بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

SERO-PREVELANCE AND RISK FACTORSOF CONTAGIOUS BOVINE PLEUROPNEUMONIA IN KHARTOUM STATE –SUDAN

الإنتشار المصلي وعوامل الخطر لمرض الإلتهاب الرئوي البلوري الساري في الأبقار في ولاية الخرطوم-السودان

By

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DEDICATION

To My Father, Mother

To My Brother Salah

To My Best Friends and Colleagues

Wish You All the Best

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LIST OF ABBREVIATIONS

CBPP: Contagious Bovine Pleuropneumonia.

*Mmm*SC: *Mycoplasma mycoides mycoides* Small Colony

c.ELISA: Competitive Enzyme Linked Immuno Sorbent Assay.

CFT: Complement Fixation Test.

CVRL: Central Veterinary Research Laboratory.

OIE: Office International des epizootic

PACE: Pan-African programme for the control of epizootics

LC: Large Colony.

PCR: Polymerase Chain Reaction.

PPLO: Pleuropneumonia like Organism.

DNA: Deoxy Nucleotide Acid.

DGIT: Disk Growth Inhibition Test.

FAT: Fluorescent Antibody Test.

AGID: Agar Gel Immunodiffusion Test.

MF.dot: Membrane Filter Dot.

GIT: Growth Inhibition Test.

IFA: Immunofluorescence Assay.

Mab: Monoclonal Antibody.

IBT: Immunoblotting Test.

LPPQ: Lipoprotein Q.

FAO: Food and Agriculture Organization

Cc: Control Conjugate.

Cm: Control Mab.

CP++: Strong Positive Control.

CP+: Positive Control.

CN- : Negative Control.

OD: Optical Density.

UK: United Kingdom.

SPSS: Social Statistical Package.

ROC: Receiver Operator Curve.

Abs: Antibody.

Abstract

Contagious Bovine Pleuropneumonia (CBPP) is one of the serious threats to the livestock in Khartoum State, Sudan.

This study was carried out to assess the prevalence, distribution and importance of CBPP using serology and risk factors epidemiology methods so as to provide useful information which supports the surveillance and control strategies of the disease in Khartoum State. Total of 386 serum samples were collected from different locations in Khartoum State and have been tested by using two serological tests; Competitive Enzyme Linked Immuno Sorbent Assay (c-ELISA) and 140 of them tested by LATEX agglutination test.

The results showed that the rate of positive reactors was 45.3% by c-ELISA.

The LATEX was found to be more successful in the diagnosis of CBPP it was found to be more effortless to be applied at the field as well. The c.ELISA was found to be useful and straight forward but showed high range of doubtful results in comparison with LATEX. The risk factors Epidemiology Approaches result indicated that sex of animal, age, breed, herd structure, herd size, number of dead animal and communities knowledge have significant association in the incidence of CBPP.

In this study high ratio of the Seroprevelance due to neglected of vaccination according to the questionnaire.

Therefore isolation and stamping out of the disease, serious surveillance, restricted of cattle movement and implementation of proper vaccination programme.

ملخص الأطروحة

يعد مرض الإلتهاب الرئوي البلوري الساري في الأبقار من أكبر المهددات للثروة الحيوانية في ولاية الخرطوم على وجه الخصوص وفي السودان بشكل عام.

تم إجراء هذه الدراسة لتقييم معدلات انتشار هذا المرض ودراسة العوامل التي تساعد على انتشاره باستخدام طريقة الفحص المصلي و دراسة وبائية عوامل الخطر التي تؤثر علي معدل انتشار المرض لتوفر هذه الدراسة معلومات مفيدة تدعم التقصي المرضي واستراتيجة التحكم في المرض في ولاية الخرطوم.

تم جمع عدد 386 عينة مصلية من مواقع مختلفة بولاية الخرطوم وتم اختبارها باستخدام إختبارين مصلييّن هما إختبار المقايسة المناعية المرتبطة بالإنزيم المتنافسة و إختبار التلازن المصلي (اللاتكس) . أظهرت النتيجة أن نسبة 45.3 % كانت موجبة لإختبار المقايسة المناعية المرتبطة بالإنزيم و 100عينة موجبة لإختبار التلازن من اصل 140عينة 30 من العينات كانت سالبة لإختبار التلازن في حين أنها كانت موجبة لإختبار المقايسة المناعية المرتبطة بالإنزيم غير أن 3 عينات كانت موجبة لإختبار التلازن و سالبة النتيجة في إختبار المقايسة المناعية المرتبطة بالانزيم .

وجد أن إختبار التلازن هو الأفضل و الأسهل عملياً في الحقل و لكنه أغلى ثمنا من إختبار المقايسة المناعية المرتبطة بالإنزيم(الإليزا) إلا أنها تظهر نسبة من النتائج المشكوك بها لبعض العينات.

وجدت النتائج إرتباطا وثيقاً بين حدوث المرض وعدد من العوامل مثل عمر الحيوان ،جنسه، تركيبة القطيع نوع سلالة الأبقار، التعرض لقطيع آخر، مشاركة قطيع آخر في المرعى والشرب، تاريخ آخر تطعيم ومن يقوم بعملية التطعيم ،مدى معرفة ووعي المجتمع بالمرض وعدد الحيوانات الميتة من القطيع.

Chapter One

Literature Review

1.1. History and distribution of disease

There is no full solid information about the time and placewhere contagious bovine pleuropneumonia (CBPP) was reported forthe first time in the world. According to Carrason (1942), the diseasewas first observed in Italy and France in the middle of the sixteenthcentury. The development of the international trade in middle of thenineteenth century helped greatly the spread of the disease to EnglandandScandinavian countries. By 1803, the disease was reported in theUnited State of America (Jasper, 1967).

In 1958 thedisease was carried by sea to Australia through infected cattle (Turner, 1954).

Due to strict control measurement at the end of the nineteenthcentury and at the beginning of the twentieth century, the disease waseradicated from the USA, 1852 and most of the European countriese.g. England, 1898 and Russia 1940 (Jasper,1967).

It was reported as late as 1958 in Portugal (Ferronha et al.,1990), further outbreaks occurred in 1961 in Spain and then in theDepartment des Pyrenee-Oruntales in France in 1967 (Provost et al., 1987) where some mortality wasrecorded in three herds (Turner, 1961).

Infection in Portugal was reported to be widespread in 1983with serological evidence suggesting that the disease had been present for months, may be years before (Ayling et al., 1999). CBPP wasendemic in north-western parts of Portugal around Porto, but outbreaks4subsequently decreased significantly. Spain began reporting cases ofCBPP from 1989. Whilst the first cases occurred around Madrid and Segovia, the majority of outbreaks were in the northern coastal areasbroadening the Bay of Biscay (Turner, 1961).In 1990 Italy reported its first outbreak for over 100 years inPiedmont in the north. The disease

quickly spread to most major cattle areas of Italy. However, as a result of abattoir surveillance, movementcontrol linked to serological monitoring, and slaughter of infected andin contact animals, no cases have been reported since September 1993.For the first time for over 20 years, no outbreaks were reported inEurope in a year 2000.The disease is still present in most African countries south of the Sahara, India and China (Hudson, 1971). Recently, there are 5reports on severe outbreaks of CBPP in Kenya, Uganda, Ethiopia, South Africa, Asia and North Portugal (OIE, 2001). In the Sudan the disease was first observed in 1875 in Darfur Province and later spread to Khartoum Province where it caused great losses among cattle (Anon, 1952). The disease disappeared during the Mahdi wars in 1889 and it reappeared again in Kordofan Province in 1912.

From there the disease spread quickly southward and eastwards of the province. In 1913 the disease was reported in Nuba Mountains, the White Nile, Upper Nile and Bahr El Gazal Provinces. In 1914 the disease reached Khartoum Province and then spread to Berber Province in 1913 and Kassala Province in 1917 (Anon, 1925).

The Pan African control of Epizootics (PACE) in 2003 showed that the disease is currently endemic in most parts of the Sudan especially in areas below 15oN latitude with varying degree of severity.

The disease is frequently reported from eastern states near Ethiopian border in Doka and Al Galabat in Gedarif State.

Al Managel (Gezira State), Al Dinder and Singa (Sinnar State), Al Rossires and Al Angasana (Blue Nile State) and Algabalin and Kosti (White Nile State) are the endemic areas in the Central States. Southern Kordofan State is also endemic area and reports were received from Al Delling, Kadogli and Abu Gebiha in the Southern 6 Kordofan State up Babanosa, Almoglad and Abeyei in the southern part of the Western Kordofan State. CBPP is widely spread in Southern Darfur State specially areas of El Dain, Buram and Radom.

Sporadic cases were reported in Khartoum State (Mageed, 2003). The disease was not reported in the northern Sudan (River Nile and Northern and Red Sea States) for more than 15 year.

1.2 Taxonomy

The mollicutes represent a class of unique cell wall-less prokaryotes that include members of the genus *mycoplasma*. According to International Committee on Systemic Bacteriology-

Subcommitte on the Taxonomy of Mollicutes, the class contain the following orders, families and genera:-

Order I :Mycoplasmatales

Family I :Mycoplasmataceae

Genus II: Mycoplasma

Order II :Entomoplasmatales

Family I :Entomoplasmataceae

Genus I :Entomoplasma

Genus II: Mesoplasma

Family II: Spiroplasmataceae

Genus I :Spiroplasma

Order III: Acholeplasmatales

Family I :Acholeplasmataceae

Genus I :Acholeplasma

Order IV: Anaeroplasmatales

Family I :Anaeroplasmataceae

Genus I :Anaeroplasma

Genus II: *Asteroplasma*

The members of this class are eubacteria that are bound by asingle membrane. The major class characteristics are the lack of cell wall, the tendency to form fried-egg type colonies on solid media, the filterability of cells through 450 and

220 nm pore-size membrane filters, the presence of A-T-rich genome, and the failure of the wall less to revert to walled bacteria under appropriate conditions. Taxonomy within orders and families is based on sterol requirement, cell and colony morphology, optimum growth temperature and requirement for oxygen. Differences at genera and species levels are based on the above mentioned parameters beside the host origin, genome size and cultural and biochemical properties.

More than 102 *Mycoplasma* species have been discovered. The most important animal pathogens belong to *Mycoplasma myciodes*cluster (Freundt and Edward, 1979).

1.3Aetiology:

CBPP is caused by *Mycoplasma mycoides*subsp. *Mycoides*(*Mmm*SC) (Nocard*et al.,* 1898). The causative agent (*Mmm*SC) is amember of the *mycoplasma mycoides*cluster which include 6biochemically and antigenically similar mycoplasmas of animalimportance (Table 1). Species and subspecies withinthis cluster are difficult to distinguish by morphological andconventional biochemical tests. Although *Mmm*SC was considered tobe host specific but it has been isolated from pneumonic lungs ofsheep and goats (Brando, 1995; Thiaucoart*et al.,* 1999). This mayconstitute a difficulty in the control of the disease in areas wherecattle, sheep and goats are raised together.

*Mycoplasma mycoides*subsp. *mycoides*was classified into small colony type (SC) and large colony type (LC) according to their morphology, cultural and biochemical characteristics (Cottew and Yeast, 1978). The large colony type activity digests casein, liquefies serum and survives at 45oC. These together with differences in growth characteristics and others permitted differentiation of the two types.

1.4 Host range:

Contagious bovine pleuropneumonia is predominantly a diseaseof the Bovidae of the kind Bos (*Bos. taurus*and *Bosindicus*). There isa variation in susceptibility to infection; in general European breadstend to be more susceptible than indigenous African breeds (Provost*et al.,* 1987). Animal less than 3 years of age are less resistant toexperimental challenge (Masiga et al., 1978). The susceptibility ofcattle to infection with *M. mycoides*depends on many factors such as the type of animal husbandry, individual resistance and other factors (Shallali, 1997). In 2002 the infection was recorded in bison and yak. Although it has been reported that the domestic buffalo (*Bubalusbubalis*) is susceptible, the disease is difficult to reproduce experimentally in this species (Provost, 1988).

1.5 Transmission of the disease

Spread of the disease from infected to susceptible animals is mainly through inhalation of infected droplets which may be carried to 10-20 meters or more by the air currents (Turner, 1954). Factors, which influence the infection rate, are closeness of contact, intensity of infection and the level of individual susceptibility (Turner, 1954). Even under extensive condition the gathering of animals at watering places will lead to outbreaks and will maintain the disease in herds (Coetzer*et al.,* 1994). The disease may not be detected for several weeks or months after infected animals have entered an area. Some animals also have a degree of resistance to the disease and those surviving CBPP are even more resistant (Coetzer*et al.,* 1994). Outbreaks usually begin as a result of movement of an infected animal into a naive herd. It is widely believed that recovered animals harboring infectious organisms within pulmonary sequestra may become active when stressed. Although this may be a factor in some outbreaks, it has not been proved experimentally (Windsor, 1977). Asymptomatically and chronically infected animals are very important in the

spread of the disease to new areas. Chronic carriers are apparently healthy animal that have a localized focus of infection sequestered in a fibrous capsule in their lungs. The organisms can persist in such lesion for months, and in time the fibrous capsule may breakdown, allow viable organisms to escape by the bronchi and so infect susceptible in contact animals. This is particularly proved to occur when chronic carrier animals are subjected to stress such as when mustered or walked for long distances. *Mycoplasma* can pass through the placenta from the infected dams to the off-spring (Stone, 1969).

1.6 Symptoms of the disease:

The incubation period of the natural disease may range from 5to 207 days. Although Turner and Campbeel (1937) reported a rangeof 29-58 days and Provost *et al.* (1987) stated 20 to 40 days. In11experimental infection ,Regalla*et al.* (1994) reported that the diseasesymptoms appear in cattle 40 days after contact .

1.7 Pathology and post-mortem findings:

Pathological lesions are confined to the thoracic cavity. Different degree of pathological changes may be found in an animal. Lesions are usually unilateral without a preference for the left or right side. The lesion is always localized in the diaphragmatic lobe. Then cranial lobe seldom affected in the acute stage. Many liters of serous fluid are usually present in the thoracic cavity. The fluid is clear yellow-brown and may contain pieces of fibrin. A thick caseousfibrinous deposit is frequently present on the visceral and parietal pleura which is a pathognomnic sign. Another pathognomonic sign of CBBP is that the interlobular septa are distended by amber-coloured serous fluid which gives the lung the marbling appearance. The hepatized lung lobules have different colours varying from relatively normal to deep red or yellow-gray due to acute fibrinonecroticm pneumonia (OIE, 2001). In the chronic stage, an adhesion connects the thickened visceral and parietal pleura. When the thoracic cavity is

opened pieces of lung adhere to chest wall. The fluid in the thoracic cavity may disappear. The lung parenchyma appears oedematous with red consolidation. The interlobular septa are distended with lymph fluid (Hudson, 1971 and Liyloid, 1970). A necrotic portion or portions of the lung are walled off by a fibrinous tissue capsule forming what is known as sequestrum (Turner, 1959 and Liyloid, 1970). Sequestra vary in size from a centimeter in diameter to 20-30 cm in their greatest diameter (Hudson, 1971). Nevertheless, the classical picture of the disease showing marbling appearance, consolidation, adhesion of the pleura and considerable amount of pleural fluid was reported in naturally infected calves (El Tahir*et al.*, 1988).

1.8 Diagnosis:

Clinical diagnosis of CBPP is unreliable as initial signs may be slight or non-existent and may be indistinguishable from any severe pneumonia. Therefore, CBPP should be investigated by pathological, microbiological, molecular or serological diagnostic methods. As the pathological lesions of CBPP are distinctive, and pathognomonic, abattoir surveillance for CBPP involving lung examination is a practical method for disease monitoring.

It is recommended to isolate and identify the causative organism in order to confirm an outbreak.(Table 1) lists the laboratory methods used for the diagnosis of CBPP. (OIE, 2014)

Table1: laboratory methods currently used for diagnosis of CBPP and their purpose

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribution to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination *
Agent detection and identification1						
***In-vitro* culture isolation (followed by species identification tests)**	+++	-	-	+++	_	_
Direct molecular test (PCR)	-	-	-	++	-	-
Detection of immune response						
CFT	+ + +	+ + +	+ + +	+ +	+ + +	_
Immunoblotting	+ +	+ +	+ +	+ +	+ + +	_
C-ELISA	+ + +	+ + +	+ + +	+ + +	+ + +	_

Key: +++ = recommended method; ++ = suitable method; + = may be used in some situations; – = not appropriate for this purpose.(OIE terrestrial manual, 2014)

1.8.1 Clinical diagnosis

Tentative diagnosis depends on clinical signs, PM findings anddemonstration of *Mmm*(SC) in pleural fluid of infected animal bydark field microscopy. But other confirmatory tests are necessary. Themethods of diagnosis have advantages and disadvantages (Table 2).

1.8.2 Differential diagnosis

In carrying out CBPP clinical diagnosis, some sources of confusion may occur. Therefore, it is very important to differentiate this disease from other diseases which may have similar clinical signs or lesions.

1.8.3 Histopathology

Early in the course of the disease, the CBPP lesion comprise abronchiolar necrosis and oedema which progress rapidly to anexudative serofibrinous bronchiolitis with extension to the alveoli anduptake of alveolar fluid into tissue spaces, lymph vessels andultimately septallymphatics (Done *et al.*, 1995). These rapidly reachsaturation and the process is extended centrifugally to thetracheobronchial lymph nodes and centripetally to the pleurallymphatics. The mediastinal, sternal, aortic and intercostal lymphnodes may then become enlarged, oedematous or even haemorrhagic.With stasis, lymph vessels become thrombosed and ultimatelyfibrosed. The pulmonary lobules become consolidated with alveolar oedema, fibrin and inflammatory cells.Coagulativenecorsis is common. *Mmm*SC can be demonstrated inthese lobules by immonohistochemistry.

Table (2) the advantages and disadvantages of the methods of diagnosis

Method of diagnosis	Some advantages	Some disadvantages	Sample type	Reservation and storage
Isolation and Identification	Very sure diagnosis	Slow, require viable pathogen agent	Lung , pleural fluid , nasal swab	Under cold in order to guarantee the viability of the pathogen agent
Serology(Detection of antibodies)	Speed and simple	Some test are not sensitive and not allow to differentiate the post infectious antibodies from vaccine antibodies	Serum or blood	Under cold to avoid degradation of the antibodies
Molecular biology	Speed, sensitive , effective even pathogen is not viable	Expensive require full equipped , suitable lab and trained person	Pleural fluid , lung ,nasal swab , liquid	Not require any particular preservation
Histopathology	Simple , no need to conservation of sample under cold temperature	Presumed diagnosis, heavy equipment and need trained person	Tissue lung	10% neutral formal saline

Razin and Tully (1981)

Perivascular organization foci or 'organizing centers', found in the interlobular septa, are considered pathognomonic for CBPP (Ferronha*et al.*, 1988). They consist of a centre occupied by a blood vessel with proliferation of connective and inflammatory cells. Two types of foci have been recognized. Type I foci contains more proliferative cells in the central zone which is larger than the peripheral zone and probably corresponds to the success of the host immune response in resolving the infection; immunoreactive antigen is associated with macrophages. In type II foci, the proliferative cells are scarce and the peripheral zone is relatively larger. Immunoreactive antigen can be seen in the central zone inside blood vessels and it is thought that Type II foci indicate failure of the immune response leading to aggravation of symptoms. In an immunocytochemical study of CBPP infected Italian cattle, Scanziani*et al.* (1997) showed that the severity of lung lesions correlated with the severity of changes in the lymph nodes. In the acute stage of the disease specific antigen was detected in the lobular periphery and in the cytoplasm of alveolar macrophages. In chronic lesions, immunoreactivity was in the fibrotic areas and in macrophages located in the lobular septa; necrotic debris and macrophages located in the inner part of the sequestra were specifically stained. Immunoreactive material was also seen in the centrofollicular areas of the broncho-associated lymphoid tissue structures and in the lymph node follicles. Furthermore, electron microscopy of the mediastinal lymph nodes of a chronically affected calf showed degenerating mycoplasmas and a few apparently intact mycoplasmas in the macrophages.

The histological section of the lung in acute stage of the disease showed odema in the lymphatics of the interlobular septa and interstitial tissue and massive infiltration of fibrin, macrophage and neutrophils into the alveolar lumen (Bygrave*et al.*, 1968). Also there was presence of lymphocytes and alveolar macrophages around the lymphatic vessels and septa margin (Jubb and

Kennedy, 1963). In chronic cases the main lesions are the sequestra and fibrinous scars (Jubb and Kennedy, 1963; Bygrave*et al.*, 1968).

1.8.4 Isolation of the organism

If the animal died, pleural fluid or affected lung can be collected aseptically for cultural examination in selective media, sterile paper can be soaked with pleural fluid or allowed to absorb fluid from lung lesion (Turner, 1954). When the animal died, pleural fluid or affected lung can be collected aseptically for cultural examination in a selective medium. The organism can be cultivated in different liquid or solid media depending on the availability of facilities in each laboratory. Different media are available for the isolation and cultivation of *Mmm*SC. Basically they are composed of two parts; the basic medium and the supplement. The basic part is a broth of lean meat heart muscle or liver (Bennett, 1932; Campbell, 1938; Dafalla, 1961; El Nasri, 1972 and Buttley, 1967). The supplement consists of equine or swine serum, yeast extract, DNA, glucose and also penicillin and thallium acetate as bacterial and fungal inhibitors. Nowadays many basic commercial media as PPLO and brucella media are available for isolation and propagation of mycoplasma.

Growth of this organism in liquid medium culture becomes apparent as uniform faint turbidity within 3-6 days after aerobic incubation at 37oC. The organism is described as pleomorphic occurring as round ovoid, ring and filamentous forms in liquid media (Turner, 1954). Colonies on solid media grow slowly in 3-6 days and are different in diameter and difficult to be seen by naked eye.

1.8.5. Biochemical tests

1.8.5.1 Glucose fermentation

Some strains of mycoplasma ferment glucose and produce acid (Erno and Stipkovits, 1973).

1.8.5.2 Hydrolysis of ariginine:

Some strains of mycoplasma, hydrolize arginine and shift the medium to alkaline (Erno and Stipkovits, 1973). This is done by the action of arginine deaminase as a character associated with nonfermentative and some of the fermentative mycoplasma.

1.8.5.3.Phosphotase activity:

It was done by incorporating phenolphthalein diphosphate inagar media as described by Eron and Stripkovits (1973). The activityis manifested by change of the colour of media to pink.This test is of diagnosticvalue and is used to indicate the lipolytic activity of certainmycoplasmas containing the enzyme lipase. Characteristic, wrinkled,pearly film with tiny black spots appears on the surface of the mediumthat contains horse serum or egg yolk. The film consist of cholesteroland phospholipids and the spots contain calcium and magnesium saltsof fatty acids.

1.8.5.4. Serum digestion:

The method was described by Eron and Stripkovits (1973). The proteolytic activity is determined by the ability of the organism to digest coagulated serum.

1.8.5.5.Terazolium reduction:

This was usually done in liquid media (Erno and stripkovits, 1973) . It shows the ability of some mycoplasma to reduce 2, 3, 5 Triphenyl

Tetrazolium chloride to brick red formozan. The ability of the strains to reduce tetrazolium was noticed to be higher under anaerobic than aerobic conditions.

1.8.5.6. Haemolysis:

The test is performed on agar or in broth culture, both showing various degree of haemolyosis due to the production of peroxide by the organism.

1.8.5.7.Hemadsorption:

The observation of the adsorption of erythrocytes tomycoplasma colonies is detected microscopically. This property isused to measure the ability of

mycoplasma to adhere to eukaryaticcells; hence it is related to the pathogenicity of the organism (Shmueland Tully, 1983).

MmmSC is sensitive to digitonin (like all members of the order Mycoplasmatales), does not produce „film and spots", ferments glucose, reduces tetrazolium salts (aerobically or anaerobically), does not hydrolyse arginine, has no phosphatase activity, and has no or weak proteolytic properties. (OIE, 2014)

For these tests, special media have been developed that include the same basic ingredients (heart-infusion broth or Bacto PPLO broth, horse serum, 25% yeast extract solution, 0.2% DNA solution), to which is added 1% of a 50% glucose solution for glucose hydrolysis, 4% of a 38% arginine HCl solution for arginine hydrolysis, and 1% of a 2% triphenyltetrazolium chloride solution for tetrazolium reduction, as well as a pH indicator (e.g. phenol red). (NOTE: a pH indicator should not be added to a medium containing triphenyltetrazolium chloride.) For demonstration of proteolysis, growth is carried out on casein agar and/or coagulated serum agar.

Once the biochemical characteristics have been checked, one of the following immunological tests can be performed to confirm the identification: disk growth inhibition test (DGIT) (Freundt*et al*., 1979), fluorescent antibody test (FAT), IFA, agar gel immunodiffusion test (AGID) (Provost, 1972), or dot immunobinding on a membrane filter (MF-dot) test (Brocchi*et al*., 1993)

1.8.6. Serology

Serological tests for CBPP are valid at the herd level only because false positive or false negative results may occur in individual animals. Tests on single animals can be misleading, either because the animal is in the early stage of disease, which may last for several months, before specific antibodies are produced, or it may be in the chronic stage of the disease when very few animals are seropositive. False-positive results can occur (2%), of which an

important cause is serological cross-reactions with other mycoplasmas, particularly other members of the *M. mycoides*cluster. The validity of the results has to be confirmed by post-mortem and bacteriological examination, and serological tests on blood taken at the time of slaughter.

Two groups of serological tests are used. Specific identification of isolated mycoplasma relies heavily of growth inhibition test (GIT) and immunofluorescence (IF).

Serological diagnosis of CBPP depends on antibody detection using different tests e.g. CFT, ELISA, Agglutination tests… etc.

The complement fixation test (CFT) and enzyme-linked immunosorbent assays (ELISAs) are recommended for screening and eradication programmes. The highly specific immunoblotting test is useful as a confirmatory test but is not fit for mass screening.

1.8.6.1. Complement fixation test (CFT)

Complement fixation test is currently the official and the most widely used serological diagnostic test. It is thought to be specific and sensitive in the acute phase of the disease. However, the test is reported to detect only about 70% of clinically infected animals and seem not to detect asymptomatic animals in the early stage of infection (Nicolas *et al.,* 1996). Although it is specific, CFT lacks sensitivity with a positive reaction result being only at 1/10 or higher. CFT is also far from robust, in a thorough examination of CFT in which over 33000 sera from healthy herds were tested between 1991-1994 in Italy. Bellini *et al.* (1998) reported that CFT was 98% specific. Regarding sensitivity, based on nearly 600 cattle with specific lesion from 11 infected herds, only 64% of animals were positive. Isolation of the causative agent from the affected animals was even more insensitive at 54%. Surprisingly, during the Italian outbreaks, abattoir surveillance detected nearly as many cases as serological monitoring, while clinical examination was much less useful (Regalla*et al.,* 1996). It followed that by using CFT as a screening test, some CBPP affected cattle, in

the early or later stages of infection were missed, accounting for the persistence of the disease in Portugal.

1.8.6.2. Enzyme linked immunosorbent assay (ELISA)

A competitive ELISA (c-ELISA) developed by the OIE Collaborating Centre for the diagnosis and control of animal diseases in tropical countries (Le Goff &Thiaucourt, 1998), has been validated internationally in accordance with OIE standards (Amanfu et al., 1998). This c-ELISA is suitable for certifying individual animals prior to movement, including for international trade. The performance of this c-ELISA method has also been validated by the French Committee for Accreditation in 2009.

Compared with the CFT, the c-ELISA has equal sensitivity and greater specificity. Advice on standard protocols and the availability of reagents can be obtained from the OIE Reference Laboratories for CBPP, or the OIE Collaborating Centre for ELISA and Molecular Techniques in Animal Disease Diagnosis (see Table given in Part 4 of this Terrestrial Manual).

Validation tests (Amanfu et al., 1998; Le Goff &Thiaucourt, 1998) that have been carried out in several African and European countries would indicate: i) that the true specificity of the c-ELISA has been reported to be at least 99.9%; ii) that the sensitivity of the c-ELISA and the CFT are similar; and iii) antibodies are detected by the c-ELISA in an infected herd very soon after they can be detected by the CFT, and c-ELISA antibody persists for a longer period of time.

To enhance its repeatability and the robustness, this c-ELISA is now provided as a ready-made kit that contains all the necessary reagents, including precoated plates kept in sealed bags. This kit can be obtained commercially and availability can be checked through the OIE Reference Laboratory in France. The kit has been especially designed to be robust and offers a good repeatability. Sera are analysed in single wells. The substrate has been modified and is now TMB (tetramethylbenzidine) in a liquid buffer and the reading is at

450 nm. The substrate colour turns from pale green to blue in the first place and becomes yellow once the stopping solution has been added. Mab controls exhibit a darker colour while strong positive serum controls are very pale. The cut-off point has been set at 50% and should be valid in every country.

1.8.6.3. Slide agglutination test

The slide agglutination test using serum (Pricestly, 1951) orblood (Newing and Field, 1953) is sensitive in early stages of thedisease and suitable for establishing of preliminary diagnosis whenlarge number of cattle is involved and for selection. So it isrecommended to be used as a herd test rather than an individualanimal.

The most important test for detecting animals particularly thosewith sequestra is CFT (Campbell and Turner, 1937; Lindley, 1960;Huddart, 1963 and Pearson, 1966). The advantage ofCFT is the low number of false positive and negative reaction(Hudson, 1971). The main disadvantage of CFT is that it fails to detectinfected animals in the incubation period of the disease.

1.8.6.4.Immunoblotting tests (IBT)

Immunoblotting test is used generally to compare between isolated *Mmm*SC strains. Gonclaves*et al.* (1998) used it to detect five different antigens (110, 98, 95, 62/60 and 45 KDa). These antigens were highly characteristic of sera from Portuguese herds affected by CBPP (170 cattle). There was 79% agreement between CFT and IBT. In a study of 88 cattle with CBPP lesions, IBT detected 80 positive animals and CFT detected 72 (Aylin*et al.*, 1991). Abdo*et al.* (1998; 2000) identified a 48 KDa protein, named LPPQ. It was found in the type strain and European, African and Australian field strains. They used the protein in an immunoblotting test for the sero-detection of *Mmm*SC in experimentally infected cattle.

1.8.7. Molecular diagnosis

A powerful diagnostic system based on PCR has been developed for rapid detection, identification and differentiation between members of the *M. mycoides*cluster. An arbitrarily primed PCR (AP-PCR) of *Mmm*SC, with Mlip 1 and Mlip 4 primers produced a fingerprint with little genomic polymorphism and thus of limited epidemiological use. Two bands of 900 bp and 100 bp for strains PGI, PO, KH3J and Fatick were produced, although the later had afaint band at 400 bp, in contrast to the five *M. Caprivolum* strains tested which produced four different patterns (Rawadi*et al.,* 1995). These test strains were designed from sequences of unknown functions or from known genes (Bashiraddin*et al.,* 1994b; Dedieu*et al.,* 1994; Hotziel*et al.,* 1996; Niserez*et al.,* 1997; Rodriguez *et al.,* 1997; Person *et al.,* 1999). With many of these tests, confirmation of the presence of *Mmm*SC-type or production of the expected amplification product was possible by the digestion of the product with specific restriction enzyme. In some cases the standard detection of PCR products by agarose gel electrophoresis was replaced with enhanced methods which improved the sensitivity.

These methods have been used for the identification and detection of *Mmm*SC-type from culture and clinical materials including nasal swab, mucous, pleural fluid, lung tissues, lymph nodes, kidneys, spleen and semen from cattle (Bashiruddin*et al.,* 1994a, 1994b; Nicholas, 1994; Bashiruddin*et al.,* 1999) and from milk and respiratory tract of small ruminants (Brandao, 1995). In some cases they had superior diagnostic sensitivities compared with conventional diagnostic tests. In particular, the sensitive nested PCR system has been used for the detection of *Mmm*SC from culture and clinical material where the target organism may be in low numbers such as in nasal swab samples (Hotzel*et al.,* 1996; Niserez*et al.,* 1997).

1.9 Disease control and eradication

Control of CBPP can be based on:

1.9.1Stamping out policy

This policy is a general method when infection is detected early. In the Sudan in which the mode of animal husbandry is nomadic this can not be done but cattle owner are persuaded to kill their clinically sick animals (Abdalla, 1975), so when the disease is reported in the herd its movement is restricted and segregated in grazing and watering areas.

1.9.2 Vaccination

Vaccination against CBPP in Sudan has been practiced since 1914 (Anon 1914). In those early days infected pleural exudates from a sick animal was inserted under the skin of the tail tip. This method usually caused severe swellings. Broth culture vaccine prepared from field strain of *M. Mycoides*subsp*mycoides*was produced in the Veterinary laboratory in Khartoum in 1925 (Anon 1925) and in 1926 (Anon 1926). F strain isolated in 1944 from pulmonary exudates of naturally infected case and was subculture in glycerine broth (Anon, 1950) was used for vaccine production in the Sudan and the recommended dose was 1 ml (Abdalla, 1975). T1 strain broth culture vaccine production originated in East Africa and was tested in the laboratory and the field in Sudan (Lindley and Abdalla, 1967). Comparative studies showed that this vaccine has superior immunizing power over F strain (Lindley and Abdalla, 1967, Daleel and Lindley, 1970). Cattle vaccinated with this vaccine were immune for more than 12 months (Daleel, and Lindley, 1970). The other alternative and most practical way is the use of lyophilized vaccine. The research for the production of a dried vaccine started in the mid fifties (Turner, 1954). Lindley (1971) emphasized that the T1 lyophilized vaccine is most valuable tool which can be used for controlling of CBPP, being more stable than broth culture vaccine (Brown *et al.*, 1965). Later Shallali*et al*. (1997) has successfully prepared lyophilized T1/44 vaccine and has been used in the field

since that time. T1/44 /SR strain is streptomycin resistant and dependant strain (Anon, 1988). Vaccine made from this strain is safe and gives satisfactory immunity for about 14 month (Provost, 1988).

1.9.2.1. Suitable age of vaccination

Colostrums are the source of antibodies which pass from calf gut to its blood stream and persist for up to 60 day (Stone, 1969). On the other hand (Turner1961) found that calves born to unvaccinated dams were highly susceptible to joint involvement within the first week of life. It was found that calves born to recently vaccinated dams showed antibodies one week post-parturition. Titers reached maximum at one month and then gradually declined. By the six months, 71% of the tested calves' sera were found negative. On the basis of these results it is suggested that six months is the suitable age to start vaccination of calves from recently vaccinated dams (Shallali*et al.*, 1998).

1.9.3.Treatment

Another method of control is chemotherapy with broad spectrum antibiotics. This is only recommended for control of severe local reaction at the vaccination site since its use on actual case of CBPP could lead to a high incidence of carrier animals with squestra in their lungs. In 1967 the FAO/OIE OAU panel unanimously opposed therapeutic treatment for the actual case of CBPP and strongly recommended that mass drug or antibiotic treatment of CBPP to be discouraged (FAO Report, 1967).

In spite of official condemnation, antibiotics are used so advice is necessary on which are the most effective. Ayling et al. (2000a) carried out an in vitro trial of the effects of five commonly used antibiotics on a number of strains of MmmSC, and concluded that tilmicosin and danafloxacin were effective both in terms of mycoplasmastatic and mycoplasmacidal activity; florfenicol and a tetracycline provide intermediate effectiveness while spectinomycin was ineffective against some strains. The use of fluoroquinalones, such as

danafloxacin, is causing concern amongst regulatory authorities that feel these drugs should be restricted to human use because of rapid increases in microbial resistance.

Chapter two

Materials and Methods

2.1 Study Area

Khartoum state 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 is composed of seven localities (Khartoum, Omdurman, Jebelawlia, Karri, Umbada, Bahry and ShargElnile).

The population is 5.7 million; 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 2017.

Surveillance of the Disease (CBPP) had been conducted in areas of high cattle population in Khartoum State in order to update the existing situation of the disease; for these sera samples and participatory data were collected (Fig.1).

2.2. Study Design and Subject:

Cross-sectional study was conducted in a period of time from November 2016 to May 2017 to determine seroprevalence of CBPP in Khartoum state taking a total number of (386) sample from ten sites in the state to be included in the study. The animals used for study were both males and females and some of them were vaccinated against CBPP and the majority of them founded none vaccinated.

The sampling frame 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. since the previous prevalence of the disease in the region was 20, 9% and

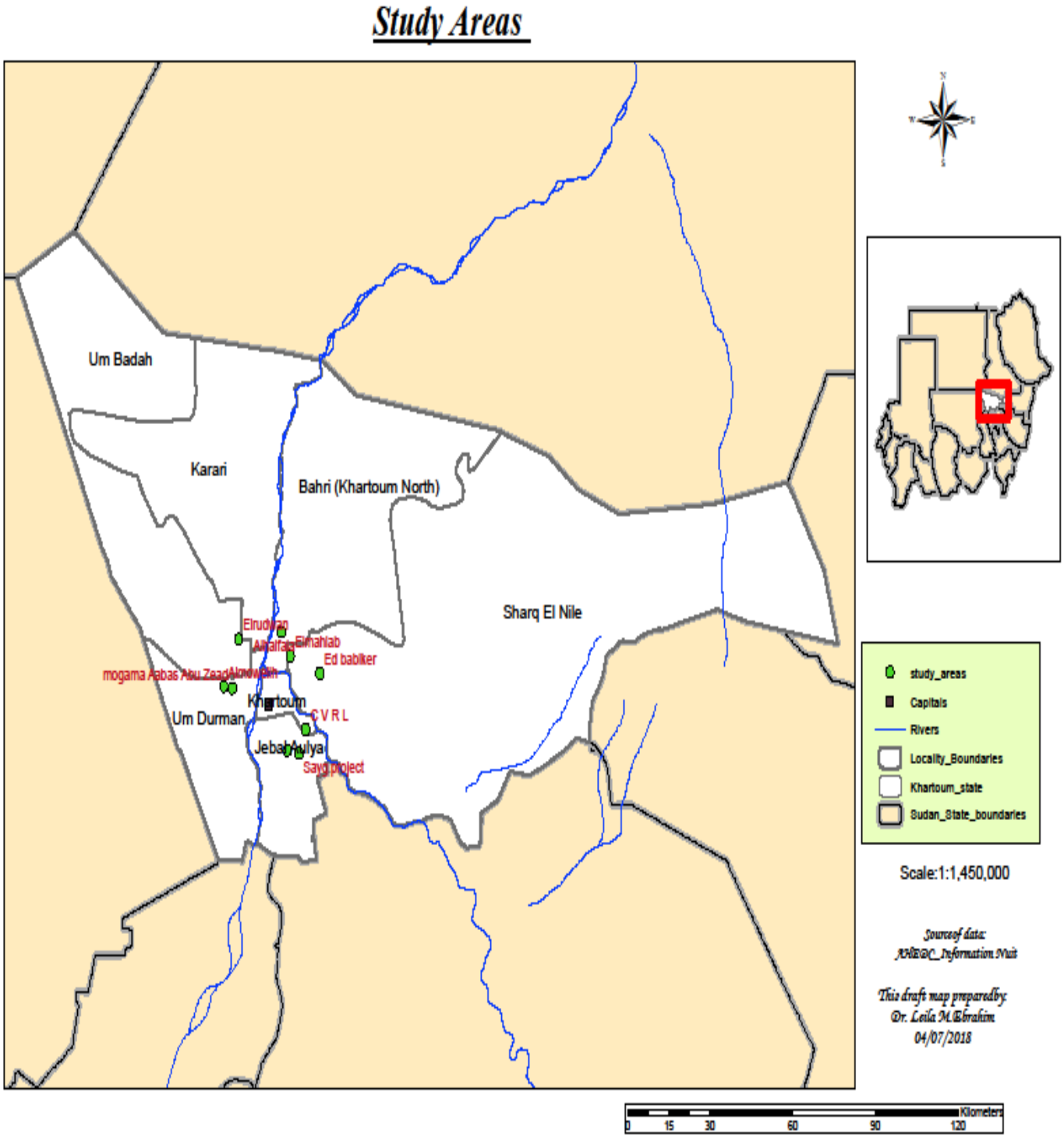


Fig. (1) Areas of study in Khartoum state

a 5% absolute level of precision was considered to calculate the number of animals to be sampled **(Thrusfield 1995)** as follows:

(Thrusfield 1995) as follows: $N = 1.96^2 \times P_{exp}(1 - P_{exp}) \times d^2 \over 0.05^2$

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

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

0.05^2

This equation reflects sample size were based on the previously founded prevalence of CBPP (20.9%) on a study of **Ibrahim (2015)** But 386 sample was taken to increase sample size for more truthfulness.

2.3Blood Samples

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 Alrudwanproject , Omdurman Almwelih market , Nifasha, Alhalfaia and Eid babeker(table 3).

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.

Table (3): The number of serum samples collected from different localities in Khartoum state

Governorate	Area	No. animals
Khartoum	West soba project	10
	Saig project	16
	CVRL	25
Omdurman	Alrudwan project	50
	Almwelih market	113
	Abuzaid market	14
ShargElnile	Mahlab2	60
	Eid babiker	9
	Nifasha	11
Khartoum north	Alhalfaia	53
Total		386

2.4. The questionnaire

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.ages, sex, type of breed, type of herd, size of animals, type of housing, sharing in water and grazing lands, occurrence of the disease in the past ,exposuring to another herd and status of that herd, passing of nomads through areas and awareness of pastoralist. The questionnaire also assessed the vaccination programs practiced; information was also obtained on the use of antibiotics for the treatment of CBPP.

2.5 Glass ware and Equipment:

- Vacutainers tubes
- Needles
- Needle holder
- Serum tubes
- Centrifuge
- Refrigerator
- Incubator at +37°c (±3°c)
- Plate agitator
- Microplate reader
- Microplate washing system that distributes 300µ per well
- (optional)
- Disposable micropipette tips
- 96-microplates for dilution

- Microplate covers (lid, aluminium, foil or adhesive)
- Vortex
- ELISA reader

2.6. c.ELISA:

2.6.1 Principle of the test

It is an assay to determine the presence of anti-CBPP antibody in serum. It is based on the competition between the anti-CBPP monoclonal antibody and the antibodies in the serum sample binding to the CBPP antigen. The presence of antibodies CBPP in the serum sample will block reactivity of the monoclonal antibody resulting in reduction in expected colour following the addition of conjugate and substrate chromogen solution. As this is a solid phase assay, wash step are required between each step to ensure removal of unbounded reagents.
Competitive ELISA kit was that of IDEXX, Institute Pourquier, Montpellier, France, CBPP serum competition ELISA. The technique described sheet fact accompanied the kit was followed.

2.6.2 The components of the kit

- Monowell coated microplates.
- Wash concentrates (20 xs).
- Dilution buffer 24.
- Control sera.
- Monoclonal antibody 117/5 (anti- Mmm SC) (Mab).
- Monoclonal anti-mouse 1gG peroxides conjugate.
- Revelation solution 3 (TMB).
- Stop solution (H_2SO_4 O.5M solution).

2.6.3 Instruments

- ELISA plates.
- Microplates covers (lid, aluminium foil).
- Adjustable micropipettes with different volumes and tips.

- An incubator with shaker.
- ELISA reader with computer.

2.6.4 Test procedure

The test was carried out according to manufacture instructions as follows:

2.6.4.1 Deposit of the sera

The samples were diluted and incubated with the monoclonal anti-Mmm SC antibody (Mab) in pre-dilution plate.

a) Dilution of the sera

- Hundred µl of dilution buffer 24 were dispensed in all wells of Pre-Plate.
- Eleven µl of the 3 control samples CP++ were dispensed in B1, B2, C1, C2 and CP+ in D1, D2, E1, E2 and CN in H1, H2.
- Eleven µl of dilution buffer 24 were dispensed in the wells A1, A2.- Eleven µl of the samples in the other wells (A3 to H12). According to the plate layout (Figure 2).

b) Reconstitution and deposit of the monoclonal antibody (Mab)

- The Mab was reconstituted with 1mL of distilled water.
- The Mab was diluted to 1/20 in dilution buffer 24.
- One hundred and ten µl diluted Mab was dispensed in all wells except A1 and A2.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cc	Cc	1									
B	CP++	CP++	11									
C	CP++	CP++	21									
D	CP+	CP+	31									
E	CP+	CP+	41									
F	Cm	Cm	51									
G	Cm	Cm	61									
H	CN	CN	71									80

Figure (2) Distribution of sera and monoclonal antibody in the microplate in competitive ELISA

Cc: Conjugate control (without serum, without Mab = 100% inhibition).

Cm: Monoclonal control (without serum = 0% inhibition).

CP++: Strong positive control.

CP+: Weak positive control.

CN: Negative control.

1: Sample no 1

2: Sample no 2

C) Incubation of the mixture serum/ monoclonal antibody

- Hundred µl of the mixture sample / Mab were transferred fromPre-plate to the coated plate.
- The plate was covered and incubated at 37oC on shaker for 1 h.

2.6.4.2 Washing:

- The wash concentrate 20x was diluted in 1900 ml distilled Water and this solution called wash solution.
- The plate was washed 3 times with wash solution and dried.

2.6.4.3 Deposit of the conjugate:

- The conjugate was diluted to 1/100 in dilution buffer 24.
- Hundred µl of the diluted conjugate were dispensed in all wells.
- The plate was covered and incubated at 37oC on shaker for 30 ±3 min.

2.6.4.4 Washing

The plate was washed 3 times with wash solution and dried.

2.6.4.5 Revelation

- Hundred µl of the revelation solution 3 were dispensed in all wells.
- The plate was incubated for 20 min at 37oC in the dark.
- The reaction was stopped by addition of 100 µl stop solution to all wells.

Finally the plate was read at 450 nm.

The reader was connected to a computer loaded with ELISA Data Interchange (EDI) software, which was used to automate the reading and calculation of percentage of inhibition (PI) values. The optical density (OD) values were converted to percentage inhibition

by using the following formula:

P1 = 100x [(optical density of monochnal control – optical density of the test) / (optical density of monoclonal control – optical density of

the conjugate control)].

P1 = 100 x [(ODcm – OD test) / (ODcm- ODCc)]

Sera showing P1 equal to or lower than 40% were considered negative and those showing P1 equal or greater than 50% were considered positive.

2.7. Latex agglutination:

2.7.1. Reagents:

The product name is BoviLAT is a latex agglutination test for rapid detection of antibodies against*Mycoplasma mycoides subsp. mycoides SC*, the causative agent of contagious bovine pleuropneumonia (CBPP).

2.7.2 Method:

The method was used according to the producer instructions (Veterinary Laboratory Agency, UK).

After the coated latex microspheres were mixed with positive test serum (from different areas) , an agglutination reaction occurred , the rapidity extent of whish depending upon the amount of antibodies .for the purposes of the present study , a four_ point scale was adopted , exhibited pictorially in (Annex 2) , and defined as heavy flocculent precipitates forming within 1 min (clear background) (3[+++]) , heavy flocculent precipitates taking 2 min to form (clear background) (2[++]) , light flocculent precipitate against mostly clear background (1 to 3 min) (1[+]) , or no precipitate , cloudy homogeneous background (0n[-]) . **(Appendix 3)**

10ml from blood or serum and the same amount from reagent then shake. After 10 sec read the result; appearance of agglutination means positive result and absence of agglutination means negative.

2.8 .Data Analysis

Beside collection of the samples questionnaire was conducted and filled by the owners of the farms and pastoralists. Data were classified, filtered, coded using MS Excel and transferred to Statistical Package for Social Sciences Software (spss) version 22. Descriptive statistics was performed to summarize seroprevalence of CBPP for all sites together and at individual level. A two-stages 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 assess risk factors at feedlots andanalyze the relationship between the supposed causal factor and the disease. Sig > 0.05 was considered statistically not significant in all cases. Any factors had a significant less than o.05 enter to analyze by using multiple logistic regression to know the association between the disease and the factors and a P. value < 0.05 considered significant.

2.9 Receiver Operating Characteristic (ROC) analysis

ROC curve is an incredibly useful tool in evaluating and can also be used to compare the diagnostic performance of two or more laboratory diagnostic tests (Griner et al., 1981). In ROC curve the true positive rate (sensitivity) is plotted in function of the false positive rate (100-specificity) for different cut-off points. Each point on the ROC curve represents a sensitivity/specificity pair corresponding to a particular decision threshold.

ROC curve was used to determine cut-off point to Assess ELISA reading result and to compare between LATEX and c.ELISA tests.

Chapter three

Results

3.1 Seroprevalence of Antibodies (Abs) against contagious bovine pleuropneumonia (CBPP):

During this study a total of 386 serum sample were collected from four projects, two markets, traditional farm and from experimental cattle in (CVRL), Therewere surveyed from three governorates and four localities in Khartoum State to assess the presence ofAbs against CBPP and to determine risk factors of CBPP.

3.2The prevalence of CBPP according to seroprevelence and risk factors of CBPP

3.2.1 Areas:

Theseroprevalence of CBPP in the four sectors is given in table (4). There was an average sero- prevalence of 45.3%. It was higher in projects (45.7%) than other sectors. The prevalence of CBPP in the other sectors was markets (27.4%), traditional farms (20.6%), CVRL (6.3%)(Fig.3).Person Chi – square test showed no significant association, the P- value was (0.162)(table 4).

3.2.2Herd structure:

Of 386 animals, 287 cattle were mixed with other animal species, about 80.0% of them were positive.

The reminder 98samples was none mixed with other animals, about19.4% of them were positive (Fig.4).

Person Chi – square test showed significant association, the P- value was (0.029) (table 4).

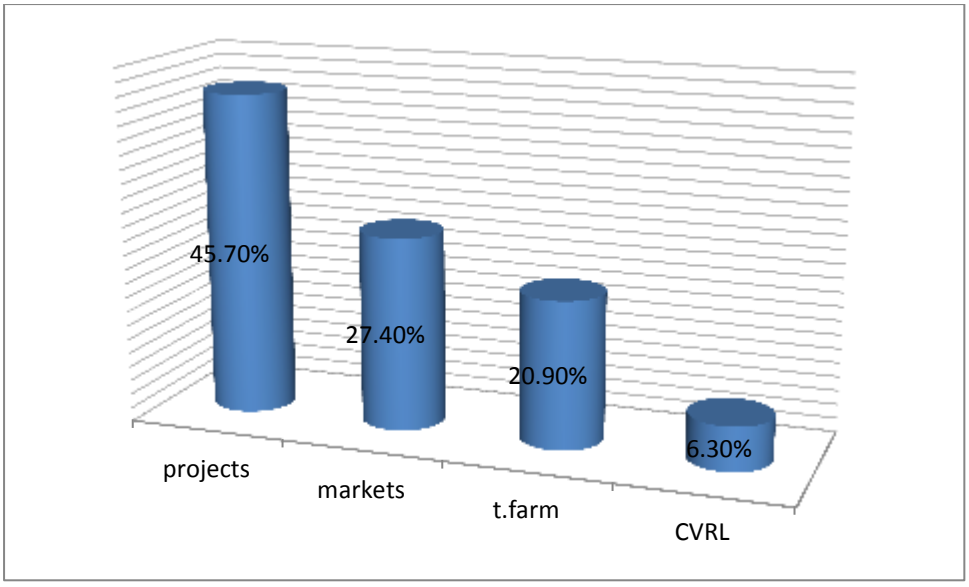


Fig. (3) Prevalence of CBPP in the four sectors

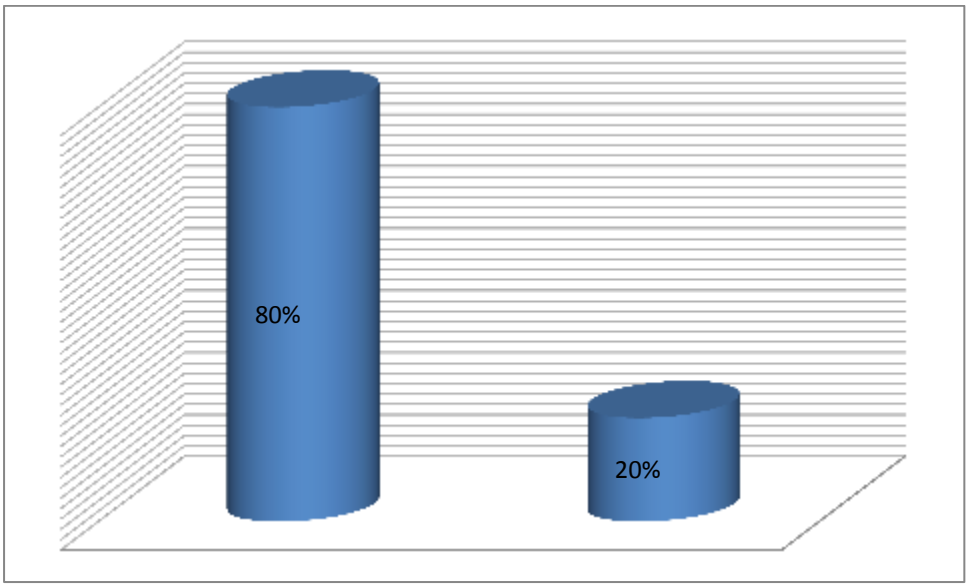


Fig. (4) Prevalence of CBPP according to the herd structure

3.2.3Breed

179 of all samples was hybrid the ratio of positive Abs incidence was 53.7% where 207 was local and the ratio of positive Abs incidence 46.3% (Fig.5). Person Chi – square test showed significant association, the P value was (0.018) (table 4).

3.2.4 Herd size

Of 386 animals, 161 sample their herds size was more than 300; the positive Abs ratio was 32.6%, 101 their herd's size was less than 100 the positive antibodies ratio was 28.6%, 124 their herd's size was (300-400) Abs ratio 38.9% (Fig.6).Person Chi – square test showed significant association to CBPP, the P value was (0.003) (table 4).

3.2.5Ages

260 from all samples (386) their ages were around 18 monthes and above, their Abs ratio was 81.1% and 126 samples their ages under 6 monthes, their positive Abs to CBPP ratio was 18.9%(Fig.7).Person Chi – square test showed significant association, the P value was (0.001) (table 4).

3.2.6Sex

Out of 386, 225 from all samples were females; positive Abs ratio to CBPP in them 68.6 % the remainder 161 samples was taken from males, Abs ratio 31.4 %(Fig.8).Person Chi – square test showed significant association, the P- value was (0.038) (table 5).

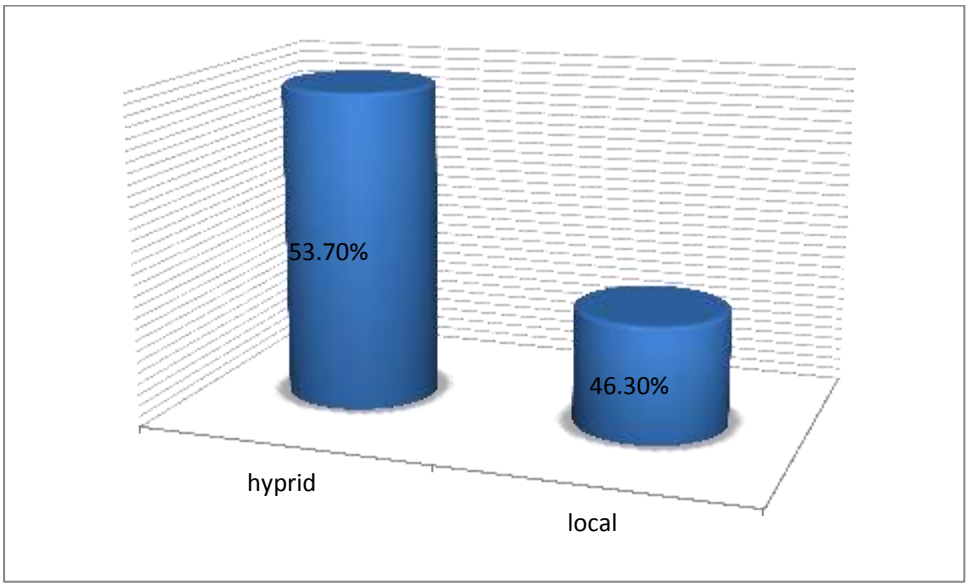


Fig.(5) Prevalence of CBPP in local and hybrid breeds cattle

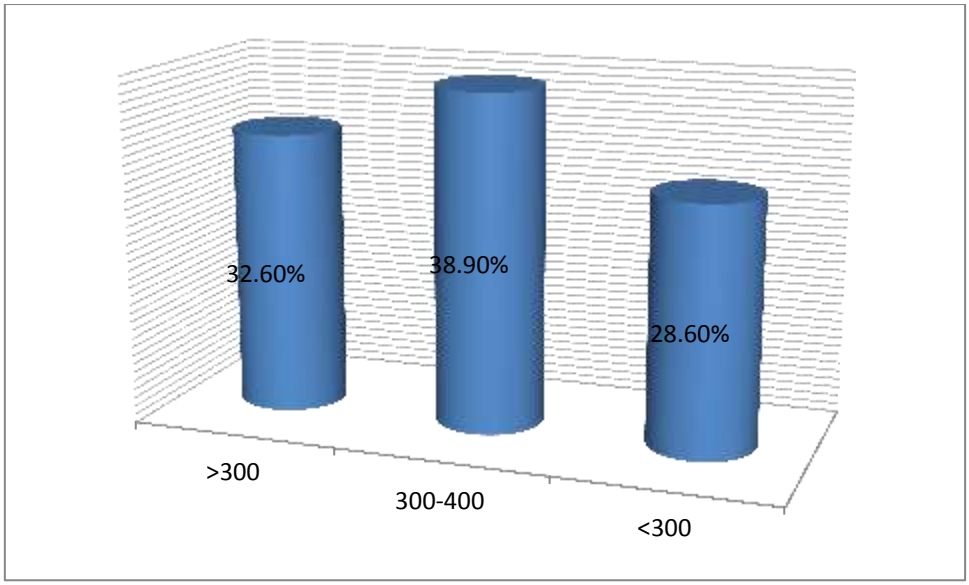


Fig.(6) Prevalence of CBPP according to the herd size

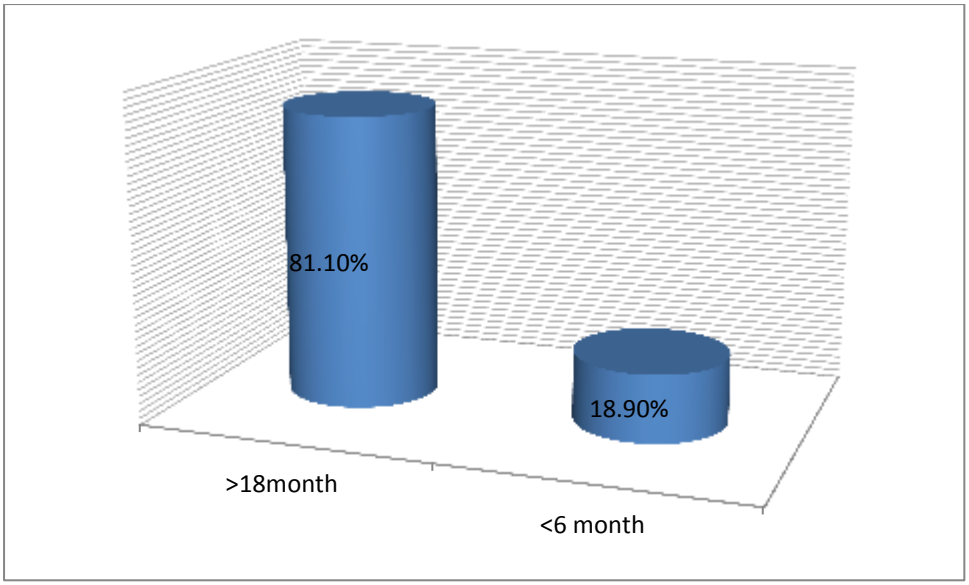


Fig.(7) Prevalenceof CBPP according to the age of cattle

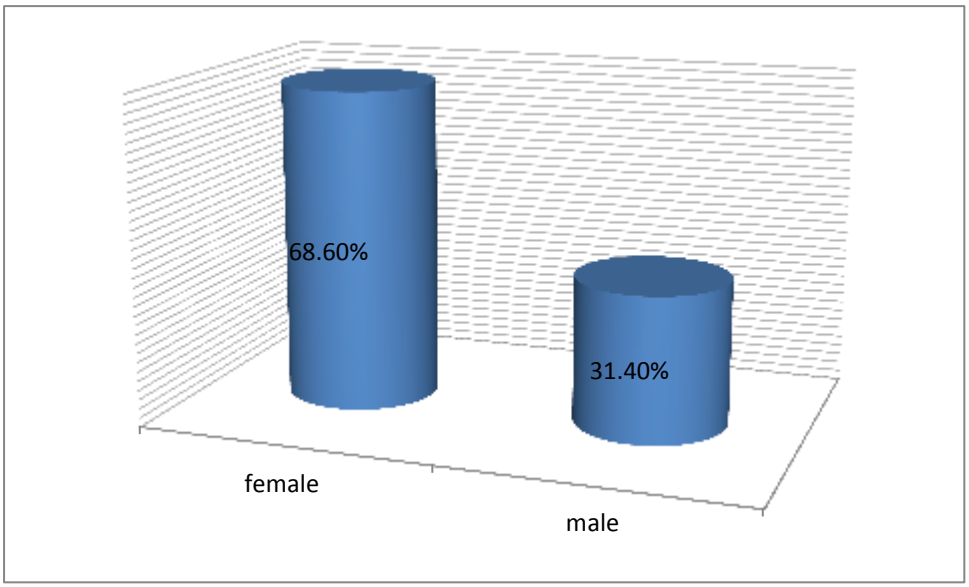


Fig.(8) Prevalence of CBPP according to the sex of cattle

3.2.7 Vaccinations

300 of all samples were not vaccinated and the positive incidences of Abs to CBPP were 78.9%. The remained samples were vaccinated and 21.1% was the positive ratio of Abs against CBPP in them (Fig.9).Person Chi – square test showed no significant association, the P value was (0.625) (table 4).

3.2.8 Last vaccination

From the 386 samples 86 were taken; their last vaccination history fall on the period of time from 6 to 12 month, and the incidence of positive Abs against in those samples was 68.0%, 174 samples their last vaccination history fall on the period of time more than 12 month, and the incidence of positive Abs against in those samples was32% (Fig.10).Person Chi – square test showed significant association, the P- value was (0.001)(table 5).

3.2.9Vacciner:

Out of 386 samples 335 samples were followed vaccination program with quality assure team 41.8% of them were positive and 58.2% of them were negative; from 51 samples which had not following vaccination program with quality assure team 68.6% of them were positive and 31.4%% of them were negative(Fig.11).

Person Chi – square test showed significant association, the P- value was (.000) (table 4).

3.2.10Introducing or entering of cattle

359 animals which introduced or entered cattle to other herds 93.1% of them had positive Abs incidence. Where 27 samples had not introduced or entered new cattle to their herd, 6.9% of them had positive Abs incidence (Fig.12). Person Chi – square test showed no significant association, the P- value was (0.923) (table 4).

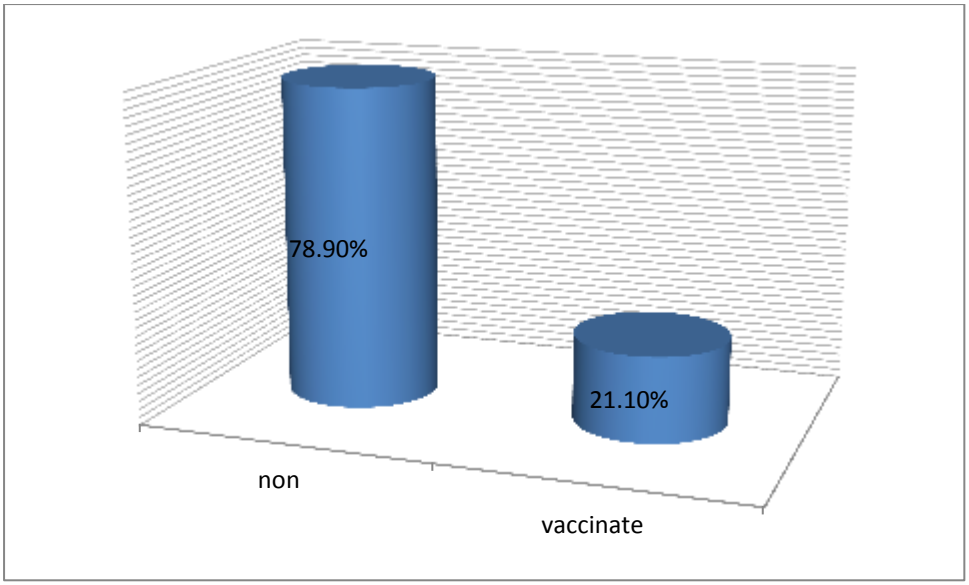


Fig.(9) Prevalence of CBPP according to the vaccination status of cattle

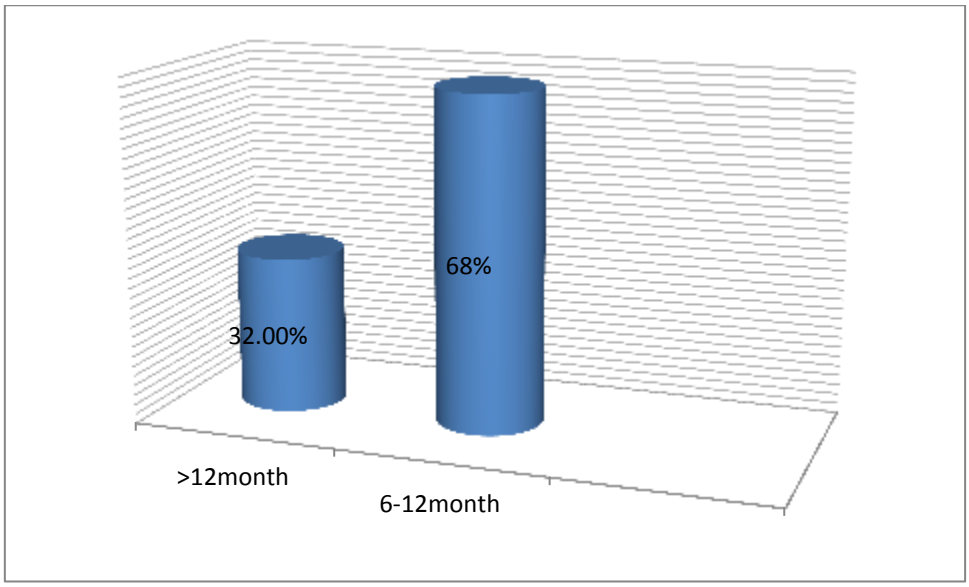


Fig.(10) Prevalenceof CBPP according to the vaccination history of cattle

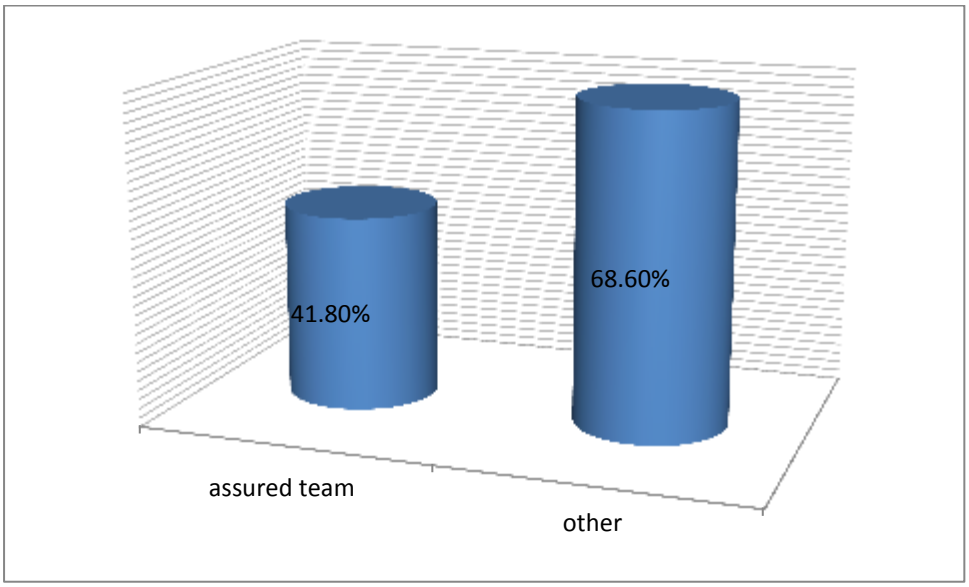


Fig.(11) Prevalence of CBPP according to the vacciner of cattle

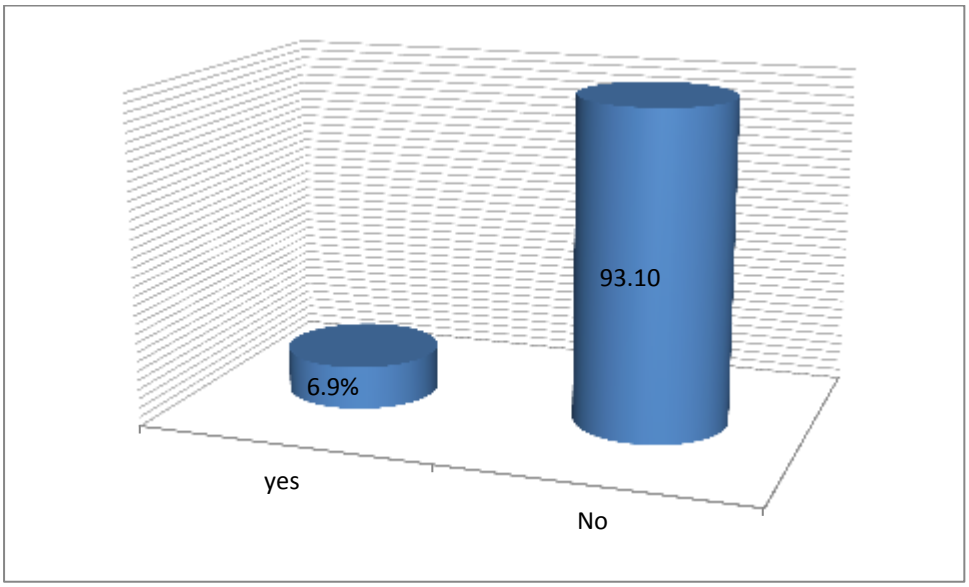


Fig.(12) Prevalence of CBPP according to the introducing of cattle to other herd

3.2.11 Exposuring

351 of all samples were exposured to another herd about 98.1% were positive Abs reading.35 of the samples were not exposed to another herd about 10.9% was positive Abs reading (Fig 13). Person Chi – square test showed no significant association, the P value was (0.265) (table 4).

3.2.12Pass of nomads

376 of the samples did not undergo regular trip or movement of nomads about 96.6% was positive Abs reading .While 10 the sample underwent regular passage of nomads about 3.4% was positive Abs reading(Fig.14),. The Person Chi – square test showed no significant association, the P- value was (0.345).

3.2.13Sharing other herd in grazing land:

From all samples 348 of animals were sharing other herd in grazing land (87.4%) animals were positive Abs reading. About 38 samples were not sharing other herd in grazing land about (12.6%) animals were positive Abs reading(Fig.15), the Chi – square test showed there is no significant association, and the P- value was (0.101)(table 4).

3.2.14Community knowledge:

204 samples of animal keepers were aware about the disease,(65.7%) animals had positive Abs reading, While 182 samples were not aware of the disease, 34.3% of their samples had positive Abs reading (Fig 16). Person Chi – square test showed significant association, the P- value was (0.001) (table 5).

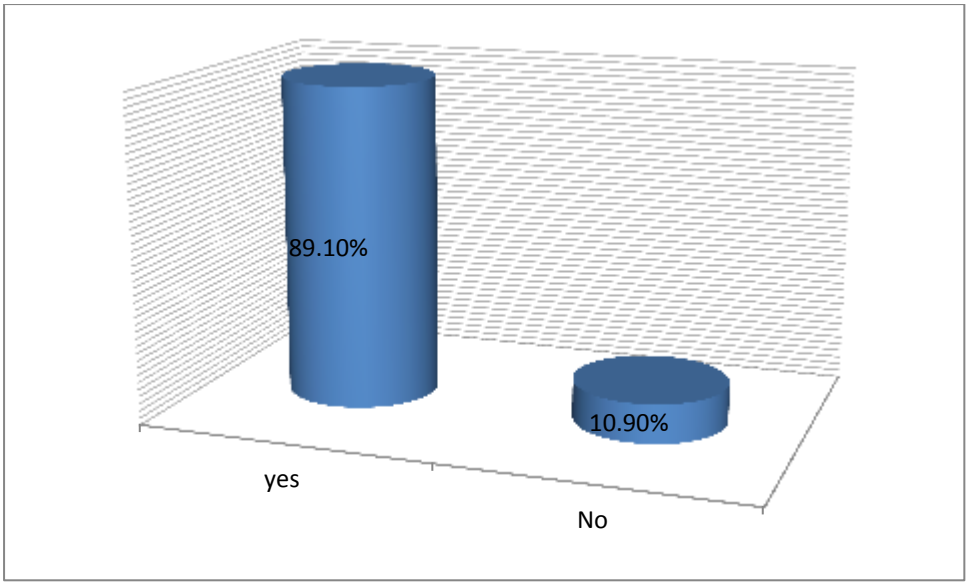


Fig.(13) Prevalence of CBPP according to the exposuring of the cattle

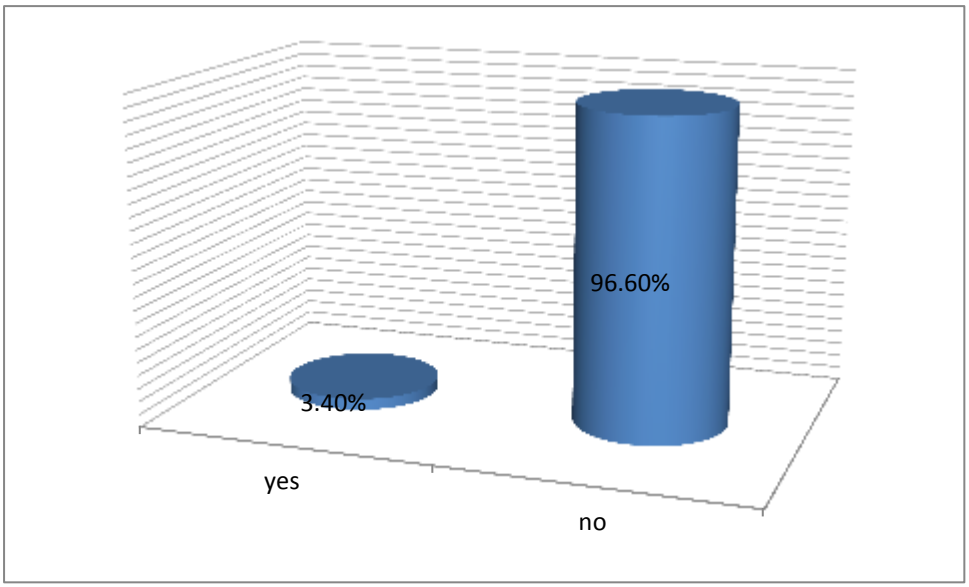


Fig.(14) Prevalence of CBPP according to the passage of nomads

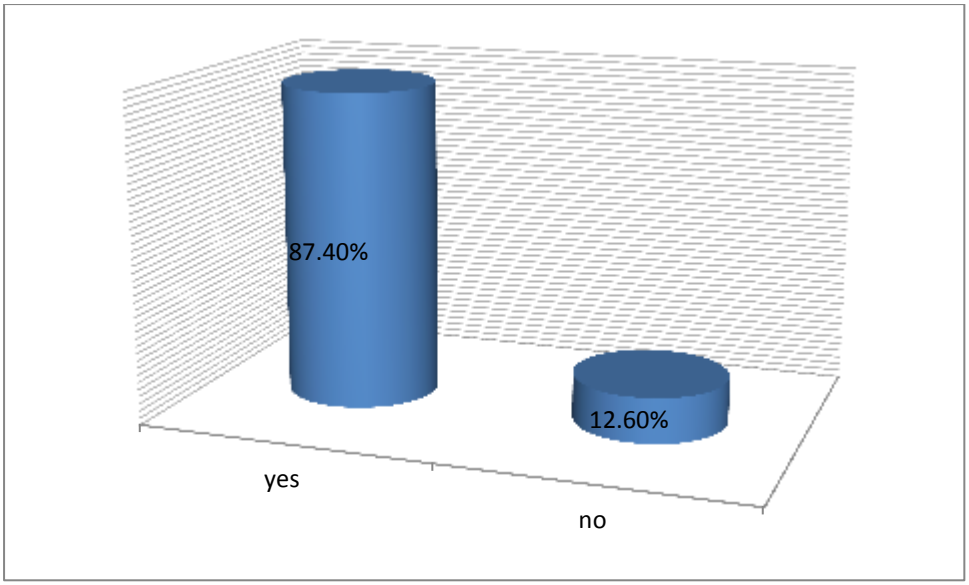


Fig.(15) Prevalence of CBPP according to the cattle sharing other herd in grazing land

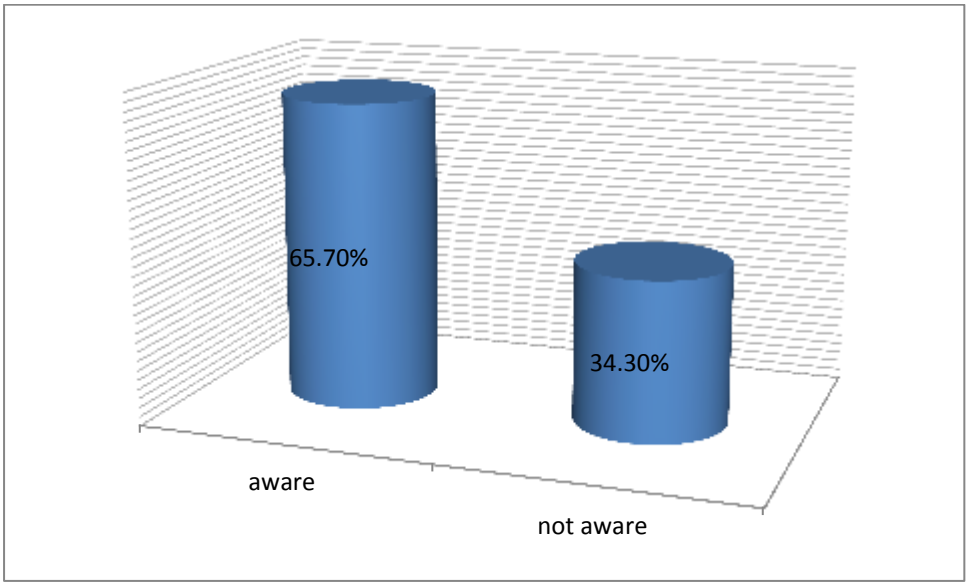


Fig.(16) Prevalence of CBPP according to the community knowledge

3.2.15 Antibiotics treatment:

360 sample of the population had received antibiotic treatment,93.7% of the treated samples had positive Abs reading, while the remainder samples 26were not treated with antibiotic 6.3% of them had positive Abs incidence(Fig.17).

Person Chi – square test showed no significant association, the P- value was (0.748) (table 4).

3.2.16 Number of sick animal:

268 samples from all population in which a number of sick animal >50% , the incidence of Abs in those samples was 70.3%. While 118 samples of the population the number of sick animal was <50% and the incidence of Abs in those samples was 29.7% (Fig.18).

Person Chi – square test showed no significant association, the P- value was (0.740) (table 4).

3.2.17 Number of dead animals:

180 samples of the population the number of dead animals was >50% and the incidence of Abs in those samples was (56.6%), while 206 samples of the population the number of dead animals was <50% and the incidence of Abs in those samples was (43.4%)(Fig.19).

Person Chi – square test showed no significant association, the P- value was (0.004) (table 5).

3.2.18 Neighbouring health Status:

123 sample of the population had poor neighbouringhealthStatus, and the incidence of Abs in those samples was 33.7% While 263 samples of the population had fair surrounding heath status, and the incidence of Abs in those samples was66.3 %(Fig.20).

Person Chi – square test showed no significant association, the P- value was (.478) (table 4).

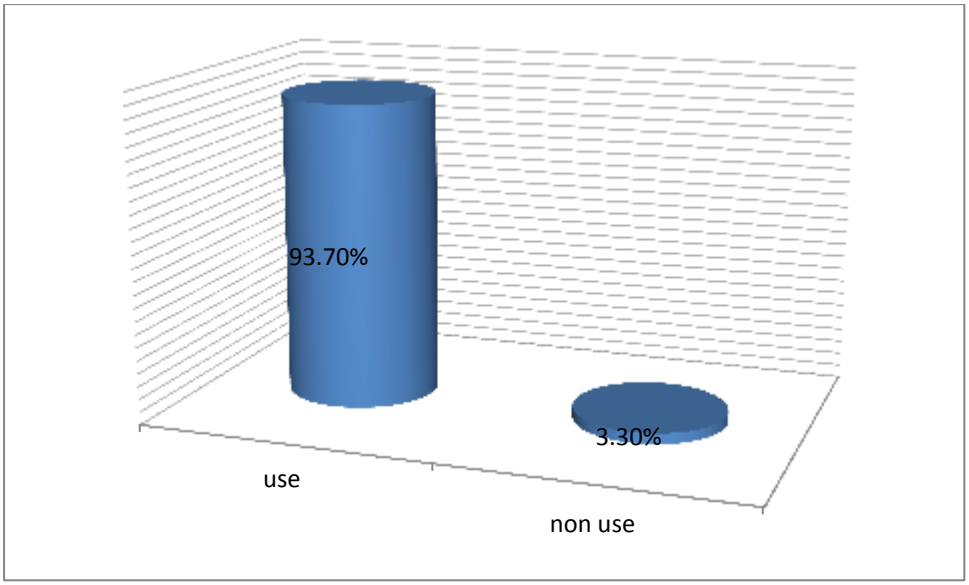


Fig.(17) Prevalenceof CBPP according to the antibiotic treatment of the cattle

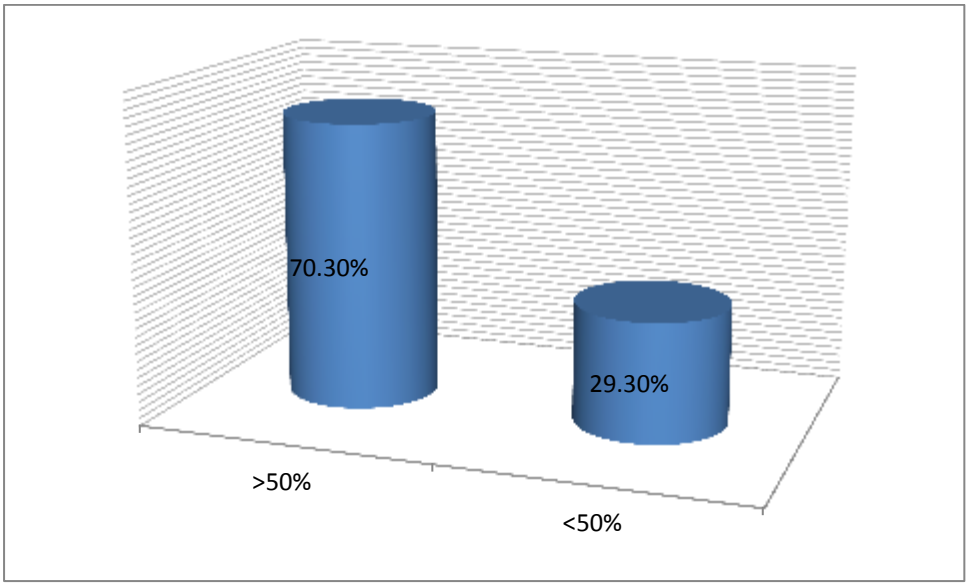


Fig.(18) Prevalence of CBPP according to the number of sick animal

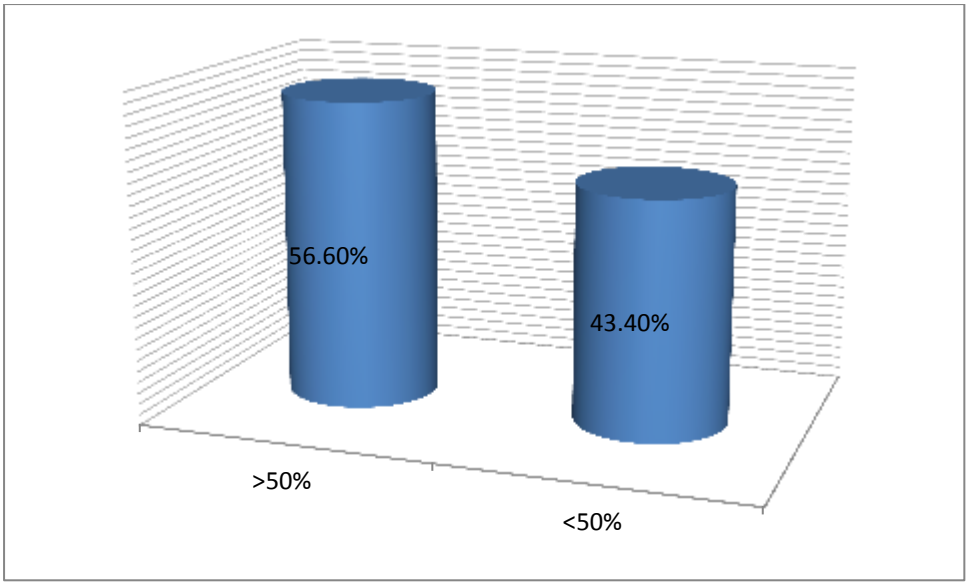


Fig.(19) Prevalence of CBPP according to the number of dead animal

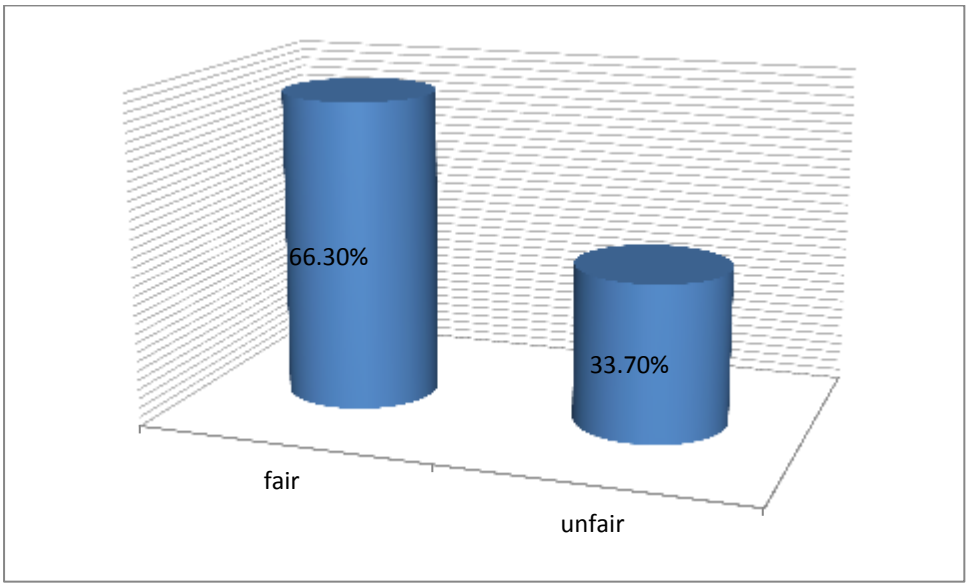


Fig.(20) Prevalence of CBPP according to the neighbouring animal health status

3.2.19 Sale or transfer:

348 sample of the population underwent sale or transfer process and the incidence of Abs in those samples was 88.6%.While only 38 sample of the population did not undergo sale or transfer process and the incidence of Abs in those samples was11.4% (Fig.21).

Pearson Chi – square test showed no significant association, the P- value was (0.341) (table 4).

3.3 LATEX result:

From 140 samples checked by latex agglutination test 100 of them were positive and the remainder were negative (Fig.22).

3.4 LATEX &c.ELISA contrast:

On 140 samples checked by two tests c.ELISA and LATEX agglutination test 107 samples had same result in two tests, 33 samples hadcontras result by comparing in two tests; since 30 samples which were negative in

LATEX testthey were positive in c.ELISA. While 3 samples showed opposite, result they were positive in LATEX and were negative in c.ELISA (Fig.23).

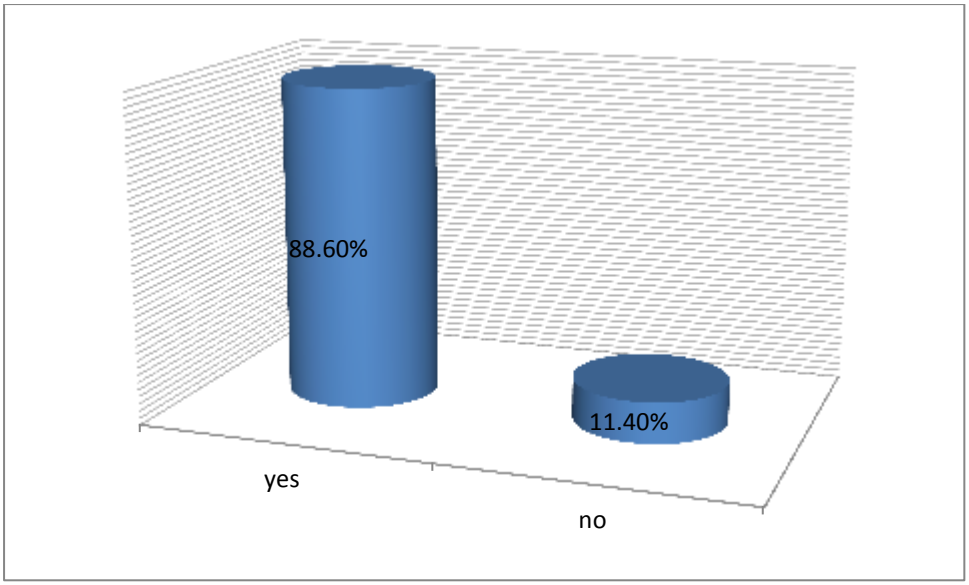


Fig.(21) Prevalence of CBPP according to the selling or transferring of cattle

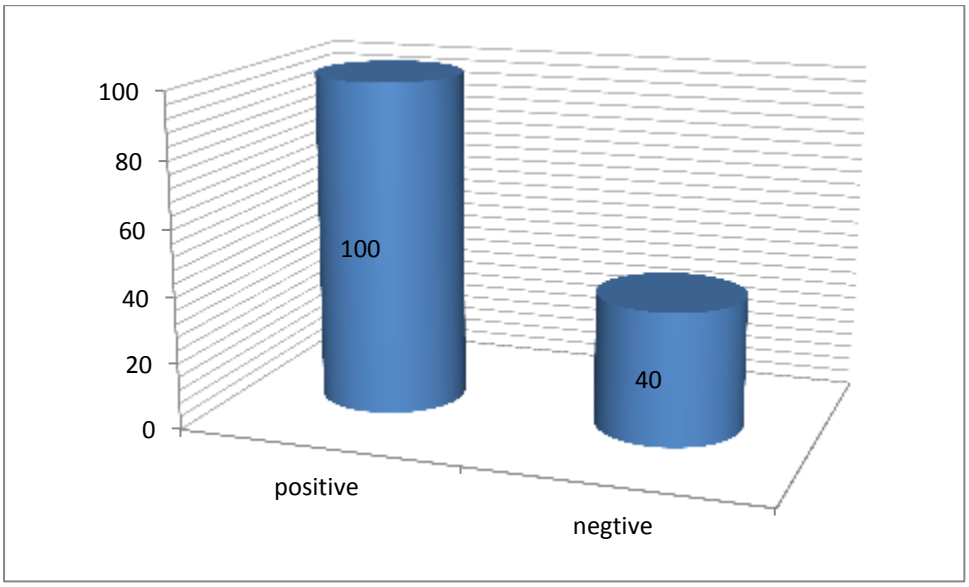


Fig.(22) Prevalenceof CBPP according to the latex result

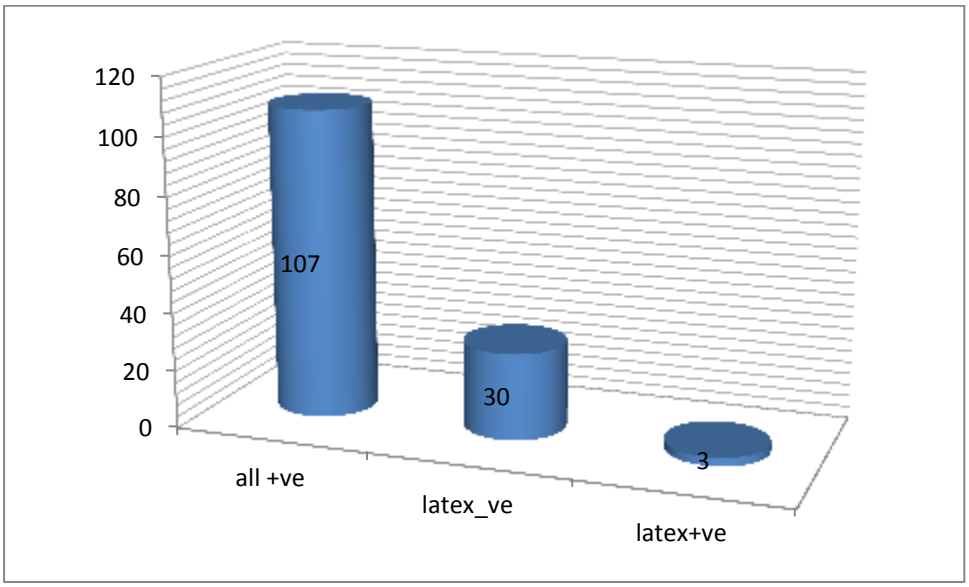


Figure (23) compromision ofc.ELISA and LATEX agglutination test

3.5 Univariate and multivariate analysis:

3.5.1 Univariate analysis:

All factors which had a significant less than 0.05 associated and increasing the risk of CBPP in Khartoum state. The result indicated that areas (sig = 0.162), herd structure (sig = 0.29), breed (sig = 0.018), age (sig = 0.01), herd size (sig = 0.003), sex (sig = 0.003), vaccination (sig = 0.625),last vaccination (sig = 001), vacciner (sig = 000) sharing in grazing land (sig = 0.101), health of introduced cattle (sig = 0.170), exposure to another herd(sig = 0.265) ,community knowledge (sig =0.001) and number of died animals (sig = 0.000) had statistically significant effect of the CBPP seroprevalence, however other factors which were antibiotic treatment, neighbouring health status, number of sick animals, introducing cattle and transferring cattle did not have significant effect on seroprevalence of the disease. (table 4)

3.5.2. Multivariate analysis:

In multivariate analysis, multiple logistic regression used, all factors had a significant less than 0.05 were entered in the analysis. The result showed that there is significant association between the disease and areas (sig = 0.034), herd size (0.135), sex (0.083), last vaccination (0.001) number of dead animals (sig =0.004). (Table 5)

Table (5): Crosstabs of ELISA test result and risk factors

Variable		No of tested	No of positive	% of positive	Chi	P value
Areas	Project	157	80	45%	5.137	0.162
	CVRL	26	11	6.3%		
	Traditional farm	76	36	20.6%		
	Markets	127	48	27.4%		
herd structure	Mix	287	140	80%	7.058	0.29
	Non	98	34	19.4%		
Breed	Hybrid	178	94	53.7%	8.0570	.018*
	Local	207	81	46.3%		
herd size	>300	161	57	32.6%	11.632	.003*
	<100	101	50	28.6%		
	300-400	124	68	38.9%		
Age	>18 month	259	141	80.6%	26.76	0.01*
	< 6 month	125	33	18.9%		
Sex	Female	225	120	68.6%	13.919	0.003
	Male	161	55	31.4%		
Vaccinations	Vaccinated	86	37	21.1%	.239	.625
	Non vaccinated	300	138	78.9%		
Last vaccinations	>12 month	174	56	32%		0.001*
	6-12month	86	58	68%		
Vacciner	Qualified	51	35	20%	12.863	0.000*
	Other	335	140	80%		

Continue table4:

introduce or buy of cattle	**Yes**	**359**	**163**	**93.1%**	**.009**	**.923**
	No	**27**	**12**	**6.9%**		
Exposuring	**Yes**	**351**	**153**	**44.4%**	**1.244**	**0.265**
	No	**35**	**19**	**45.6%**		
Sharing other herd in grazing land:	**Yes**	**348**	**153**	**87.4%**	**2.682**	**0.101**
	No	**38**	**22**	**12.6%**		
Community knowledge:	**Aware**	**204**	**115**	**65.7%**	**21.262**	**0.001***
	Not aware	**182**	**60**	**34.3%**		
antibiotics treatment:	**Yes**	**360**	**164**	**93.7%**	**0.103**	**0.748**
	No	**26**	**11**	**6.3%**		
number of sick animal:	**>50**	**268**	**123**	**70.3%**	**0.110**	**0.740**
	<50	**118**	**52**	**29.7%**		
number of dead animal:	**>50**	**180**	**99**	**56.6%**	**12.708**	**0.000***
	<50	**206**	**76**	**43.4%**		
Neighbouring health Status:	**Fair**	**263**	**116**	**66.3%**	**0.504**	**0.478**
	Poor	**123**	**59**	**33.7%**		
sale or transfer	**Yes**	**348**	**155**	**88.6%**	**0.905**	**0.392**
	No	**38**	**20**	**11.4%**		

*significant association between factor and disease

Table (5): logistic regression analysis results

Variables	No tested	%of positive	Sig.	95% C.I	
				Lower	Upper
Area			.034		
Project	157	45%	.503	.133	61.321
CVRL	26	6.3%	.009*	.041	.634
Traditional farm	76	20.6%	.019*	.037	.747
Herd structure			.325		
herd structure(mix)	287	80%	1.000	.000	.
herd structure(non)	98	19.4%	1.000	.000	.
Breed			.641		
Breed(hybrid)	178	53.7%	1.000	.000	.
Breed(local)	207	46.3%	1.000	.000	.
Herd size			.135		
herd size(>300)	161	32.6%	.146	.278	1.209
herd size(<100)	101	28.6%	.083*	.239	1.092
Age			.001*		
age(>18 month)	259	80.6%	.264	.007	3.944
age(< 6 month)	125	18.9%	.765	.025	15.069
sex(Female)	225	68.6%	.038*	1.043	4.619
Last. Vaccination			.001*		

Continue table 5:

Last. vaccination(>12 month)	**174**	**32%**	**.973**	**.250**	**4.198**
Last. vaccination(6-12 month)	**86**	**68%**	**.005***	**1.926**	**36.258**
vacciner(qualified)	**51**	**20%**	**.917**	**.235**	**4.999**
health of introduce cattle(fair)	**88**	**26.3 %**	**.259**	**.777**	**2.554**
Exposure to another herd(yes)	**351**	**44.4 %**	**.999**	**.000**	**.**
Share in grazing drinking(yes)	**348**	**87.4 %**	**.999**	**.000**	**.**
Community knowledge(aware)	**204**	**65.7 %**	**.268**	**.290**	**1.411**
Number of died(>50)	**180**	**56.6 %**	**.004***	**1.716**	**18.732**

*significant association between factor and disease

Chapter four

Discussion

During this study a total of 386 serum sampleswere collected from four projects, two markets, traditional farms and from experimental cattle in (CVRL),thesurvey was done in the three governorates and four localities in Khartoum state.

The prevalence of CBPP in the four sectors is given in (table 4). There was an average sero- prevalence of 45.3%. It was higher in projects (45.7%) than other sectors. The prevalence of CBPP in the other sectors was markets (27.4%), traditional farms (20.6%), CVRL (6.3%) by using c.ELISA test and it was higher than those reported in previous study, where the prevalence of CBPP in Khartoum state was 20.9% (Ibrahim, 2016) and 17.19 % (IbtisamElsadig, 2012). The present study showed high percentage in projects sectors that may be due to owner's opinions and difficulty to exclude with permanent coexist of infected cattle.

In this study there was positive association between herd structure and the present prevalence of the disease, the P- value was (0.029).

Also there was positive association between disease and breed since inhybrid the ratio of Abs incidence was 53.7% where 207 was in local breed and the ratio of Abs incidence 46.3%, the P- value was (0.018).this means the local breed had more tolerance to the disease .

This study also showed that high herd size had positive association with the disease since, of 386 animals, 161 sample their herds size was more than 300 the Abs ratio was 32.6%, 101 their herds size the antibodies ratio was 28.6%, 124 their herds size was (300-400) Abs ratio 38.9%, the P- value was (0.003); this is in contest with last study which indicated that the prevalence of the disease is reduced when the herd size increased. (IbtisamElsadig, 2012)

The present study showed that the age of the animals was associated with the CBPP, the disease had low occurrence in animals less than six month and the disease prevalence increase with age ; since the result showed 260 from all samples their ages around 18 monthes and above their Abs ratio was 81.1%,And 126 samples their ages under 6 monthes their Abs ratio was 18.9%, the P- value was (0.001).this result agrees with last study which supposed that there was association between the disease and the immunity of the animal which acquired it from his dam, also may indicate that the duration of exposure to the causative agent is too longer in older animals.

The present result showed statistically significant association of sex, since it was found 225 from all samples were females Abs ratio in them 68.6 % the remainder 161 samples was taken from males, Abs ratio 31.4%, the P value was (0.000); this result agrees with last study which found result females were more affected than males with percentage of 21.14% and 13.6% respectively (Ibrahim, 2016).

The present study showed that the prevalence of the disease is higher in animals which were reported to have previous CBPP vaccination compared with animals that were not vaccinated; since300 of all samples were not vaccinated and the ratios of Abs were 78.9%. The remained samples were vaccinated and 21.1% was the ratio of Abs in them, the P value was (0.625).this agrees also with last study which reported (25%) with previous CBPP vaccination compared with animals did not vaccinated (14.58%)(IbtisamElsadig, 2012)

The result agrees with the suggestion that the owner did not vaccinate their animals regularly so, the immunity may reduced and animals become susceptible to infection, the immune responses measured by c- ELISA following vaccination wane after three months(Shallali ,1998). The other reason may be the dosage which given to animals was not accurate or due to bad storage or transportation of the vaccine and this result is in agreement with previous study in Kenya (Niwael, 2009). this suggestion supported with two

following factors which intended in this study while it was found 86 samples were collected from animal; their last vaccination history fall on the period of time from 6 to 12 month, and the incidence of Abs in those samples was 68.0% ,174 samples their last vaccination history fall on the period of time more than 12 month, and the incidence of Abs in those samples was 32%,; the P- value was (.001),the other factor studied was the vacciner since which founded that of 386 samples 335 samples followed vaccination program with quality assure team, 41.8% of therewere positive and 58.2% of there were negative; from 51 samples which did not follow vaccination program with quality assure team 68.6% of them were positive and 31.4%% of them were negative, this agrees with last study which pointed out to awareness which should be created among animal owners on the protection period of the vaccine and the need for at least annual vaccination. A high number of animals that reacted adversely to vaccination were treated with antimicrobials. There was no revaccination of animals treated for post-vaccination reactions; this could lead to a high proportion of non protected animals following the killing of the vaccine by the antimicrobials used for treatment. This category of animals should be revaccinated (Ibrahim, 2016).

The present result showedno statistically significant association with introducing of cattle, health of introduced cattle, exposuring and passing of nomads where the p-value was (0.923),(0.170),(0.265),(0.345) respectively .

The present result showed low statically significant association with sharing other animals in grazing land (p-value 0.101) this disagrees with last study of IbtisamElsadig (2012).

The present result showed statically significant association with community knowledge (p-value 0.001) and number of dead animals (p-value 0.004).

There were no statically association with antibiotic treatment (p-value 0.748) this agrees with suppose which say that veterinarian have supported the use of

antibiotic for the control of (CBPP)for animal owners use but accompanied with other control methods **(Amanfu ,2007) & (Mariner *et, al*.2006)**.

Conclusions and recommendation:

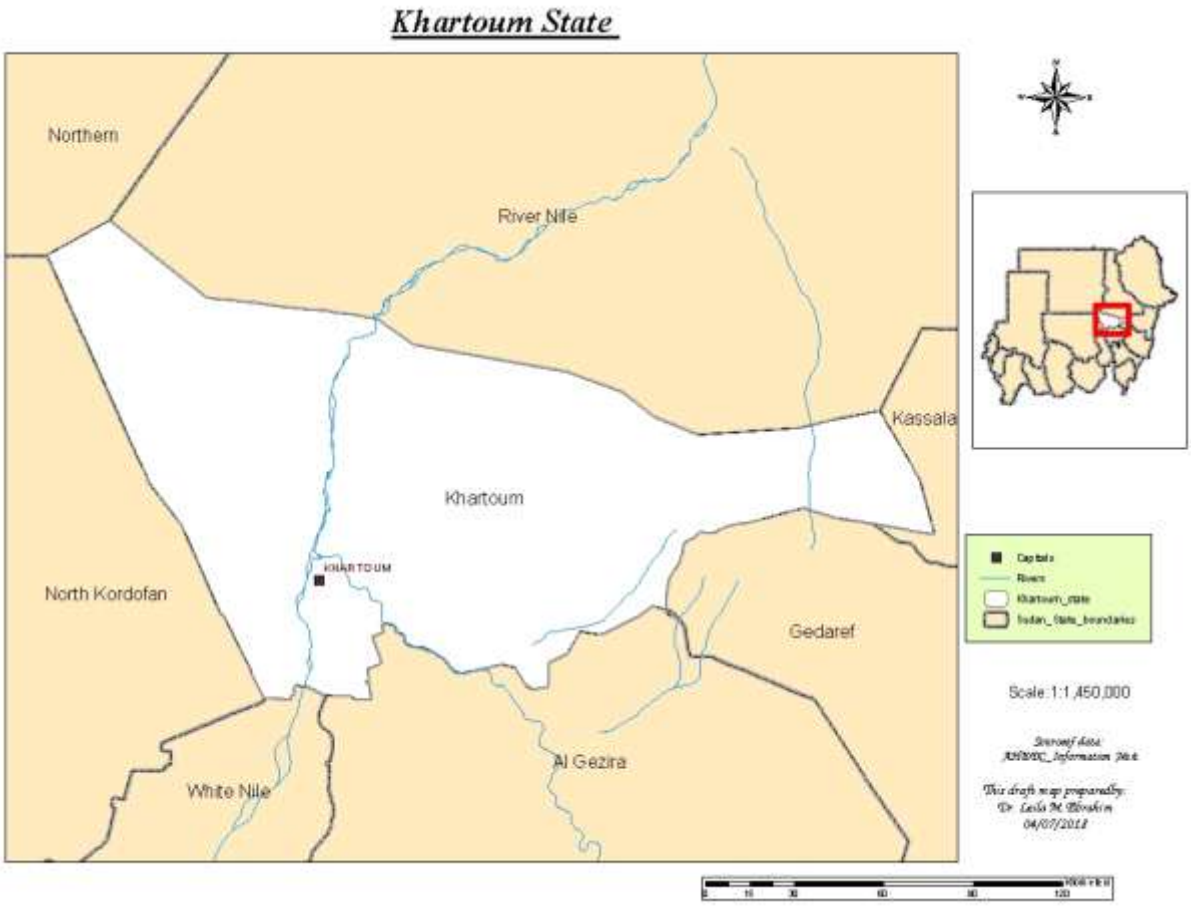
Conclusions

- Prevalence of CBPP was found 45.3% in Khartoum state.
- The significant risk factors were sex of animal, age, breed, herd structure, herd size, number of dead animal and communities' knowledge.
- Latex agglutination test was more specific in diagnosis of CBPP.

Recommendations

- Annual Survey of CBPP in the Khartoum state is recommended.
- Punctual diagnosis of CBPP with restriction of cattle movement.
- Stamping out of infected cases is very mandatory.
- Mass vaccination program should be implemented.
- More study about antibiotics treatment of CBPP should be done.
- Latex agglutination test is more specific should be adopted rather than c.ELISA.
- Encouragement of local breed production which has more tolerance to CBPP than hybrid.

THEAppendixes:



Appendix(1) Khartoum state map region of study

Appendix(2)

Questionnaireabout recognizing contagious bovine pleuropneumonia

When CBPP is suspected, the questions asked should include the following:

1) What species of animals are present on the livestock holding facility (or village)

Cattle ☐ sheep ☐ goats' ☐ animals ☐

2) How many of each is present

Less than 100 ☐ 100-200 ☐ 200-300 ☐ more than300 ☐

3) What ages of cattle/domestic buffaloes are affected?

Under 6 months ☐ over 18 months ☐

4) A. Have the cattle been vaccinated against CBPP or other epidemic diseases

Yes ☐ **no** ☐

B. If yes, when did the last vaccination take place?

6-12 month ☐ more than 12 month ☐

D. Who conducted the vaccination?

Team of quality-assured CBPP vaccine

Other ☐ determine ……☐…

8) A. Have other cattle been bought or introduced for any reason during the six months before the disease was first noticed?

Yes ☐ no**.**

9). was the herd exposed to another herd, during the six months before the Disease was first noticed?

Yes ☐ no ☐

10) A. Do nomadic herds pass through the area?

Yes ☐ no ☐

11). Are grazing lands, water holes, and drinking troughs or dipping tanks

Shared with other nomadic or sedentary herds?

Yes ☐ no ☐

12). Does the community know the disease and does it have a local name?

Yes ☐ no ☐

14).A. Have the infected animals been treated with antibiotic(s)?

Yes ☐ no ☐

15). How many animals are clinically sick out of the total?.....

16). How many animals have died since the outbreak occurred?.....

17). what is the health status in neighbouring herds?.....

18). Have any animals been sold, transferred or given on loan in the last six Months, e.g. for ploughing or as gift (dowry)?

Yes ☐ no ☐

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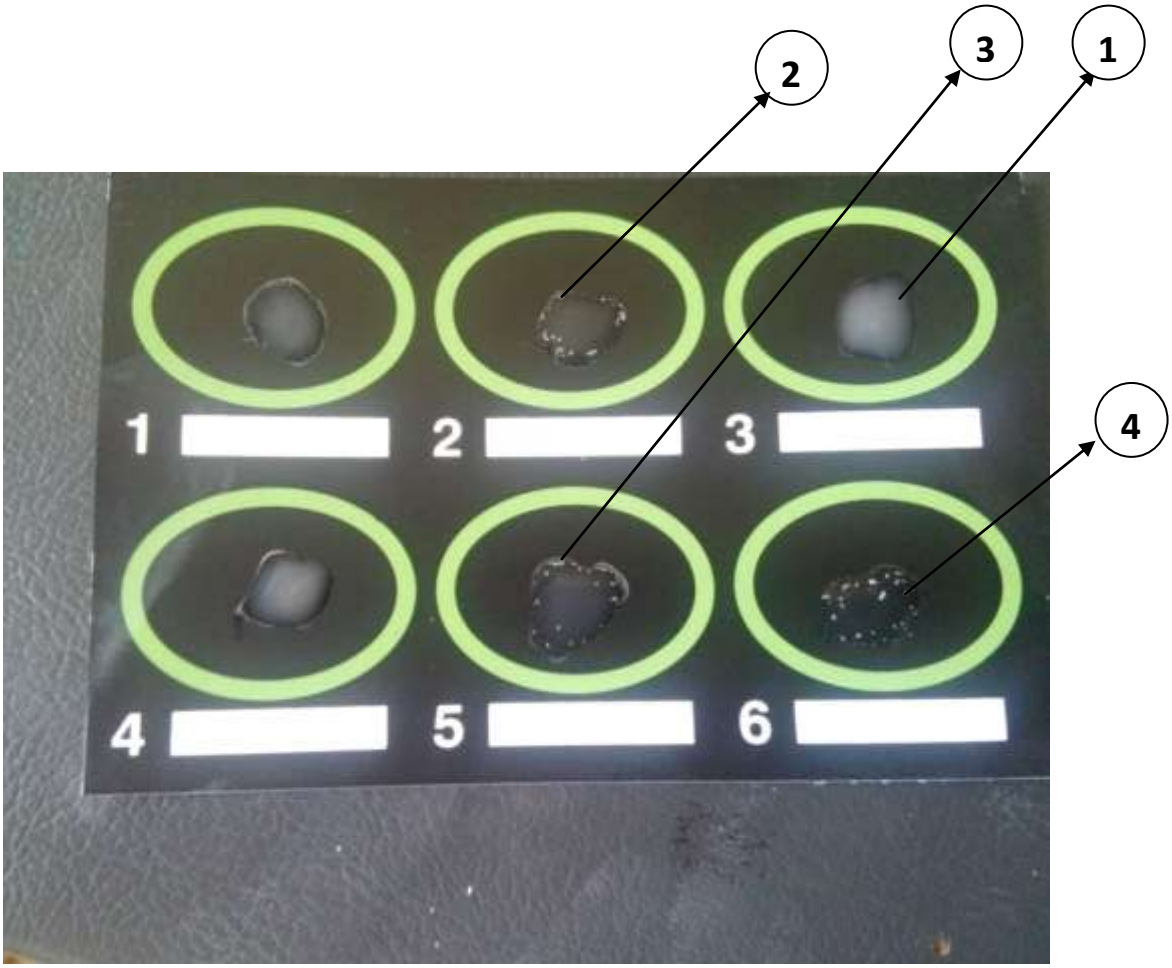

1(=negative), 2=(+positive), 3=(++positive), 4-=(+++positive)

Appendix(3) the result of latex test

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