2 billion US$ per year due to death of livestock from the disease.

Ethiopia is a tropical African country in which mobile pastoralism is dominant in the arid and semi-arid areas in the eastern, northeastern, and southeastern parts of the country (Tegegne et al. 2009). This practice facilitates the transmission of the disease from one herd or area to another and the establishment of the disease in the country (Ezanno and Lesnoff 2009). Currently, CBPP is one of the most important cattle diseases and impediments to livestock development in Ethiopia (MOA 2003; Amanfu 2009). Studies undertaken on CBPP so far revealed the existence of the disease in different parts of the country with prevalence that vary from 4.3 % in Jijiga (Gedlu 2004) to 96 % in Western Gojjam (Yigezu and Roger 1997). CBPP has been causing significant economic losses on the agricultural sector and the national economy. It accounts for a loss of over 205.6 million Ethiopian birr per year (Laval 1999). Thus, over the last decades, the country has lost a substantial market share and foreign exchange earnings due to frequent bans by the Middle East countries (Belachew and Jemberu 2003). Although the disease brings such a high economic loss in the livestock industry, there is not enough information and research works regarding its predisposing factors and prevalence in export quarantine centers as a priority disease in the country. Therefore, the objectives of this study were to determine the seroprevalence of CBPP in export bulls under different export quarantine centers and identify the potential risk factors associated with CBPP occurrence.

Materials and methods

Study area

The study was conducted at four export quarantine farms of beef animals located in Adama and its surroundings. Adama city is located in the Oromia Regional State, 99 kms East of Addis Ababa. The average annual temperature is about 21 °C, with altitudes ranging from 1,600 to 1,700 m above sea level and average rainfall of 760 mm.

Study animals

The study animals were local breed bulls that are quarantined and ready to be exported. Bulls of any age groups and no previous history of vaccination against CBPP were included in the sample population. A total of 2,203 and 908 bulls originating from Borana and Bale, respectively, were included in the study from the four export quarantine farms. In all farms, the animals were well-managed with good watering and feeding, and have similar management pattern among the farms.

Study design

A cross sectional study was conducted from October 2010 to March 2011 to determine the seroprevalence of CBPP on export bulls under different export quarantine farms, namely, Bekero, Jogo, Kedir, and Dera farms by using c-ELISA. Data on the potential risk factors associated with the occurrence of CBPP were collected by using recording formats. Information regarding the origin of the animal, vaccination status, stay time in quarantine center, transportation condition, duration of transportation, management system of the quarantine center, confinement level of the animals in the quarantine center, and other conditions related to the quarantine center management system were recorded and assessed. Age of the animals was estimated based on De Launata and Habel (1986) dental table, and the animals were grouped into three age categories: ≤4, 4–7, and ≥7 years. To evaluate the degree of confinement, the area of the quarantine centers was measured in square meters (m^2) and divided for the total number of animals on each farm. Blood samples were collected when the animals were ready for export, and the test for CBPP using c-ELISA was performed at National Veterinary Institute.

Sampling and testing procedure

Sampling procedure

It is a requirement that all animals being exported to Arabian countries are tested for CBPP; therefore, all bulls being exported were considered for sampling. About 5 ml of blood sample was collected from the jugular vein of each animal by using plain vacutainer tube. The collected blood samples were labeled and transported to the National Veterinary Institute as soon as possible and then allowed to clot by placing them overnight at room temperature. The decanted sera were pipetted into labeled sterile tubes and stored at −20 °C until tested.

Serological test procedure

Sera were tested using the CBPP c-ELISA (OIE 2008) following the manufacturer's instructions. For economic reasons, the tests were interpreted as negative for results below 45 % and positive above 45 %.

Data management and statistical analysis

The data collected during sampling and laboratory findings were entered and stored in MS-excel. Stata 11 software package was used to perform the statistical analysis. Contagious bovine pleuropneumonia prevalence was calculated as percentage by dividing the number of animals positive for *MmmSC (*positive using c-ELISA) to the total animals sampled. Pearson chi-square (χ^2) and Fisher's exact tests were employed to assess the existence of association between occurrence of CBPP and different potential risk factors considered in the study. For this analysis, P values <0.05 were considered significant whereas P values >0.05 considered non significant.

Results

From the 3,111 cattles tested, 124 (4 %) were seropositive to CBPP. The highest prevalence of CBPP was recorded in Bekero export quarantine farm (4.7 %) and the lowest in Kedir (2.5 %). However, there was no statistically significant (P>0.05) difference in the occurrence of CBPP among the four farms.

Risk factors including transportation condition, confinement in the quarantine center, origin of the animal, age, and stay time in quarantine center were assessed, and the result was as indicated here in Table 1. Among the three age categories, the highest CBPP prevalence was recorded in age group ≥7 years (9.5 %) followed by age group 4–7 years (5.2 %), and age group ≤4 years (3 %), and the difference was found statistically significant (P<0.05) (Table 1).

The animals that stayed in the export quarantine center for 1 month had a higher prevalence (4.7 %) than those that stayed longer (Table 1). However, the difference was not statistically significant (P>0.05).

As the animals transported from 1 to 2 days, they were exposed for different transportation conditions such as high environmental temperature, 48 h transportation without feed, rainy conditions for 3 h, and normal environmental temperature. Of these transportation conditions, high prevalence (4.4 %) was recorded in those animals that have been transported in rainy environment (Table 1). Origin wise, higher prevalence (4.1 %) was found in animals originated from Borana and the lower from Bale (3.7 %) (Table 1). However, there was no statistically significant (P>0.05) difference in the occurrence of CBPP among the transportation conditions and between the two animal origins.

The confinement level were found to be 2, 2.5, and 2.8 m^2/animal for Bekero and Jogo farms, Dera farm, and Kedir farm, respectively. Relatively high seroprevalence

Table 1 Seroprevalence of CBPP in export bulls with respect to different possible risk factors

Factors	Classification	No. of samples, no. of positive samples (in bracket), and seroprevalence as % from each farm				
		Bekero	Dera	Jogo	Kedir	Total
Age	≤4 years	606 (28) 4.6	210 (6) 2.9	629 (14) 2.2	307 (4) 1.3	1752 (52) 3
	4-7 years	349 (17) 4.9	393 (16) 4.1	495 (29) 5.9	80 (6) 7.5	1317 (68) 5.2
	≥7 years	0 (0) –	10 (2) 20	26 (2) 7.7	6 (0) 0	42 (4) 9.5
Confinement	2 m^2/animal	955 (45) 4.2	0 (0) –	1150 (45) 3.9	0 (0) –	2105 (90) 4.3
	2.5 m^2/animal	0 (0) –	613 (24) 3.9	0 (0) –	0 (0) –	613 (24) 3.9
	2.8 m^2/animal	0 (0) –	0 (0) –	0 (0) –	393 (10) 2.5	393 (10) 2.5
Transportation condition	High env'tal. T°	0 (0) –	0 (00 –	632 (26) 4.1	0 (0) –	632 (26) 4.1
	48 h transportation	955 (45) 4.7	0 (0) –	0 (0) –	393 (10) 2.5	1348 (55) 4.1
	Normal env'tal. T°	0 (0) –	429 (16) 3.7	518 (19) 3.7	0 (0) –	947 (35) 3.7
	Rainy for 3 h	0 (0) –	184 (8) 4.4	0 (0) –	0 (0) –	184 (8) 4.4
Stay time in quarantine center	1 month	0 (0) –	0 (0) –	506 (24) 4.7	0 (0) –	506 (24) 4.7
	1.5 months	0 (0) –	0 (0) –	644 (21) 3.3	0 (0) –	644 (21) 3.3
	3 months	955 (45) 4.7	0 (0) –	0 (0) –	393 (10) 2.5	1348(55) 4.1
	3.5 months	0 (0) –	613 (24) 3.9	0 (0) –	0 (0) –	613 (24) 3.9
Origin	Bale	0 (0) –	184 (8) 4.4	519 (20) 3.9	205 (6) 2.9	908 (34) 3.7
	Borana	955 (45) 4.7	429 (16) 3.7	631 (25) 4	188 (4) 2.1	2203 (90) 4.1

env'tal. T° environmental temperature

(4.3 %) was observed in highly confined animals (i.e., 2 m^2/animal), but there was no statistically significant ($P>0.05$) difference among the confinement levels (Table 1).

Discussion

In this study, a total of 3,111serum samples were collected from four export quarantine farms and tested with c-ELISA for CBPP antibody. Of these, 124 (4 %) were found positive for CBPP. This finding is in agreement with that of Gedlu (2004), who reported 4.3 % in Jijiga. It is also in line with the prevalence of CBPP across Africa which varies from 2.8 % in Kenya (Wanyoike 1999) to 0.01 % in Nigeria (Alawa et al. 2011). However, the result of this study is not in agreement with that of Niwael (2009), who reported 11 % seropositivity from Loita and Mara herds, in the Maasai ecosystem of South Western Kenya; and Takele (1998) who reported 17.3 % seropositivity in the Awi and West Gojjam Zones of Amhara Region. The prevalence discrepancy observed between the current and the previous studies might be due to the variations that exist in the epidemiology of the disease as well as the cattle production system in the study areas. Furthermore, the animals brought to the quarantine centers were purchased based on their good body and health condition; the probability of including severely affected and recently recovered animals from the disease would be low, so this might be one of the reasons that make the overall prevalence of this study low.

Seroprevalence of 4.1 and 3.7 % was obtained from unvaccinated bulls originated from Borana and Bale range land, respectively. However, there was no statistically significant ($P>0.05$) difference in the occurrence of the disease between animal origins. The current finding is low when compared with the report of Yigezu and Roger (1997) and Ahmed (2004), who reported a seroprevalence of 74 and 12 % in Borana rangeland, respectively. This shows that the prevalence of the disease is decreasing progressively from time to time and this might be due to CBPP control measures exercised in the area. Even if the prevalence of the disease in these areas is low, due to the practice of pastoral and agro-pastoral production system in the areas, there might be a potential danger of spreading the disease to the other cattle production areas. It is a well-understood fact that cattle movement is responsible for the transmission of CBPP from one herd, region, or country to another (Thomson 2005; Amanfu 2009; Ezanno and Lesnoff 2009).

Results of the current study revealed that there is a significant difference ($P<0.05$) among the age groups in the occurrence of the disease, in which high prevalence was recorded from age group ≥7 years. In contrast to Gedlu (2004), who reported no significant difference of CBPP occurrence in different age groups, we determined that there was a significant difference, which is in line with the claim that CBPP is a disease of older animals (Andrews et al. 2004). This might be due to long time exposure and life span of the older animals than the younger ones and the persistency of sequestrum for a long period of time in CBPP recovered animals.

Despite the fact that the results of this study are supported by findings in the literature, this study has its own limitation. This study used only c-ELISA test to categorize the export cattle as CBPP seropositive and negative. It is well understood that c-ELISA is more sensitive in detecting cattle with chronic stage than any other test, and it is more prone to miss individual animals at the early stage of infection (Muuka et al. 2011; Schubert et al. 2011). Since there is no single serological test which detects all stages of CBPP infection (Muuka et al. 2011; Schubert et al. 2011), use of an additional test could be considered in future studies.

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Ethical standards We declare that the undertaking of this study was complied with the current laws of Ethiopia in which it has been undertaken.

Conflict of interest We declare that we have no conflict of interest.

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