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Investigation of Contagious Bovine Pleuropneumonia Vaccination in Khartoum State, Sudan 2016-2017

Omer, E. A. D.[1]and Elsemaih, N.M.E[1], Hamid, F.M.A[1], Tageldeen, M.T.I[2] and Mohammed, G.E[2]

1. Animal Resources Research Corporation, Central Veterinary Research Laboratory.
2. College Of Veterinary Medicine Sudan University Of Science &Technology

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ABSTRACT

This study was aimed to determine the impact of CBPP vaccination as a risk factor in the prevalence of CBPP in Khartoum state. Cross-sectional study was conducted from November 2016 to May 2017 in various pastoral areas in Khartoum state assessed firstly using well designed questionnaire from the animal owners and the pastoralists. A total of 386 sera were examined for the incidence of specific antibodies against *Mycoplasma mycoides* subsp. *mycoides* small colony (*Mmm*SC), using a competitive enzyme-linked immunosorbent assay (c.ELISA). The result showed From 300 non vaccinated samples tested by c.ELISA 46% were positive& 54% were negative while the 86 vaccinated sample 43% were positive & 57% were negative result. This obliges the implementation of appropriate vaccination programme and control measures to reduce the economic losses associated with CBPP.

INTRODUCTION:

Contagious bovine pleuropneumonia (CBPP) is a disease of ruminants (Bos and Bubalus genuses) caused by *Mycoplasma mycoides subsp. mycoides SC* (MmmSC; SC = small colony). It is manifested by anorexia, fever and respiratory signs such as dyspnoea, polypnoea, cough and nasal discharges in bovines. Diagnosis requires the isolation of the aetiological agent. The main problems for control or eradication are the frequent occurrence of sub acute or subclinical infections, the persistence of chronic carriers after the clinical phase and the lack of extensive vaccine coverage. **(**OIE terrestrial manual 2014).Since the beginning of the 20th century, many vaccines against CBPP have been described (e.g. killed vaccines, and heterologous vaccines), but none of them has proven to be really satisfactory or cost effective. Various attenuated *Mmm*SC strains have been used in the past and have been neglected, such as the KH3J or V5 strains. Two strains are now used for preparing CBPP vaccines: strain T1/44, a naturally mild strain isolated

in 1951 by Sheriff& Piercy in Tanzania (Sheriff & Piercy, 1952), and strain T1sr (Wesonga & Thiaucourt, 2000; Yaya *et al*., 1999). The 44th egg-passage of strain T1 called T1/44, was sufficiently attenuated to protect cattle without post-vaccinal severe reactions, however such reactions may still occur in the field although rarely. Their frequency is unpredictable. Cattle breeds should be assessed for their sensitivity before mass vaccination. It should be noted that when given by intubation, the vaccine can produce CBPP lesions (Mbulu *et al*., 2004); however, as the vaccine is to be injected subcutaneously, this should not create a serious disease problem (Hubschle *et al*., 2002). T1/44 and T1sr vaccines can effectively protect herds when vaccinations are regularly performed; once a year for T1/44 and twice a year for T1sr. They can be used for CBPP control on a wider scale; national or regional, but they cannot lead to CBPP eradication when used alone.

In Sudan vaccination against CPBP has been practiced since 1944 by using infected pleural exudates from sick animal and inserted in under the skin of the tail tip but this method was not safe to animal it causes severe swelling. Broth culture vaccine by using F strain which isolated in1944 from Darfur province used for vaccine production in Sudan (Abdalla 1975). It is used till 1970. At present, only one *Mmm*SC attenuated strain is produced in Sudan for CBPP vaccination; T1/44 Prior to 2005 the vaccine produced was in broth culture form but this type has some limitations (Daleel, 1970). Alternative of that practical way is the use of T1/44 live attenuated freeze dried CBPP vaccine was used (FAO, 2005).

The master seed used for vaccine production should be as close as possible to the original vaccinal strains. Grand parental stocks of these strains are kept at the OIE Reference Laboratory in France and at the OIE Collaborating Centre for Quality Control of Veterinary Vaccines in Ethiopia. It is recommended to cultivate the master seed in a suitable medium that does not contain any preservative such as antibiotics so as to allow it to be shown that the master seed stock is pure. The freedom from extraneous agents should be tested according to international or national guidelines. Strain T1/44 has a known residual virulence that may vary according to local conditions, Post-vaccinal reactions are characterised by a localised inflammatory reaction that develops at the site of injection (Willems‟ reaction). It may be noticed as early as 1 week post-injection. In many cases this local reaction will wane naturally but in some instances it may become extensive and lead to the death of the animal if no suitable antibiotic treatment is administered. Storage temperature shall be indicated for the freeze-dried product and also for the final product once reconstituted in the appropriate diluents (OIE manual 2014). For all above this study was conducted to assess the influence of the CBPP vaccination programme in Khartoum state on the CBPP incidence.

MATERIALS AND METHODS:

Description of the study area: The study was carried out in Khartoum state which is the Capital of Sudan bordered North East by River Nile state, North West, by Northern state, East by South Eastern state of Kassala and Gadaref and Gezera states. It is located between 31 to 34 east longitudes and 15 to 16 latitudes north, Area of Khartoum State estimated as 22.736 square kilometres, and an average elevation of 1352 feet above sea level. Khartoum state composed of seven localities (Khartoum,

Omdurman, Jebelawlia, Kararie, Umbada, Bahry and Sharg Elnile). The livestock sector (the resident animals) was estimated at about 1.321.852 according to census 2017.

Investigation of (CBPP) vaccination had been conducted as an important risk factor for CBPP in Khartoum state in four projects including West soba, Alrudwan, Mahlab 2 and Saig project which are distributed in the three localities of the state, from two major animal markets including Almwelih and Abu-zaid market, experimental calves samples taken from Central Veterinary Research Laboratory and the reminder of samples taken from traditional farms.

Study population: The study population comprised cattle of both local and cross-breeds in the study area. consisted of number of areas which are associated cattle populations in the selected regions. The sampling methods were based on the areas activities, markets, projects, traditional farms experimental laboratory farm.

Study design &questionnaire data: A cross-sectional study was carried out in Khartoum State.

Vaccination data taken from owners and pastoralist follow investigation of CBPP data that mentioned in FAO Animal Health Manual (2002).

Sampling frame and sample size determination: The sampling frame Since the previous prevalence of the disease in the region was 20, 9% and a 5% absolute level of precision was considered to calculate the number of animals to be sampled **(**Thrusfield 1995) as follows:

$$N = \frac{1.96^2 \times 0.209(1-0.209)}{0.05^2} 100 = 246$$

This equation reflect sample size that should be taken based on the previously founded prevalence of CBPP (20.9%) on a study of Ibrahim (2015) But 386 sample was taken because there were no strong evidence of vaccinated number of cattle in relation to the incidence of CPBP in the population area of the study the equation was calculated by 50% expected prevalence and a 5% absolute level of precision was considered to calculate the number of animals to be sampled (Thrusfield 1995) as follows:

$$N = 1.96^2 \times P_{exp}(1-P_{exp}) \times d^2$$

N = required sample size; = expected prevalence; d = desired absolute precision.

$$N = \frac{1.96^2 \times 0.5(1-0.5)}{0.05^2} 100 = 384$$

Sample collection: A total of 386 serum samples were collected randomly from dairy farms and cattle herds in Khartoum State(between November 2016 – May 2017) from different areas(west soba project, Omdurman abuzaid market, Saig project, Mahlab 2 eastern Nile province, Omdurman Alrudwan project , Omdurman Almwelih market , Nifasha, Alhalfaia and Eid babeker. For collection of serum, 10 ml of blood samples were collected in sterile plain vacationers. Samples were left for 1h at room temperature then kept overnight in refrigerator at 4ºc. Samples were then centrifuged at 3000 rpm for 10 min. The separated serum was aspirated with sterile pipette, transferred into sterile containers and stored at -20ºc till used. Parallel to each sample, the sample code, age, breed, vaccination, revaccination and sex of every animal were recorded.

Serological testing: c.ELISA was conducted as recommended by (IDEXX, Institute Pourquier, Montpellier, France) was used. It is

based on a monoclonal anti-MmmSC antibody named Mab 177/5 as previously described (LeGoff & Thiaucourt 1998). The specificity of the diagnostic test used was, respectively, 99.7%. Serum samples were diluted in dilution buffer in un coated plates mixed with specific monoclonal antibody (Mab 117/5) then transferred into the *Mmm*SC-coated microplate and incubated with gentle agitation for 37 °C for 1 h, After washing, anti-mouse IgG serum–conjugated horse radish peroxidase was added then incubated in for 37 °C for 1/2 h . After a series of washings, the horse radish peroxidase substrate (TMB) was added after 20 minutes forming a blue composite that turned yellow when the reaction was stopped. The optical density was read in an ELISA reader at 450 nM (StantFax 630 USA) and the cut-off point was calculated to validate the results. All sera with percentage inhibition (PI) > 50% were considered positive. Sera with PI between 40% and 50% were considered doubtful and those sera with PI less than 40% were negative.

RESULTS:

The overall vaccinated cattle against CBPP in the study area were 22.3% (86 cattle) the reminder were nonvaccinated 77.7% (300 cattle).From 300 non vaccinated samples tested by c.ELISA 46% were positive. Where 54% were negative result; since the 86 vaccinated sample 43% were positive and 57% were negative result (Table 1).

Table (1): Incidence of anti-bodies (Abs) presence against *Mmm*SC concerning the status of vaccination

	N	Percentage	+ve%	-ve%
Non vaccinated	300	77.7%	46%	54%
Vaccinated	86	22.3%	43%	57%

From the 386 samples 309 (80.1%) were taken; their last vaccination history fall on the period of time from 6 to 12 month, 51 (13.2%) samples their last vaccination history fall on the period of time more than 12 month. 26 samples which represent 6.10% their last vaccination history fall on the period of time less than 6 month .With regard to the samples that have been taken from the cattle that were vaccinated for more than one year 70.6% were positive and 29.4% were negative. By observe to the samples that taken from the cattle that were vaccinated for more than 6 month and less than one year 38.5% were positive and 61.5% were negative. With view to the samples that taken from the cattle that were vaccinated for less than 6 month (recently vaccinated) 76.9% were positive and 23.1% were negative (Table 2).

Table (2): incidence of Abs presence against *Mmm*SC concerning history of last vaccination

Vaccination history	N	Percentage	+ve%	-ve%
More than 12 month	51	13.2%	70.6%	29.4%
6- 12 month	309	80.1%	38.5%	61.5%
Less than 6 month	26	6.7%	76.9%	23.1%

From all samples 230(59.6%) samples were taken; rate of animal vaccinated number in their areas was less than 25% , 24 (6.2%) samples the rate of animal vaccinated number was 25% in their areas , 17 (4.4%) samples the rate of animal vaccinated number in their areas was 75% and 115 (29.8%) samples the rate of animal vaccinated ratio was 100%. among the samples

that have been taken from the cattle that the animal vaccinated number percent was 100% the positive percentage 41% and 59% were negative, With regard to the samples that taken from the cattle that the animal vaccinated number percent was 75% were 35% were positive and 65% were negative, With regard to the samples that taken from the cattle that the animal vaccinated number percent were 25% where positive and negative in same percentage 50% (Table 3).

Table (3): incidence of Abs presence against *Mmm*SC concerning number of vaccinated animal

Animal vaccinated number	Number	Percentage	+ve%	-ve%
100%	115	29.8%	41%	59%
75%	17	4.4%	35%	56%
25%	24	6.2%	50%	50%
Less than 25%	230	59.6%	47.8%	52.2%

Out of 386 samples 335 samples were follow vaccination program with quality assure team 41.8% of them were positive and 58.2% of them were negative; from 51 samples which were not follow vaccination program with quality assure team 68.6% of them were positive and 31.4%% of them were negative (Table 4).

Table (4): incidence of Abs presence against *Mmm*SC concerning the vacciner who done vaccination

Vacciner	N	Percentage	+ve%	-ve%
Quality assured team	335	86.8%	41.8%	58.2%
Other	51	13.2%	68.6%	31.4%

DISCUSSION:

In this investigation a total of 386 serum samples were tested from traditional farms, three projects, two markets and 26 from experimental cattle in CVRL in Khartoum state. The overall CBPP associated with vaccination seroprevalence observed in this study was 45.3% by using c.ELISA test and it was high than previous study reported that prevalence of CBPP in Khartoum state was 20,9% (Ibrahim Osman. 2015).

In this study The overall vaccinated cattle against CBPP in the study area was 22.3% and 77.7% was nonvaccinated this low rate result ensure the suggestion mentioned in previous study which found prevalence associated with CBPP vaccination (25%) compared with animals did not vaccinated (14.58%) suggested that the owner did not vaccinated their animals regularly (Ibtisam Elsadig, 2012).

From the vaccinated sample 43% was positive and 57% was negative result this was not agree with in previous study which found prevalence associated CBPP vaccination (25%) compared with animals did not vaccinated (14.58%) (Ibtisam Elsadig,2012) but the high of ratio in all scale that may refer to the immune responses measured by c- ELISA following vaccination diminish following three months, The other cause may be due to missing in use of vaccination process like insufficient dosage which given to animals or due to bad storage or transportation and fail in dissolve of vaccine and this result conformity with previous study in Kenya (Niwael.J.2009) also with this study which found high association

with individual who do vaccination. we found sample which were follow vaccination program with quality assure team 41.8% of them were positive ELISA and 58.2% of them were negative compare with samples were not follow vaccination program with quality assure team 68.6% of them were positive ELISA and 31.4%% of them were negative

The result showed statically significant association with last vaccination history and regulatory; we found increasing of disease in the samples that have been taken from the cattle that were vaccinated for more than one year 70.6% were positive and 29.4% were negative and declining of ratio in the samples that taken from the cattle that were vaccinated for more than 6 month and less than one year 38.5% were positive and 61.5% were negative. We notice also high ratio in the samples that taken from the cattle that were vaccinated for less than 6 month (recently vaccinated) 76.9% were positive and 23.1% were negative we suggests that due to immune response to the vaccine this result agree with(Shallali,1997).

In this study signify the disease was waning when the ratio of animal vaccinated number in herd was increase in the cattle that the animal vaccinated number percent was 100% the positive percentage 41% and 59% were negative. With regard to the samples that taken from the cattle that the animal vaccinated number percent was 75% were 35% were positive and 65% were negative. With regard to the samples that taken from the cattle that the animal vaccinated number percent were 25%were positive and negative in same percentage 50%.

CONCLUSION:

The result concluded that the vaccination programme has strong responsibility in incidence of Abs against *Mmm*SC but this role limited by other factors such as vaccinated population, how done the vaccination and the regularity of vaccination. The presence of statistically important differences in the prevalence of CBPP among the study areas suggests that variation in vaccination factors favours the incidence and extend of the disease. so, it is recommended, as a short-term intercession, that annual quality vaccination programme with control of animal movement should be started in CBPP-seropositive areas, as well as awareness creation among the pastoralists about the means of transmission of the disease and its high economic importance.

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