CHAPTER TWO

MATERIAL AND METHOD

2.1 Materi

2.1.1 Study Area :

The study was conducted from January 2011 to may 2011 in private farms distributed in the state and from the markets in Khartoum state, geographically, Khartoum is one of the largest state in the Sudan, it lies in the semi desert zone between latitude and longitude 16.54 – 15.8 north and 25.3 – 31.45 east, covering an area of about 20971 Km square.

It is surrounded by sixth states including Gazira, Kassala, Gadarf, Rive Nile, White Nile and Northern state.

Khartoum state composed of seven localities (Khartoum, Jebelawlia, Karri, Umbada, Bahry and Sharg Elnile).

The population is 5.7 millions, there are 1.5 millions cross the Khartoum state every year moving from state to another, the live stock sector (the resident animals) was estimated at about 1.321.852 according to census 2009.

1.1.2 Glass ware and Equipment:

1\ vacutainers tubes

2\ needles

3\ needle holder

4\ serum tubes

5\ centrifuge

6\ refrigerator

7\ incubator at +37°C (±3°C)

8\ plate agitator

9\ microplate reader

10\ microplate washing system that distributes 300µ per well (optional)

11\ disposable micropipette tips

12\ 96-microplates for dilutions

13\ microplate covers (lid, aluminium, foil or adhesive)

14\ vortex

15\ ELISA reader

1.1.3 Samples collection :

According to the data from the Ministry of Animal Resources & Fisheries – the information unit the estimate of livestock population in Khartoum state was 1321852, there are about 236909 cattle in the study area. A Cross-sectional survey was carried out in Khartoum state. 192 samples were collected from the state and from each governorate 64 samples were collected. Sample size was calculated according to the formula :

$$N = \frac{4PQ}{L^2}$$

P ≡ Prevalence

Q ≡ 1- P

L ≡ allowable error

(Wayne Martin, 1988)

$$= \frac{(4\times41\times959\times10000)}{25\times1000\times1000} = 64 \text{ animals}$$

Study animals were selected from this population by simple random sampling. Study area were divided to three governorates, Khartoum, Omdurman and Khartoum north. The samples were selected randomly from 22 farms and three markets from each herd 10% of animals were selected using the random sampling procedure. Blood samples were collected from the jugular vein, then blood were centrifuged and sera were collected from the blood, sera were preserved in sterile bottle at low temperature (- 20C°) after labeling each sample in order to diagnose them. c ELISA test used for serological diagnostic test to measure antibodies concentration in the serum sample (table (1).
Samples detected in the central laboratory –soba by using a competition ELISA based on a monoclonal anti-MmmSC antibody.

1.1.4 Chemicals and Reagent:

1\ wash dilution
2\ dilution buffer 24
3\ strong positive control
4\ weak positive control
5\ negative control
6\ monoclonal anti-MmmSC antibody (Mab 117/5)
7\ anti-mouse IgG

8\ TMB substrate solution 3
9\ stop solution

Table (3): Estimated sample size:

No	Description Number	Number
1	Number of governorates	3
2	Number of Localities	5
3	Total Number of farms	22
4	Total Number of Markets	3
5	Total Number of samples collected	192
6	Percentage of samples /herd	10

Table (4):The number of the samples from different localities in Khartoum state:

governorate	locality	No. animals
Umdorman	Karari	23
	Umbada	41
Khartoum	Jebelawlia	64
Khartoum North	Sherg Elnle	32
	Bahry	32

2.2 Method:

2.2.1 Method of the cELISA test:

the wells of the polystyrene micro titer plates were coated within Mmm SC lysate. Serum samples to be tested were diluted and incubated with the specific monoclonal antibody (117/5)in a pre-plate. This mixture was then transferred into the MmmSC coated micro plate. Any antibody specific to Mmm SC in the serum will form an Mmm SC/bovine antibody immune complex, which effectively masks the Mmm SC sites. In this case the monoclonal antibody can not bind to the corresponding epitope.

After washing, an anti-mouse-IgG antibody coupled to peroxidase was incubated in the wells. In the presence of specific Mmm SC antibodies in the serum that is being analyzed, the monoclonal antibody (117/5) is not fixed in the plate and the conjugate cannot bind in the wells. On the contrary, the conjugate can bind to the monoclonal antibody.

After washing, the enzyme substrate (TMB) was added to the conjugate, forming a blue compound becoming yellow after blocking. The intensity of the color is an inverse measure of the proportion of anti- Mmm SC antibodies in the serum sample tested.

The cut-off was calculated by using the results obtained from a monoclonal control (Cm) and a conjugate control (Cc).

The positive and negative controls were delivered with the kit. They were added to each micro plate and the results were validated.

2.2.2 Statistical Analysis:

Beside collection of the samples questionnaire was conducted and filled by the owners of the farms. The questionnaire was containing information about animals, farms and environment in order to analyze it by using statistical analysis to detect the factors which is associated with the CBPP. The questionnaire contained closed ended question to facilitate data analysis, minimize variation and improve precision of response.

The data which collected comprised information concerning mainly the following articles; localities. Appearance of CBPP signs, ages, sex, type of breed, type of herd, size of animals, the purpose of production, body condition, type of housing, sharing in water body and grazing lands, insect population and house cleaning. The questionnaire also assessed the vaccination programs practiced, information was also obtained on the use of antibiotics for the treatment of CBPP.

Data were analyzed by using SPSS program (SPSS version 15). A two- stage statistical process was applied. In the first stage chi- square test and simple regression, in the second stage multivariable logistic regression was used.

Chi-square test used to analyze the relationship between the putative causal factor and the disease. Sig > 0.2 was considered statistically not significant in all cases.

Any factors had a significant less than o.2 enter to analyze by using multiple logistic regression to know the association between the disease and the factors and a P value < 0.1 considered significant.

The questionnaire

Serial No ……..

State ……..Locality ……

Owner name …….phone ……..

1\ Did you observe in your farm any signs of cough , difficult breathing or discharge ?

Yes No

2\ If yes did you treat it by antibiotic ?

Yes No

3\ Have the cattle been vaccinated against CBPP ?

Yes No

4\ What type of breed in your herd ?

Local mix exotic

5\ What ages of cattle affected ?

<6 month 6 – 12 > 18 month

6\ Sex :

Male Female

7\ Type of herd :

Resident Nomadic Trade

8\ The No of the herd size :

< 20 20 – 40 > 40

9\ The purpose of production :

Milk Meat Dual

10\ Body condition :

Healthy Not healthy

11\ Type of housing :

Open system Semi open Close

12\ Type of feeding :

Good Poor

13\ Sharing with other herds in water body :

Yes No

14\ Sharing with other herds in grazing lands :

Yes No

15\ Insect population :

Heavy Low

16\ House cleaning :

Daily Every 7 days Every 30 days

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