ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
AND
FREIE UNIVERSITAT BERLIN
FACULTY OF VETERINARY MEDICINE

EPIDEMIOLOGICAL SURVEY OF CBPP IN AWI & WESTERN GOJJAM ZONES OF AMHARA REGION & COMPARISON OF CFT & C-ELISA FOR THE DIAGNOSIS OF CBPP

GASHAW TAKELE

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Faculty of Veterinary Medicine

FREIE UNIVERSITÄT BERLIN
Fachbereich Veterinärmedizin

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A thesis submitted in partial fulfilment for the degree of
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BY

GASHAW TAKELE

Board examiners:

Dr. Yilma M.	
Prof. Dr. Hellmann	

Academic Advisors:

Prof. Dr. Staak	

Dedication

This paper is dedicated to my parents: Bazet Alehegn and Takele Teferi, my girl friend Mulu Tadesse.

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Abbreviations

Abs	Antibodies
ABTS	2,2-Azino-di-(3-ethyl-bencethiazoline Sulphate)
Ag	Antigen
C	Complement
C-ELISA	Competitive enzyme linked immunosorbent assay
CBPP	Contagious bovine pleuropneumonia
Cc	Conjugate control
CFU	Colony forming units
CFT	Complement fixation test
CIRAD/EMVT	Centre de cooperation internationale en recherche agronomique le développement/département d'élevage et de médecine vétérinaire
DF	Degree of freedom
HRPO	Horse radish peroxidase
Ig	Immunoglobulin
MmmSC	Mycoplasma mycoides subspecies mycoides small colony
MmmLC	Mycoplasma mycoides subspecies mycoides large colony
MAbs	Monoclonal antibodies
MOA	Ministry of agriculture.
NHS	Normal horse serum.
OD	Optical density
PARC	Pan African Rinderpest eradication Campaign
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PCV	Packed cell volume
PI	Percent inhibition
PV	Post vaccination
SDS	Sodium dodecyl sulphate
Vs	Versus

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ABSTRACT

For assessing the prevalence and distribution of contagious bovine pleuropneumonia (CBPP) in North-Western Ethiopia, a cross-sectional questionnaire survey and sero-epidemiological investigation has been conducted in 11 selected districts of Awi and Western Gojjam zones of Amhara region. The questionnaire survey and serum sample collection was conducted from February to August, 1997.

The purpose of the questionnaire was to assess the production system, prevalence and economic importance of major cattle diseases, occurrence of bovine respiratory diseases and measures taken to deal with it. The questionnaire was given to a total of 39 farmers.

According to the questionnaire, the farmers have described bovine respiratory disease using various local names: Samba (21%), Sal (59%), Woziwuz (10%), Zihon-Wotetie (5%) and Aslig (5%) and it was ranked as a major veterinary problem. About 41% of the interviewed have reported the seasonal occurrence of the respiratory disease problem. The major sources of the disease outbreak were reported as grazing contact (51%), social factors involving cattle movement (3%), while others (about 41%) did not specify them. Measures taken in case of outbreaks: Treatment using antibiotics (69%), vaccination (23%) and traditional measures (8%).

A total of 2140 serum samples have been collected for the sero-prevalence study and tested with the standard CFT. The mean sero-prevalence for villages and districts was found to be 19.3 + 12.0% and 22.2 + 24.6% respectively and the mean for all samples was estimated to be 17.3 + 1.6%. The minimum and maximum sero-prevalence observed for villages and districts was 0 and 77.5% and 7.3 and 66.3%, respectively.

The mean sero-prevalence for Awi and western Gojjam zones was found to be 27.3 + 3.2% and 12.0 + 1.7% and this difference was statistically highly significant ($P<0.001$). The mean sero-prevalence for highland, medium altitude and lowland agro-climates was recorded as follows: 8.1 + 3.2%, 13.7 + 2.2% and 27.3 + 3.2% respectively and these differences were statistically significant: lowland Vs highland ($P<0.001$), lowland Vs medium altitude ($P<0.001$) and medium altitude Vs highland ($P<0.01$).

Analyses of the results based on the official CBPP outbreak report was made. The mean sero-prevalence for outbreak areas, for suspected areas and for areas considered free was 27.3 + 3.2%, 17.5 + 3.9% and 10.1 + 1.8% respectively and this difference was found statistically significant: Outbreak area Vs suspected ($P<0.001$), outbreak Vs free ($P<0.001$) and suspected Vs free ($P<0.001$).

Further analysis based on the year of last CBPP vaccination recorded and statistically significant difference was found between years up to 1991 and after 1991 ($p<0.001$). The mean sero-prevalence for 1990/91, 1992/93, 1994/95 and 1996 onwards was found 7.3 + 3.2%, 16.2 + 2.5%, 21.5 + 2.8 and 17.5 + 5.3%.

There was no statistically significant difference ($p>0.05$) recorded in the various age groups, but there was statistically significant difference ($P<0.05$) between males and females, males were found sero-positive more frequently than females.

To follow the objective of assessing the efficiency of T1-44 freeze dried monovalent vaccine and the level and duration of post vaccinal antibodies, a vaccination trial has been conducted on 77 cross-bred cattle in Debre Zeit research farm. Using CFT, antibodies due to vaccination have been detected seven days after vaccination, reached peak values at day 28 and declined from day 35 onwards up to day 49 of the experiment and none of the animals reacted on day 56 and 63. The maximum sero-conversion recorded was 80% with CFT. On the other hand, the C-ELISA detected vaccinal antibodies on day 14 PV and antibodies persisted up to day 21. None of the animals was showing positive reaction on day 28 PV until the end of the experiment. The maximum proportion of sero-positive animals recorded with this test was 64% on day 21.

Comparing CFT and C-ELISA, a total of 3150 serum samples including samples collected for sero-prevalence study, samples collected during vaccination trial and PV samples from Andassa and Metekel cattle breeding ranches were tested using both tests. Comparison was made on confidence interval of means, calculation of their observed proportional agreement (concordance) and agreement beyond chance (kappa values) of the two tests. About 85% of the village means and 73% of district means were found overlapping indicating that the variation in this respect was much due to chance. The mean concordance for all samples was 85.3 + 1.2% and the mean kappa value was found to be 0.30 + 0.20. The minimum and maximum kappa value recorded was 0 and 0.78 respectively.

Based on the results of this study, CBPP seems to be a major concern in North-west Ethiopia and hence implementation of immediate control measures, such as vaccination have been proposed. In addition, further study on the prevalence and distribution of the disease in the country, comparison of the serological tests, improvement of the national veterinary infrastructure and education of livestock owners are suggested.

1. INTRODUCTION

Agriculture is the mainstay of the Ethiopian economy. Over 80% of the population is engaged in agriculture. Agriculture accounts for about 45% of the gross domestic product (GDP) and 85% of export earnings. Livestock contributes 16% of the total GDP, 30% of agriculture and 12-16% of export earnings comes from skins and hides.

Livestock plays a critical role for the majority of the Ethiopian population. Domestic animals are mainly used as draught animals, for crop production and transport, for production of milk, meat, hides and skins and manure. Apart from this, domestic animals serve as risk diversion, and accumulation of wealth among the rural community.

It has been said that Ethiopia's cattle population is the largest in Africa, ranging tenth in the world. However, livestock productivity is marginal due to poor genetic potential, traditional management system and diseases. Contagious bovine pleuropneumonia (CBPP) is a major killer disease prevalent in the country.

CBPP is regarded as the second most important disease of cattle and the incidence of the disease is increasing in the continent of Africa, mainly in East Africa (Nathathe, 1992; Sylla *et al.*, 1995). The disease has been reported in 24 countries in Africa including Ethiopia and is considered to be the main hazard next to Rinderpest. Although a thorough epidemiological investigation has not been undertaken and the magnitude of the damage is not yet established, disease outbreak reports from 1991 to 1995 indicated that several epizootics in the south, south-west, west and north-west regions of the country have been registered each year.

The highest record was in 1993, when 29 outbreaks were reported (MOA, 1997). Due to the insidious nature of the disease (*iceberg phenomenon*) such official data do not necessarily convey the extent of the problem caused by CBPP in Ethiopia.

The epidemiology of CBPP is characterized by the occurrence of sub-acute and symptomless infections which is insidious by nature with disseminated foci. The disease does not spread rapidly and the persistence of latent carriers or "*Lungers*" i.e. infected animals that have been recovered but still harbor the pathogen in lung abscesses or "sequestra". Spread of the disease is predominantly associated with cattle movement that makes its control difficult in Africa (Masiga and Domenech, 1995; Masiga *et al.*, 1996 and Tulasne *et al.*, 1996).

Research carried out on CBPP in Europe and Africa provided a detailed knowledge concerning its dynamics, aetiological agent, and basic immunological principles involved. Development of new vaccines based on attenuated *MmmSC* strains helped to carry out disease control activities by wide-spread vaccination campaigns. Vaccination using the attenuated strains is still the only available tool for the control of CBPP in Africa (Masiga *et al.*, 1996; Tulasne *et al.*, 1996).

Attempts to eradicate CBPP from the African continent faces many obstacles:

- The available vaccines are conferring immunity up to 6-8 months in vaccinated subjects (Abdalla, 1975), up to 12 months (Atang, 1968 and 1969; Dyson and Smith, 1975), up to two years (Masiga and Read, 1972; Masiga and Windsor, 1975; Masiga and Domenech, 1995).

- Occasionally vaccinated animals contracted CBPP as a direct effect of vaccination. Vaccination may induce several unwanted local reactions in vaccinated animals. In some areas anaphylactic reactions occurred in cattle that were being vaccinated with egg adapted vaccine applied for the second and third time (Davies and Gilbert, 1969a and 1969b; Masiga and Mugera, 1973; Chalmers, 1975; Dyson and Smith, 1975).
- Regarding practical and efficient techniques to detect the latent carriers, CFT is more specific but less sensitive to detect chronic carries and the technique is cumbersome (Bashiruddin *et al.*, 1994). ELISA on the other hand is said to be more sensitive but less specific (Onoviran and Taylor-Robison, 1979).
- A high percentage of CBPP infected animals have no detectable clinical signs and therefore, the disease is frequently misdiagnosed and mishandled (Atang, 1969; Blancou, 1996).
- Poor quarantine and cattle movement control legislation.
- Lack of data on the economic impact of the disease (Masiga *et al.*, 1996).

OIE recommended the Campbell and Turner complement fixation test (CFT) as a dependable serological diagnostic test for CBPP. Recently, a competitive enzyme linked immunosorbent assay (C-ELISA) employing monoclonal antibodies (MAb) has been developed by CIRAD-EMVT to substitute the former. The test is currently under evaluation in different African countries.

Vaccination has been considered as a strategy for the control of CBPP in Ethiopia. Despite concerted effort by the veterinary department of the MOA to control the disease through vaccination, CBPP still persists in several regions probably due to nomadism and transhumance, limited vaccination coverage, financial constraints and also absence of effective policies for regional disease control program.

Current survey data in North-west Ethiopia revealed that CBPP causes considerable economic losses to cattle production through morbidity and mortality and warranting for serious attention.

The objectives of this study were:

1. To assess the prevalence and distribution of CBPP in Western Gojjam and Awi Zones of Amhara Region, Ethiopia.
2. To measure the response, level and duration of vaccinal Abs in cattle vaccinated with T_1 - 44 freeze dried monovalent vaccine.
3. To compare CFT and C-ELISA for the diagnosis of CBPP.

2. LITERATURE REVIEW

2.1. Description of the organism

2.1.1. Taxonomy

The mycoplasma causing CBPP is belonging to the class Mollicutes, order Mycoplasmatales, family Mycoplasmataceae, genus Mycoplasma and species Mycoplasma mycoides sub species mycoides small colony variant (*MmmSC*) (Carter and Changeappa, 1991; Nicholas and Bashiruddin, 1995).

2.1.2. Morphology and Staining characteristics

The mycoplasma organisms have no cell wall and consequently are plastic and highly pleomorphic. The cell is confined by a lipoprotein plasma membrane and occurs in a variety of forms including spherical or pear-shaped cell and filamentous structures (Egwu *et al.*, 1996). Mycoplasmas in smears have the shape of coccobacilli, cocci, rings, spirals and filaments.

They stain poorly with the Grams stain although Giemsa staining is useful. They range in size from 0.2 to 0.8 μm in diameter, with variable length depending upon the form. The genome is a double stranded DNA molecule (Provost *et al.*, 1987; Egwu *et al.*, 1996).

2.1.3. Cultural features

Nutritionally, the mollicutes are an extremely fastidious group of organisms, being dependent on their host for a large variety of organic nutrients such as vitamins, nucleic acid precursors, amino acids, fatty acids and lipids. The large number of amino acids required by mollicutes reflects the limited synthetic capability of this organism. The organism requires media supplemented with uracil, thymine and guanine for nucleic acid synthesis (Nicholas and Bashiruddin, 1995; Egwu *et al.*, 1996).

In addition the organism is unable to synthesize or alter the chain length of the fatty acids required for phospholipid synthesis, which therefore, must be provided by the growth medium. Sterol, which makes up 20% of the membrane lipids, is required by all mollicutes except for species of Acheloplasma and Astroplasma.

Cholesterol is the sterol usually provided in the medium, but others such as cholestanol and ergosferol can be substituted. Glycerol is required by mycoplasmas for the synthesis of α-glycerophosphate and thus glycerides. The polyamines spermine and spermidine, although not essential, appear to stimulate the growth of mycoplasmas in defined medium (Carter and Changeappa, 1991; Nicholas and Bashiruddin, 1995; Egwu *et al.*, 1996).

Most mollicutes, including *MmmSC* grow faculatively in anaerobic and aerobic environments at a pH of 7.6 - 7.8. They usually grow well in sealed liquid broth cultures, especially if the broth level is a few inches deep to allow the presence of oxygen or air gradient. Mycoplasmas ferment glucose with the production of acid from anaerobic metabolism, but other energy sources, including fructose, are utilised as well. Glucose supported higher growth rates than did other sugar sources, even though these sugars were present in saturating concentrations. It therefore appears that glucose may have a role in metabolism, in the regulation or synthesis of other sugars, in addition to providing an energy source (Nicholas and Bashiruddin, 1995).

The *MmmSC* unlike *MmmLC* strains, do not ferment sorbitol, are less resistant to streptomycin and their growth is less stimulated by the presence of fermentable substrates. *MmmLC* strains digest casein, liquefying inspissated serum and surviving longer at 45°C. Unlike small colony strains, the *MmmLC* posses the enzymes α-glucosidase and ornithine transcarbamylase (Nicholas and Bashiruddin, 1995).

2.2. Contagious bovine pleuropneumonia (CBPP)

2.2.1. Definition

CBPP is a disease of cattle characterized by extensive sero-fibrinous pleurisy and oedema of the interlobular septae. Septicaemia is frequently followed by localization in the thorax with extensive supportive lesions involving the lungs, pleura and pericardium (Provost *et al.*, 1987; Seifert, 1996). It is one of the list "A" diseases ("A_6") registered by the OIE (OIE, 1996).

2.2.2. Economic significance

CBPP is one of the major plagues of cattle causing heavy losses in many parts of the world. In affected countries enormous losses are experienced each year from the morbidity and mortality of animals, reduced production and working performance, reduced fertility, cost of control programmes i.e. vaccination campaigns, quarantine and restriction on cattle trade etc. Mortality ranges from 30-70% and morbidity up to 70% (Provost *et al.*, 1987; ter Laak, 1992). Losses due to the chronic illness are difficult to assess. It should be noted that economic evaluation of losses due to CBPP has not been performed systematically throughout Africa (Masiga and Domenech, 1995; Masiga *et al.*, 1996).

2.2.3. Clinical picture

The incubation period is uncertain and can last from a few days up to several months depending on the resistance level of the animal and intensity of exposure. The disease takes an acute, sub-acute to chronic course. The acute course is followed by a chronic stage which may last for years (lunger).

The majority of cases are sub-clinical. The clinical symptoms start with the characteristic short, dry cough which becomes more and more painful. This is a consequence of multiple serous cellular bronchopneumonic foci in the lung and it is accompanied by a moderate rise of body temperature (40°C). The respiration becomes more frequent and aggravated, feed intake and milk production are reduced, the animals are listless with a staring, lustreless coat and they become emaciated (Seifert, 1996).

According to Provost *et. al.* (1987), the incubation period is poorly defined in healthy cattle in contact with infected cattle and ranges from 20 to 123 days. Masiga *et al.* (1996) reported for natural infection incubation periods of three to six weeks or longer and about three weeks for experimental infection.

In endemic regions, 13% of cases are of hyperacute form, 20% of the acute form and 46% of the sub-acute form. Approximately 21% of animals are resistant to the disease (Masiga *et al.*, 1996).

Hyperacute form

This form occurs during the onset of an outbreak and death may be all that is seen. In some cases the animal may die after one to three days with no signs of pneumonia. Death may result from asphyxia, toxaemia or heart attack (Masiga *et al.*, 1996).

Acute form

This is the form that is usually observed, particularly in the early stage of an outbreak. The first abnormality noted may be an increase in body temperature and moderate respiratory signs, such as polypnoea and a dry painful irregular cough. The posture of the animal may be stiff. After a few days, the temperature rises to 40°C or higher, accompanied by a drop in milk yield, anorexia and cessation of rumination. At this stage, chest pain is evident.

Affected animals are reluctant to move, and stand with the elbows abducted and the back arched, the head extended and the nostrils dilated. Breathing becomes short and rapid and a moist cough may be present. In severe form of the disease, the mouth remains widely open and may contain foam and mucoid discharge from the nostrils may occur.

Exercise may aggravate the respiratory distress. In rare cases diarrhoea occurs. Auscultation reveals pleuritic friction sounds during the acute inflammatory phase and then, during the later stages, fluid sounds or moist gurguling rales are observed (Masiga *et al.*, 1996).
The mortality rate varies between 30 and 70% depending on the level of resistance of the respective breed. Shaw (1971) in Zambia has reported 70% morbidity, 60% mortality and 15% recovery with an incubation period of eight weeks. Similar opinion was reported from India (Karishna, 1969).

Sub-acute and symptomless forms

Subacute and symptomless forms are very frequent and are characterized by mild signs, or no clinical signs at all. It is known that animals with these forms are able to transmit the infection and so it is possible that they are the most dangerous of all (Masiga *et al.*, 1996).

Chronic form

The common signs revealed are: mild respiratory distress on exercise, violet and prolonged cough, intermittent fever and chronic emaciation (Masiga *et al.*, 1996; Blancou, 1996). Regeneration of the lesions may occur after several weeks, but full recovery is exceptional. Sequestral lesions and pleural adhesions remain. On the other hand Masiga *et al.*, (1996) have reported that after about 36 months the lung lesion may get sterile and depending on its size there might be complete healing.

2.2.4. Lesions

Macroscopic

The essential lesions are located in the lungs and pleura. They are almost invariably unilateral, without predilection for one side or the other, and are often in the posterior part of the chest. The pleura is thickened at the stage of dry pleurisy. Acute inflammation is accompanied by serofibrinous exsudate (up to 30 litres), the colour of which is amber and blood tinged.

The exsudate coagulates readily upon contact with air. "Omeletes" of fibrin are often found floating in this fluid, or attached to the parietal pleura and the lung surface. Hepatisation, hypertrophy of bronchial lymph nodes, and infiltration of connective tissue have been reported (Provost *et al.*, 1987; Egwu *et al.*, 1996; Blancou, 1996 and Masiga *et al.*, 1996).

Microscopic

Dilatation of the perilobular space, intra-alveolar oedema, thrombosis, perivascular organization, necrosis were considered pathognomonic, infiltration of neutrophils and macrophages have been reported (Provost *et al.*, 1987; Egwu *et al.*, 1996).

2.2.5. Epidemiology of CBPP

Aetiology

CBPP is caused by *MmmSC*, which belongs to one of the six closely related mycoplasmas that make up the group called "the Mycoplasma mycoides cluster". This includes *MmmSC*, *MmmLC*, *Mycoplasma mycoides subspecies capri (Mmc)*, *Mycoplasma capricolum subspecies capricolum (Mcc)*, *Mycoplasma capricolum subspecies capripneumonaiae* (MCCP) and bovine serogroup 7 (Hotzel *et al.*, 1996).

The Mycoplasmas in this group are pathogens of cattle, sheep and goats and share many immunological, biochemical and genetic properties, which leads to difficulties in diagnosis. *MmmSC* and bovine serogroup 7 are pathogens of cattle and *MmmLC*, *Mmc*, *Mcc* and *Mccp* are pathogens of sheep and goats (Dedieu *et al.*, 1994; Hotzel *et al.*, 1996) (Table 1).

Strains of the causal agent vary in pathogenicity and infectivity in different epidemics, and as well in the same epidemic depending on the moment of isolation (Provost *et al.*, 1987). *MmmSC* and *MmmLC* are generally indistinguishable by in vitro serological tests but they differ in their sensitivity to heat and streptomycin, in their proteolytic activity, ability to ferment sorbitol and *MmmLC* posses the enzymes α-glucosidase and ornithine-transcarbamylase (Smith, 1984; Egwu *et al.*, 1996).(The differentiation between *MmmSC* and *MmmLC* is indicate Table 2).

Table 1. Members of the mycoid cluster group pathogenic to cattle, sheep and goats.

Group	Name	Type strain	Disease	Major host	Other host
I	*MmmSC*	PG1	CBPP	Cattle	Goat, sheep, Buffalo)*
	MmmLC	YG	peritonitis		
			septicaemia	Goat, sheep	Cattle
			polyarthritis		
	Mm capri	PG3	pneumonia	Goat	
			arthritis		
	Mm capricolum	C-kid	pneumonia		
			arthritis	Goat, sheep	Cattle
			mastitis		
II	MCCP		CCPP	Goats	
	Serogroup-7	PG50	arthritis	Cattle	
			mastitis		
			calf pneumonia		

Source: Nicholas and Bashiruddin, 1995; * artificial infection

Table 2. Some differences between *MmmSC* and *MmmLC* types

Characteristics	SC type	LC type
Mean colony size	0.99 mm	2.26 mm
Broth turbidity: optical density	0.14	0.4
Liquefaction of inspissated serum	weak	+
Casein digestion	-	+
Sorbitol fermentation	-	+
Ornithine transcarbamylase	-	+
α-Glucosidase	-	+
Thermal stability at 45°C	Sensitive	Resistant
Streptomycin resistance	+	+++
Mycoplasmaemia in mice	+	-*
Electrophoresis of AsnI digests of PCR products with primers 450/451	Two bands	Three bands
Digestion of DNA by MboI	+	-
DpnI	-	+

Source: Bashiruddin *et al.*, 1994, Nicholas and Bashiruddin, 1995, * Most strains

Modes of transmission

Source of infection

The main source of infection under natural conditions is the excretion of flugge-type droplets by the coughing animal. Newly infected cattle can harbour the mycoplasma in the pharyngeal region and act as source of infection. Clinical cases are responsible for the spread of infection; although some believe that "lungers", that is chronic carriers, may break down and shed organisms into the bronchus and then into the environment. Urine, foetal fluids and nasal discharge of sick animals can present a source of contagion (Masiga *et al.*, 1996).
Transplacental infection of calves was reported in East Africa (Atang, 1969). Windsor *et al.* (1972) have reported the isolation of *MmmSC* from the fetus of unvaccinated pregnant dams which had been in contact with experimentally infected animals and this shows that there was transplacental transmission of the organism. Additionally, Regalla *et al.* (1996) have reported the isolation and identification of *MmmSC* from bull semen and sheath washings and suggested its great importance in the transmission of the disease.

The chief type of infective material is the pleuropneumonia ''lymph'' which exudes from the cut lung or accumulates by exudation within the pleural cavity. In acute forms of the disease, the excretion of infective material is considerable but in sub-acute and chronic forms, excretion is less abundant, irregular and sometimes absent.

While respiratory excretion is the prime source of transmission, there is a certain degree of mycoplasmaemia during the early febrile stage, which renders other organs infective such as the brain, liver, kidney, lymphnodes, uterus, fetus and fetal membranes. In this respect urine may also be important, for considerable numbers of *MmmSC* have been recovered from urine (Bygrave *et al.*, 1968; Masiga *et al.*, 1972b; Provost *et al.*, 1987; Seifert, 1996).

Masiga *et al.* (1972b) have reported another method of transmission quoted as "urinary tract to nose transmission" and have indicated that the sexual behaviour of cattle is a predisposition for this type infection. During micturition splashing of the urine gives rise to small droplets which can be inhaled in the same way as droplets of bronchial secretion.

Sequestra are usually closed, but an occasional pulmonary sequestrum may open into a draining bronchus, with the dangerous epidemiological consequences of excretion of the causal agent. The cycle of infection is completed when the carrier emits aerosols causing infection of disease free animals in contact with the carrier (Lloyd and Etheridge, 1983).

Invasion of the blood suggests the possibility of transmission by tick vectors, with the creation of a natural reservoir of infection and the possibility of repeated initial sensitization of cattle, the disease then being elicited by direct contact (Shifrine *et al.*, 1970). However, this initial sensitization was reported beneficial as it induces better production of protective antibodies when the host is exposed to future infection.

Cattle fed on hay experimentally infected with the Gladysdale strain had developed complement fixing antibodies and a positve reaction to the comparative intradermal test but no animal died from CBPP. When they were killed, some animals had unequivocal lesions of the disease and isolation of the *MmmSC* was made. Contact transmission to other susceptible animals from these hay fed animals was also reported (Windsor and Masiga, 1977; Masiga and Domenech, 1995). However Seifert (1996) has reported that neither ingestion of infected fodder nor direct exposure to diseased organs will cause transmission of *MmmSC*.

Modes of infection

The disease is transmitted by direct contact, over small distances by infected coughing animals, emitting flugge-type droplets from the mucous membrane of pharynx, trachea and bronchi and from saliva. Microdroplets from infected urine may be transported by an air current.

Natural transmission of *MmmSC* occurs through droplet infection either from cattle with clinical disease or from sub-clinical carriers (lungers) which pass the organism on to susceptible animals in close contact. The droplets may reach as far as 20 meters or more (Provost *et al.*, 1987), 50 to 200 meters (Masiga *et al.*, 1996) and infection is facilitated by crowding the animals around watering places or in a pen or paddock (Seifert, 1996; Masiga *et al.*, 1996).

Pathogenesis and pathogenicity

The *MmmSC* is a pneumotropic microorganism strictly adapted to bovines. Its pathogenicity can be measured accurately only by administration in aerosol form into the bronchi of animals previously unexposed and kept under well-controlled conditions. In this case the strains can be classified as hyper-virulent or hypo-virulent, requiring inocula of 10^2 and 10^9 mycoplasmas per ml of culture, respectively. In addition, a test for mycoplasmaemia in mice may be a guide, particularly in identifying vaccine strains (Provost *et al.*, 1987).

However, inoculation experiments have to take into account a certain number of virulence factors inherent in the organism itself. These concern the strain, colonial morphology, number of subcultures and age of the culture. Certain severe and rapidly spreading epidemics are caused by hypervirulent, lentogenic strains. It seems that virulence declines as the disease evolves. In contrast to exaltation of virulence by passage, this is an instance of spontaneous attenuation, perhaps engendered by the acquisition of spontaneous immunity formed during indistinct and inapparent infections. Strains thus show different degrees of virulence from the moment of isolation (Provost *et al.*, 1987).

The mechanisms by which mycoplasmas cause disease is poorly understood. It is thought that a galactan produced by *MmmSC* has a pathogenic role like the endotoxin of gram-negative bacteria. It is also suggested that accumulation of mycoplasmal metabolites may contribute to cytopathic effects and tissue damage (Nicholas and Bashiruddin, 1995). Masiga and Domenech (1995) have suggested that the development of lesions are an effect of the direct multiplication of inhaled infective droplets and immunological reactions.

Gourlay and Shifrine (1965) and Kakoma *et al.* (1973) have considered the antigenic similarity between the normal bovine lung tissue and *MmmSC* galactan leading to auto-immunity. It was suggested that the bovine lung is immunologically tolerant to a galactan-containing immunogen; if this tolerance breaks up some degree of auto-immunity should be expected. Evidence suggests that galactan itself is toxic, or is a component of a soluble toxin, and capable of causing necrosis and striking connective tissue reactions in cattle in the absence of the mycoplasma. This induced tissue response, similar to that seen in sequestra in chronically infected animals, may protect the mycoplasmas from the host defenses.

It was suggested that the amount of galactan produced by a strain of *MmmSC* was directly proportional to its virulence and that the KH_3 J Vaccine strain was avirulent because it produces less galactan than the virulent Gladysdale strain (Nicholas and Bashiruddin, 1995). Garcia *et al.* (1989) as cited by Nicholas and Bashiruddin (1995) have recorded that *MmmSC* strains were generally more cytotoxic than *MmmLC* strains, particularly on bovine endothelial and embryonic lung fibroblasts.

It is assumed that a diffusible toxin produced by *MmmSC* stimulates fibrinous granulation tissue proliferation resulting in capsule formation around infected necrotic tissue. A carbohydrate galactan, the major Ag of *MmmSC* increases similarly to those of the endotoxins of gram-negative bacteria. Apparently immunologically induced cell damage and autoimmune hypersensitivity reactions are both involved in the development of lesions. Additionally agglutinating antibodies were as well suspected to cause local lesions (Seifert, 1996).

An essential part of the pathogenesis is thrombosis of the pulmonary vessels, probably prior to the development of pneumonic lesions (Lloyd *et al.*, 1975). Thrombosis, perivascular organization and necrosis are pathognomonic lesions of CBPP. Death results from anoxia and/or toxaemia. The induction of auto-immunity and hypersensitivity reactions seems essential for the development of lesions (Masiga *et al.*, 1996). The fibrinous exsudate present in infections protect them from antibody and antimicrobial drugs and the removal by mucosal secretions contributes to chronicity.

Tulasne *et al.* (1996) have reported about the mechanism by which *M. mycoides subspecies capri* invades the lung alveoli and have suggested that this invasion took place as a result of the interaction of this organism with the ciliary activity of the epithelial cells. It produces hydrogen peroxide (another pathogenic factor) which inactivates the ciliary activity of epithelial cells and hence the organism is able to adhere to alveoli macrophages without being engulfed. Rosendal *et al.* (1995) on the other hand have reported the production of cytokines and citric oxide by stimulated macrophages during infection with *MmmLC*.
In short, the pathogenicity and pathogenesis of *MmmSC* can be summarized as below:

- The majority of the mollicutes are cell-surface parasites which rarely invade or enter the circulation and are generally confined to the mucosal or epithelial surfaces lining the oral cavity, synovial tissue or urogenital tract, as well as lungs of adult cattle.
- Thrombosis as a result of an unknown endotoxin have been suggested.
- Activation of host immune system leads to auto-immunity thereby causing immunopathological damages (Nicholas and Bashiruddin, 1995).
- *MmmSC* produces mycoplasmaemia and can be found in numerous sites including the kidneys, liver, lyphnodes and brain and can cause high morbidity and mortality (Nicholas and Bashiruddin, 1995).
- Accumulation of metabolites leads to cytotoxicity.
- Hydrogenperoxide production during glucose and glycerol oxidation leads to the speculation of oxidative damage (Miles, 1983; Tulasne *et al.*, 1996).
- Galactan itself is toxic or a component of soluble toxin, and capable of causing necrosis and a striking connective tissue response in cattle in the absence of mycoplasmas (Nicholas and Bashiruddin, 1995).

Antibodies against *MmmSC* cross-react with pneumogalactan isolated from normal bovine lung and it was suggested that the phenomenon might play a part in the pathogenesis of CBPP (Gourlay and Shifrine, 1965 and 1966). A study conducted by these authors indicated that cattle which received immune/hyperimmune serum prior to the inoculation of virulent *MmmSC* did not develop "Willems" reaction but they did show lung lesions; indicating that possibly the antiserum had "sensitized" the lungs to subsequent infection with *MmmSC*.

"Willems reaction": This is a subcutaneous swelling which develops into a hard fibrotic mass, which can grow into a horn like structure after vaccination with attenuated *MmmSC* vaccine. It has been called "Willems reaction" when a Belgium veterinarian named Willems has observed and reported his finding in 1850 after subcutaneous inoculation of CBPP infected macerated lung tissue into a bovine animal. This tissue reaction has been considered as unwanted result of vaccination against CBPP. On the other hand, it has been reported as an indication of the immunogenic capacity of CBPP vaccine (Tulasne *et al.*, 1996).

Factors associated with epidemiology

Agent factor

The organism can survive for a year in frozen, infected lungs. Preservation in liquid medium is difficult to quantify, because it depends on pH, as a result of the harmfulness of acid for mycoplasmas. As a rule, broth cultures can be stored for 2-4 months at 4°C. Freezing of cultures has no immediate harmful effect. Paradoxically, freezing may even enhance the titre of cultures, as consequence of rupture of pseudomycelia.

A temperature of -20°C, or better -30°C or -70°C enables the organism to be kept for several years, although the titre falls by 1 or 2 log units. It is also worth noting that repeated freezing and thawing destroys mycoplasmas suspended in water, but it has no effect on mycoplasmas suspended in normal saline or other isotonic or hypertonic solution. Ultraviolet radiation inactivates cultures with a loss of 10^6 $\log_{10}$ in 15 minutes. (Provost *et al.*, 1987).

Despite the lack of a cell wall, placing the organisms in a hypotonic solution or even distilled water is not followed by immediate lysis at freezing point , although it does occur at 37°C . Inversely, the organism can tolerate an osmotic pressure six times its own, with only slight loss in viability of the culture. The presence of bivalent and trivalent cations has a protective effect (Egwu *et al.*, 1996). The relative resistance to osmotic shock is attributable to the high cholesterol content of the cytoplamic membrane, which forms bridges between phospholipid molecules, facilitating deformation of the membrane.

Wetting agents, saponin and digitonin lyse *MmmSC*, as do bile and bile salts (including sodium desoxycholate, at a concentration of 3 x 10^{-5} M). All the ordinary antiseptics are active, 1% phenol solution for 3 minutes, 0.5% formaldehyde solution for 30 seconds, 0.01% mercuric chloride solution in 1 minute, calcium hydroxide solution in less than 5 minutes, ether in less than 5 minutes, 0.004% mericurichrome in one hour. From its activity as a wetting agent, alcohol has no effect, and the same is true for boric acid.

Other factors which affect viability of *MmmSC* vaccine, have been stated by Lloyd, *et al.* (1974). Some types of rubber stoppers were highly toxic to *MmmSC* vaccines. Loss of viability was greater when the vaccine was stored in smaller bottles and this was related to the relatively greater air space.

Environmental factors

The resistance of *MmmSC* to environmental factors is low, and it is capable of surviving for only 2-3 days in tropical regions, and rather longer (1-2 weeks) in temperate regions. The tenacity of *MmmSC* is rather low, sun radiation and unusual disinfectants destroy it quickly. In 'lymph' it is inactivated within 240 minutes at 45°C, 60 minutes at 50°C, 5 minutes at 55°C and 2 minutes at 60°C. Suspended in normal saline, it is inactivated in 2 hours at 45°C and immediately at 47°C (Provost *et al.*, 1987). The relative sensitivity of fluid cultures to heat is important in the case of live CBPP vaccines in fluid form, because they must not be exposed to excessive heat, particularly in tropical countries (Egwu *et al.*, 1996).

Intercurrent diseases

An intercurrent disease, such as Rinderpest in Africa, frequently brings about the same type of synergistic-pathogenic combination. Subsequent vaccination runs the risk of producing a similar situation. Thus, the use of caprinised Rinderpest virus vaccine considerably increased the reactions to CBPP. Similarly, anthrax vaccine can "reactivate" a weak Willems's reaction induced by an attenuated strain of *MmmSC*, with the development of a fatal Willems reaction at the site of the first inoculation, despite the presumed existence of immunity.

Efficacy of bisec vaccine (CBPP vaccine with Rinderpest vaccine) was compared with that of monovalent vaccines, and there was no evidence that the serological response to the dual vaccine (Rinderpest and CBPP) was in any way less than that of either agent given alone and no clinical disease was detected after incontact challenge.

After subcutaneous challenge the dual vaccinated groups were similarly susceptible like animals of unvaccinated control groups and unlike animals of the group vaccinated only with *MmmSC*. This would indicate that the Rinderpest virus component of the dual vaccine interfered with the ability of the *MmmSC* component to induce a fully effective immune response (Jeggo *et al.*, 1987). It was suggested that the Rinderpest component of the bisec vaccines has an immunosuppressive effect to the local lymph nodes of the site of inoculation and this can be avoided by application of the two vaccines separately at different sides of the body.

The application of combined vaccines has a significant disadvantage. It has been proven that the required concentration of antigen has to be increased in proportion to the components of the antigen contained. Consequently, this vaccine should contain three times the amount of antigen of each single component. This requirement is limited by the desirable volume of dose and possible side reactions (Windsor *et al.*, 1972). Brown and Taylor (1966) have reported a similar observation regarding a lack of immunity when both Rinderpest and CBPP vaccines were inoculated in a vaccine cocktail and that protective immunity similar to inoculation of either vaccine was obtained only when they applied at different inoculation sites.

Host factors

Species affected

Under natural condition CBPP affects species of the genus: *Bos taurus* (cattle) and *Bos indicus* (zebu) and both are equally susceptible (Masiga and Windsor, 1974, and 1975; Masiga *et al.*, 1996). It was considered exceptional to detect the infection in bison (*Bison bison*) and yaks (*Bopoephagus grunniens*) in zoos (Shifrine and Domermuth, 1967; Shifrine *et al.*, 1970).

Egwu *et al.* (1996) have reported that goats and sheep are susceptible only under experimental condition. However, on the other hand, Brandao (1995) and Regalla *et al.* (1995) have reported the isolation and identification of *MmmSC* in goats from cases of pneumonia and rarely in mastitis of sheep under natural infection. But the role of small ruminants as a reservoir for CBPP has not been demonstrated.

It has been reported that the domestic buffalo (*Bubalus bubalis*) is susceptible, and that outbreaks occur among this species in Assam (India). The African wild buffalo (*Syncerus caffer*) is not susceptible, but experiments on the feral buffaloes of Northern Australia have shown that introduction of infective material directly into the bronchi is followed by a respiratory disease, but the subsequent course of the disease was found to be abortive, and the animals were not infective for other buffaloes.

A unique case, and thus of doubtful validity, was the provocation of "Willems reaction" in young roan antelope (*Hippotragus equinus*). Despite the detection of complement fixing antibodies to *MmmSC* in the gnu (*Gorgon taurinus*), subcutaneous infection of these antelopes was not followed by any reaction (Provost *et al.*, 1987). On the other hand, under natural condition, detection of complement fixing antibodies in wildebeest and hippopotami was also reported (Shifrine and Domenech, 1967).

However, Seifert (1996) has reported that under natural conditions antibodies to *MmmSC* have also been found in African buffalo (*Syncerus caffer*), Impala (*Aypeceros melampus*) and in camels. Egwu and Aliyu (1997) have reported that out of 58 camels serologically tested with dot enzyme immunoassay and western blot tests 7 (12.1%) and 6 (6.8%) of the camels were found sero-positve, but none of them were positive with the standard CFT.

Breed factor in natural disease and following vaccination

Among zebu, some breeds are remarkably resistant to CBPP, and these include Somba, the breed of coastal lagoons of Benin, and the small Côte d'Ivoire breed. The Massai breed of Tanzania is equally resistant, and 80-85% of them recover without treatment. whereas the European breeds and their crosses are more susceptible.

In Zambia there is a high degree of infection among Barotse and Mashulumbive breeds, while in the Sudan, by contrast, almost 40% of zebus are resistant to experimental infection. Among cattle, it was found that the Dutch and Flamish cattle of Europe were more susceptible than Swiss, while at present day, Kenyan Jersey cattle are affected more often than Friesians. N'Dama cattle of Guinea seem to be more susceptible than the zebu and cross bred N'Dama of Senegal (Provost *et al.*, 1987; Seifert, 1996).

In Australia, dairy cows of Anglo-Normandy breeds were more susceptible to experimental infection than Hereford breed cattle. As a rule, taurine cattle are more susceptible than zebu, at least in Africa. Thus breeds of the Benin coast and the Côte d'Ivoire, reported to be resistant to trypanosomoses, develop particularly severe reactions to the inoculation of infective 'lymph', or strains of *MmmSC* which are still pathogenic. This finding has led some laboratories to prepare separate vaccines differing in residual virulence for the two breeds.

Nevertheless, Curasson (1936) as cited by Provost *et al.*, 1987 expressed the opposite opinion: application of Willems's method in both West Africa and Europe at the end of the last century was followed by high post vaccinal losses among zebu, up to 5% compared with barely 1% in Europe. Kouri cattle of Lake Chad proved to be no more sensitive than the zebu of the same environment. While vaccination in Central Africa showed that N'Dama and Baoule cattle did not develop greater reactions than zebus inoculated with a relatively pathogenic egg-culture vaccine, provided that the inoculation was performed correctly. Therefore, it is inadvisable to draw up strict rules.

The apparently high resistance of zebus has been ascribed to contamination of the environment, which confers on progeny a certain acquired resistance, sustained by successive reinfections. No immunogenic proof has been offered to conform this supposition. The concept of an average level of breed susceptibility remains useful, with important consequences for vaccination campaigns (Provost *et al.*, 1987; Seifert, 1996).

Age factor

In the natural disease, the susceptibility curve is sigmoid in shape with three phases. An initial phase of susceptibility in unweaned animals which develop only minor lesions of tendons and joints, and absence of the severe pulmonary form, a subsequent phase of moderate susceptibility, gradually increasing until 12-18 months of age, and a final phase of full susceptibility, which explains the choice of cattle over two years of age for experiments on the virulence of *MmmSC*. The mechanism of this variation is unknown, and serological testing does not provide a satisfactory explanation of the acquisition of spontaneous, occult immunity by animals in contact with infected animals.

Similar variations were found in both, experimental infection and vaccination. It is true, as stated by Willems (1854) and cited by Provost *et al.* (1987) that calves up to six months of age respond poorly to the inoculation of virulent lymph, developing no more than slight, transient oedema and numerous cases of polyarthritis develop as a consequence. To this may be added cases of coronary valvular disease and myocarditis, encountered among calves less than three months old. At a later age, heifers respond weakly to vaccination, but nevertheless become more resistant to experimental infection than adult cows. Analyses of baseline data in the Sudan indicate that older animals were found more sero-positve using CFT than the younger age group (Zessin *et al.*, 1985).

Masiga and Windsor (1978) have recorded in two separate experiments that cattle over three years of age were more resistant to CBPP than younger animals. A similar finding was recoded when different age groups were experimentally challenged and death was considered as sole criterion of response. The explanation given was that aged animals completing their physical development and hence can resist better than younger animals.

Masiga and Domenech (1995) on the the other hand have reported an age difference in clinical disease and noted that young animals suffer from articular forms and adult animals are more susceptible to pleuropneumonia. Provost *et al.* (1987) as cited by Nicholas and Bashiruddin (1995) have reported the higher sero-prevalence in aged animals than in younger animals. The explanation suggested was associated with maturation of the immune system as age increases.

Individual factor

In Australia, it has been found that aged dairy cows are more sensitive to both vaccination and challenge infection than steers. However, this rule include numerous exceptions from group to group in each type of cattle. Thus some adult dairy cows were as sensitive as steers, while, inversely, certain steers were as sensitive as those cows classified as being least sensitive. Such variation in sensitivity of a group is far from remaining constant, and seems to follow unpredictable cycles (Provost *et al.*, 1987). Immunity in CBPP depends upon an interplay of humoral and cell mediated immune factors:

1. Both types of immunity have been used for diagnostic purposes: serological tests, mainly CFT for detection of humoral antibodies, and skin testing for demonstrating cell mediated immunity (CMI) (Lloyd., 1967; Etheridge *et al.*, 1976; Garba *et al.*, 1987a and 1987b and Regalla *et al.*, 1996).

2. Immunosuppression caused by trypanosoma infection has been reported. Cattle infected with T. vivax produce less CBPP complement fixing antibodies and 50% succumbed to experimental CBPP infection when compared to uninfected controls (Boarer, 1973; Windsor *et al.*, 1978; Osiyemi *et al.*, 1985).

Type of husbandry

Type of husbandry affects both epidemiology and aetiology, and may be the crucial factor, since CBPP is predominantly related to the movement of animals. Areas completely free from infection can adjoin endemic areas if there is no movement of cattle between them. It is both true and false that CBPP is a disease of nomadic or at least transhumant cattle. In fact, in tropical Africa the disease occurs especially in that segment of farming populations and breeders, who have little experience of livestock husbandry (Provost *et al.*, 1987).

Newly introduced cattle are likely to pay a heavy toll to diseases revealing the existence of carriers in the nomadic cattle, and thus sparking off the disease in the recipient herd. This establishes a surprising contrast between the apparent mild course and low incidence of the disease among nomadic cattle, on the one hand, and the severity and catastrophic nature of the disease in areas populated by farmed cattle.

Local husbandry practices in sahelian Africa involve compact grouping of herds during grazing, mixing with other herds at watering points, and confinement at night within small enclosures and such conditions are eminently favorable for infection. The intensity of infection is low in sahelian regions, where herds are spread out, the air is dry, and the sexes are kept separate. Under these conditions the disease may persist for a long time, only becoming evident when the animals are moved, new stock introduced or animals exchanged between tribes or traded (Seifert, 1996).

Climate and season

These conditions are more important for the way in which they affect the type of husbandry than any direct effect on the disease. According to Turner (1953) as cited by Provost *et al.* (1987), a dry climate diminishes the risk of spread, because infective aerosols from infected cattle evaporate rapidly, and the pathogen is inactivated by ultraviolet rays. In regard to the disease itself, once established and regardless of racial susceptibility, its severity is identical in dry or humid climates. Season seems to play a role in stimulating infections, particularly the rainy season, when the animals are exposed to cool down pours while feeding.

Dennis (1986) has reviewed the importance of sudden changes in weather condition and noted that changes in temperature and relative humidity brings a change in the survival and virulence of the organism and changes on the resistance of the host. The role of feeding has not been examined experimentally as an aetiological factor. In general, nutrition seems to have only an ancillary role (Provost *et al.*, 1987).

Geographical distribution

CBPP was widely spread until the middle of the 19th century: Its area of distribution has since been reduced considerably by successful application of control measures. The only areas which have never been affected are South America and Madagascar. Numerous countries have been able to eradicate the disease, such as the USA in 1892 and Australia 1973 (Provost *et al.*, 1987; Masiga and Domenech, 1995; Masiga *et al.*, 1996). CBPP is now confined to the three continents: Europe, Asia and Africa (Table 3).

Europe

The oldest data on the prevalence of CBPP go back to 1713 when the disease occurred in Germany and Switzerland. In the 18th century, CBPP was introduced into most European countries and there were great economic losses. In the 19th century, Veterinary services were established in many European countries, including the Netherlands, because of the presence of CBPP and Rinderpest (Martel *et al.*, 1983 as cited by ter Laak, 1992). Europe had been free from the disease since the beginning of the century, but it has returned in the form of persistent outbreaks in southern Europe namely Italy and Portugal and at the border of Spain and France (Blancou, 1996; Masiga *et al.*, 1996). A CBPP episode occurred after the first world war in Germany by imported oxen from Romania (Hussel, 1962).

Asia

CBPP is endemic in the Middle East and certain regions of East Asia. According to ter Laak (1992) and Masiga *et al.* (1996) CBPP is spread from Australia to Asia and was reported in Mongolia, China (Tibet), Bhutan, Nepal, India, Bangladesh, Myanmar and Kampuchea. Sporadic outbreaks in imported cattle occur in the Middle East: Yemen, United Arab Emirates, Saudi Arabia, Kuwait, and Lebanon.

Africa

It has been reported that CBPP reached Africa during the colonization period where it continues to occur with changing extension in a belt which reaches from 18°N down to about the equator where it spreads into the hot, humid regions of the continent (Seifert, 1996). CBPP has been introduced into South Africa in 1854 and from there it extended to other countries.

The origin of CBPP in Central, West and East Africa is obscure and it has been suggested that the infection was introduced by zebu cattle when they first migrated to the African continent. There is a suggestion that CBPP was introduced into East Africa from India by the army of Field Marshal Napier when he invaded Ethiopia in 1867-1868 (Masiga *et al.*, 1996), while Tulasne et al. (1996) have reported that the traditional practice of provoking "Willems reaction" was rediscovered by Willems in 1854. This indicate that CBPP had been existing in Africa before 1854.

According to Masiga and Domenech (1995) and Masiga *et al.* (1996), CBPP was present in 24 African countries and the epidemiology of CBPP in Africa is dominated by four factors:

- cattle are the only species affected, there is no reservoir in wild animals,
- clinical cases or chronic carriers are the usual sources of infection,
- direct contact and cattle movements play a very important role in the maintenance and extension of the disease.

CBPP has a number of supporting factors: nomadism, pastoralism, geographic, social and economic factors (Bölske *et al.*, 1995; Masiga *et al.*, 1996)

Table 3. Summary of world wide distribution of CBPP

Continent	Countries with CBPP report
Europe	Italy, Spain, Portugal,
Middle East	Yemen, United Arab Emirates, Saudi Arabia, Kuwait, and Lebanon.
East Asia	Mongolia, China, Bhutan, Nepal, India, Bangladesh, Myanmar, Kampuchea, and Tibet
Africa	Angola, Benin, Botswana, Burkina Faso, Cameroon, Chad, Côte d'Ivoire, Eritrea, Ethiopia, Ghana, Guinea, Kenya, Mali, Mauritania, Namibia, Niger, Nigeria, Rwanda, Somalia, Sudan, Tanzania, Togo, Uganda and Zaire.

Source: Nicholas and Bashiruddin 1995; Masiga and Domenech 1995.

Ethiopia

According to disease outbreak reports, CBPP is widely spread in different regions of Ethiopia. Outbreaks and enzootic situations in the western, south-western and north-western parts of the country were reported for several years (Yigezu *et al.*, 1996; MOA,1997). A total of about 76 new outbreaks and 594 deaths due to CBPP have been registered by the MOA between 1991 to 1995. A total of 330,350 vaccinations have been performed: 265,276 doses to control outbreaks and 65,225 doses as prophylactic measures (MOA, 1997).

2.3 Control and eradication

2.3.1 Diagnosis

Clinical diagnosis

Consideration of species affected, CBPP is to be suspected in an endemic zone whenever general illness in cattle is coupled with pleuropneumonia. Sudden onset of high fever, fall in milk yield, anorexia, cessation of rumination, severe depression, lag behind a travelling group supports this suspicion. Coughing, at first only on exercise and chest pain are evident, disinclined to move, standing with the elbows out, the back arched and head extended, shallow respiration which is rapid and accompanied by expiratory grunting are typical signs for CBPP. Pain is evident on percussion of the chest (Blerk *et al.*, 1966; Bygrave *et al.*, 1968; Blood *et al.*, 1983; Provost *et al.*, 1987; ter Laak, 1992; Seifert, 1996; Egwu *et al.*, 1996; Blancou, 1996; Masiga *et al.*, 1996).

However, Regalla *et al.* (1996) have reported that the clinical symptoms may be masked and the classical signs may not be manifested especially in Europe where the management of cattle is more intensive with good housing, feeding and excessive use of antibiotics.

Post mortem diagnosis

The pathognomonic lesion is the characteristic type of lung hepatisation found in this disease. Its significance is always supported by finding extensive pleural lesions and abundant pleuritic fluid. Lesions are confined to the chest cavity. There is thickening and inflammation of the pleura often with heavy deposits of fibrin and one or both lungs may be partially or completely affected with marked consolidation. Affected lobules show various areas of gray and red hepatization and the inter lobular septa are greatly distended with serofibrinious exsudate, the classical 'marbled' lung of this disease. Under the microscope the vascular lesions of thrombosis, perivascular infiltration and necrosis are pathognomonic (Blerk *et al.*, 1966; Bygrave *et al.*, 1968; Karishna, 1969).

Laboratory diagnosis

Isolation of *MmmSC* is followed by identification of the pathogen by examining its biochemical properties with serological confirmation, by growth inhibition and/or immunofluorecence tests with monospecific conjugates. Tests for circulating antibodies or antigen (galactan) in serum, pleuropneumonic lymph or organ homogenate can be considered for the diagnosis of CBPP (Atang, 1968 and 1969; Bygrave *et al.*, 1968).

Samples for identification of the agent

The samples to be taken from live animals are nasal swabs, broncho-alveolar washings or pleural fluid obtained between the 7^{th} and 8^{th} ribs by puncture. Samples to be taken at necropsy are lung lesions, lymph nodes and pleural fluid. Direct examination of the exsudate or smears is possible, but requires great skill.

When dispatching the samples to the laboratory it is advisable to use a transport medium that will protect the mycoplasmas and prevent proliferation of other bacteria (heart infusion broth without peptone and glucose, 10% yeast extract, 20% serum, 0.3% agar, 500 iu/ml penicillin, thallium acetate 1:7,000). The samples must be kept under cold conditions: + 4°C for a few days or frozen at or below -25°C for a longer period (Buttery, 1967; Carter and Changeappa, 1991; OIE, 1992).

Examination of pathological samples

Direct diagnosis can be performed on the exsudate or on lung smears, either without staining under a phase contrast-microscope or after May-Gruenwald-Giemsa (MGG) staining. This direct examination requires great skill and should be considered as indicative only. An indirect fluorescent antibody test (IFAT) can also be performed on the smears using hyper-immune serum against *MmmSC* and labeled anti-IgG.

The test is satisfactory when applied to pleural fluid smears but less so with lung smears due to considerable non-specific fluorescence. However good results can be obtained using lung smears counterstained with Erichrome black (Nicholas and Bashiruddin, 1995). An agar gel immunodiffusion (AGID) test can detect the specific circulating Ag (galactan) present at the surface of *MmmSC*.

Microbiological diagnosis

Culturing

As culturing of *MmmSC* takes several days, desiccation of the solid medium should be limited by closing the petridishes hermetically or by using controlled humidity incubators. The media should contain a basic medium based on heart infusion or peptone (Tryptose from Difco or from Oxoid), Difco yeast extract to provide group B vitamins (1%), antibody-free horse serum 10%, and several other components such as glucose, glycerol, DNA, L-cysteine and fatty acids. To avoid growth of other bacteria, inhibitors are necessary: Penicillin and thallium acetate. The media can be used as broth or solid medium with 1.0 to 1.2% agar (Buttery, 1967; OIE, 1992; Tulasne *et al.*, 1996).

Serological diagnosis

The specific Ag (galactan) which occurs on the surface of *MmmSC* can be detected readily during the clinical stage of the disease, while distributed widely throughout the body, including blood serum, lymph, lung fluid, pericardial fluid and urine. For this purpose the double agar gel immunodiffusion test is the test used most often. The result is read after about 24 hrs. Poor results are to be expected in case of chronic carriers (Kakoma and Stone, 1974, Provost *et al.*, 1987).

Tests for antibodies

A number of serological tests have been reported used for the serological diagnosis of CBPP, some of which are: complement fixation test (Etheridge and Buttery, 1976, LeGoff and Thiacourt, 1995), serum slide agglutination test, slide whole blood agglutination test reported by Lloyd (1967), Etheridge *et al.* (1976), plate CFT (Karst, 1970; Clay and Lloyd, 1975), indirect haemagglutination test (Bygrave *et al.*, 1968; Atang, 1969) and ELISA. Of these, CFT is the only standardized serological test accepted by the OIE for international cattle trade.

Passive haemagglutination and the latex particle agglutination test have also been applied for the detection of antibodies in CBPP infected animals. When the sensitizing Ag is a lysate of whole organisms, the erythrocytes have to be pre-treated with formaldehyde or glutaraldehyde when galactan Ag is used, which becomes adsorbed to the surface of erythrocytes spontaneously. Use of the last-named Ag provides good correlation with CFT titres.

Nevertheless, the sensitivity threshold is always difficult to establish because non-specific cross-reactions occur with low dilution of serum. The technique is, therefore, not recommended for diagnosis, and is useful only in the study of particular serum samples. The slide whole blood agglutination test is another test performed by using stained Ags. This test provides a qualitative or semi-quantitative diagnosis within two minutes. However, correct results can only be expected during the acute stage of the disease.

The complement fixation test (CFT)

Antibodies are detectable from about 10 days after the onset of the disease and during the subsequent few months. During the clinical stage of the disease the rate of detection is variable, and practically no sick animal will give negative result. However, for animals entering the chronic stage the percentage of false negative results increases. It detects only about 72% (Shifrine and Gourlay, 1967) or 74% (Bashiruddin *et al.*, 1994) of chronically infected animals. Another disadvantage of CFT reported was that there is false positive reaction from Actinobacillus species (Gee, 1975).

In countries where vaccination is practiced, a presumptive diagnosis of CBPP can not be made because the CFT can give positive results for 3-6 months after vaccination owing to the presence of vaccinal antibodies. No test is capable of detecting infected animals at every stage of the disease (Masiga *et al.*, 1996).

ELISA

Immuno-enzyme techniques are inherently sensitive and are already being used for diagnosis of CBPP. The automation of each step including reading of results, makes ELISA readily reproducible, provided that all the components are strictly defined and tested. Whereas the CFT detects mainly the anti-galactan-Abs, enzyme immunoassay has the advantage of detecting all antibodies to CBPP when the antigen consists of a lysate of *MmmSC*. Despite this difference, there is good correlation between the results of the two techniques. Another potential advantage, which has not yet been demonstrated, would be to improve the detection of chronic cases, thanks to the high sensitivity of the method which is capable of detecting traces of antibody (Provost *et al.*, 1987).

Onoviran and Taylor-Robison (1979) have reported that ELISA detected antibodies still 19 months after natural infection and 23 months after vaccination and they concluded that the test is sensitive.

Egwu and Aliyu (1997) have reported their serological assessment on camels using dot enzyme immunoassay, western blot and CFT: 12,1% of the camels were positive for dot enzyme immunoassay, 6.8% for western blot and none were positive for CFT. This indicated that the higher sensitivity of ELISA over the other tests.

On the other hand Nicholas *et al.* (1996) have reported their finding that ELISA was the least sensitive as compared to CFT, western blot and dot blot. Nowadays a C-ELISA using specific monoclonal antibodies (MAb) to specific determinant epitope to *MmmSC* Ag as to rule out cross-reaction with antibodies from other mycoplasma species is under investigation (FAO/IAEA, 1995).

Molecular epidemiology

Polymerase chain reaction (PCR)

The standard methods for identification of Mycoplasmas rely on serology. However, serological cross-reactivity between species and strains often hinder the identification of Mycoplasma isolates, particularly in the mycoides cluster. Recent advances in diagnostic techniques have produced a number of PCR based assays, which are specific, sensitive and rapid (Dedieu *et al.*, 1994; Hotzel *et al.*, 1996).

Diagnosis based on molecular biological techniques have been developed recently for the identification of antigenic material. The majority of these technics are based on PCR with different primers to allow for the identification of the "mycoides cluster" (Rawadi *et al.*, 1995) or the differentiation of the different members within this cluster (Hotzel *et al.*, 1996).

2.3.2 Methods of Control and eradication

The main problems for control or eradication are the frequent occurrence of sub-acute or symptomless infections and the persistence of chronic carriers after the clinical phase. CBPP remains endemic in Africa till today for a variety of reasons mainly socio-cultural, geographic and economic situations. The control measures which have led to eradication of CBPP from numerous countries, and which are still being applied in Europe are impracticable in Africa. Systematic serological detection of infected cattle and cattle harboring lung sequestra is impossible in regions where extensive husbandry is the main type of cattle keeping (Masiga and Domenech, 1995; Masiga *et al.*, 1996).

There are three methods of control: Test and slaughter with compensatory payment, control of cattle movement and vaccination. But in Africa there is no real possibility to prohibit the movement of herds, or to slaughter infected animals. This entails the annual vaccination of cattle (Minor, 1967; Garba *et al.*, 1987a; Masiga and Domenech, 1995; Egwu *et al.*, 1996; Masiga *et al.*, 1996).

The measures that have successfully used elsewhere may not be feasible in the African situation at this point. It seems that vaccination is the only realistic method of choice for the control of CBPP in endemic zones in Africa. Apart from the aborted JP-28 program in West Africa, there has been no systematic and well coordinated vaccination campaign against CBPP in Africa (Tulasne *et al.*, 1996). The following general approach should be considered to control CBPP.

Quarantine

Any procedure which brings CBPP suspected animals together should be avoided, especially in the early stage of the disease. Passage through the milking shed, collection for inspection, bleeding and vaccination all facilitates the spread of the disease. Droplet infection is more likely to occur under humid conditions. Strict quarantine of the infected and in-contact herds must be maintained until all residual reactors have been eliminated. Usually 12 weeks after the removal of the last reactor and/or clinical case is sufficient. Animals in quarantine should be kept under constant surveillance, so that, clinical cases may be observed (Provost *et al.*, 1987)

Infected animals should be removed from the herd as soon as possible. The CFT is adequate to identify infected animals and it should be carried out in conjunction with clinical examination. Since animals during incubation and the early stage of the disease may give negative reactions it is necessary to have two completely negative tests two months apart before the herd is classified as negative. After vaccination a positive reaction occurs but it usually disappears within two months (Staak, 1974), although, in rare cases it may persist as long as 5 months. All positive and suspicious reactors and clinical cases should be killed or transported under close control to abattoirs (Nicholas and Bashiruddin, 1995).

Vaccination

Types of vaccines

According to the Tulasne *et al.* (1996) vaccination against CBPP has been implemented as follows:

- Willems method using pleuritic or pulmonary "lymph" or the secondary lymph resulting from a subcutaneous Willems reaction. This is the traditional African procedure which was rediscovered in Europe in 1854 by Willems.
- Attenuated vaccines (broth culture vaccines, egg culture vaccines), inactivated vaccines of various types (Brown, 1966; Shifrine and Beech, 1968; Garba *et al.*, 1986, 1987a and 1987b) have been reported as used in the control of CBPP.

Factors to take into account for CBPP vaccination

Vaccines against CBPP need to comply with three fundamental rules, derived from experience: Viability, immunogenicity and route of injection. Vaccines should contain a minimum of 10^7 cfu per dose. The vaccine should compromise between retention of immunogenicity and minimal local reactions acceptable to cattle owners (Tulasne *et al.*, 1996).

Permissible vaccination sites are those parts of the animal body having dense connective tissue of low reactivity in order to limit adverse local and generalized reactions (Garba *et al.*, 1989). The current trend of vaccination is to inoculate the vaccine subcutaneously posterior to the shoulder blade, using a vaccine of acceptable or absent residual virulence. Vaccination against CBPP is never harmless. Apart from local, even generalized reactions, vaccination may enhance latent infections or infestations in cattle having a previous physiological balance prevalent under tropical conditions, such as trypanosomiasis and piroplasmosis (Jeggo *et al.*, 1987; Tulasne *et al.*, 1996).

Breed:
In general taurine cattle breeds are more susceptible to vaccination accidents than zebu, at least in Africa. Dairy breeds are more susceptible than beef breeds, but exceptions occur in both cases (Provost *et al.*, 1987).

Group:
Within the same species and same breed, there are individual fluctuations in sensitivity to vaccination and also to infection.

Sex:
Pregnant females in the terminal stage of gestation should not be vaccinated. Pregnancy makes the animal more susceptible to infection (Windsor *et al.*, 1972).

Age:
Stone (1969) as well as Masiga and Domenech (1995) have reported about the passive transfer of colostral antibodies and its protective effect for about 60 days from experimental infection with virulent *MmmSC*. Although calves respond to vaccination with sufficient serological reactions, the value of calf hood vaccination is limited because arthritis, myocarditis and valvular endocarditis may occur 3-4 weeks after vaccination if calves were less than 2 months old.

Vaccination of calves after this age is recommended because it avoids the occasional deaths which occur after vaccination of calves. In general, vaccines made from *MmmSC* grown in broth culture produced less severe reactions but a correspondingly briefer immunity of about 6-10 months and required annual revaccination.

The bovine placenta, because of its structure does not enable the transfer of maternal serum globulins across the placenta. Calves are dependent on colostrum, with a high concentration of immunoglobulins. Absorption in the intestine is rapid and non-selective. In calves born from recently vaccinated dams, the response of complement fixing antibodies was suppressed by colostral antibody for at least 60 days (Gilbert and Stone, 1970).

With any CBPP vaccination there is a risk of creating semi-resistant cattle which can become chronic carriers after natural infection, maintaining the disease. Hence, CBPP vaccination must be extensive in area and continuos in time to be effective. Partial and not renewed vaccination of a susceptible herd may simply maintain the disease at a low level. All vaccines contain live attenuated *MmmSC* strains.

Over the years, the vaccines used were based on four strains namely T_1, KH_3J, F-strain and V_5, either as liquid or lyophilized preparations. The T_1 strain was isolated in Tanzania and currently there are two types of strains recommended and widely used: T_1 -44 and T_1-SR. The T_1-44 strain induces at least 12 months protection (Abdulla, 1969; Tulasne *et al.*, 1996).

The T_1 strain cell culture vaccine is the one more in use in the nomadic cattle herds of Africa. It provides long-term immunity of at least 2 years duration (Masiga and Domenech, 1995) and avianized vaccines were developed which increase duration of immunity to 3-4 years (Windsor *et al.*, 1974; Windsor and Masiga, 1977). However, more susceptible breeds such as the *Bos taurus* exhibit severe reactions and they should not be vaccinated with this strain.

T_1-SR strain presents several advantages: it may be used to produce combined vaccines (e.g. Rinderpest) and its resistance to streptomycin may be used as a marker when testing the vaccine for purity (Provost *et al.*, 1987; Tulasne *et al.*, 1996). The T_1 strain cell culture vaccine is the one more in use in the nomadic herds of Africa. According to Abdalla (1969) this vaccine provides one year protection.

The KH_3J and F-strains were isolated in the Sudan. They do not have any residual pathogenic effect and may be safely used in all breeds. However, immunity after vaccination is not absolute and does not last for more than 6 to 8 months (Shifrine and Domermuth, 1967; Abdalla, 1969).

Vaccination can only have maximum effect if the vaccine is potent, stored properly, administered by competent personnel and the correct route of vaccination is used (Garba *et al.*, 1987, 1989). Comparative vaccination trials brought the following results regarding the relationship of virulence; $T_1 > V_5 >> KH_3J$; the same sequence is true for the immunogenic capacity (Blood *et al.*, 1983).

The appearance of local reactions after vaccination is partly considered to be a prerequisite for good immunogenicity. The appearance of lungers is also a problem which can be a consequence of the application of live vaccines (Davies and Gilbert, 1969). The protective effect of vaccines varies between 60 and 70% depending on the production technique, storage time and transport conditions.

Currently all vaccines in use in Africa are living preparations, and their use is always subject to the suspicion that they may spread the disease. The modern trend in vaccine production is to remove the risk of introducing live organisms into the animal. This has necessitated research into developing inactivated vaccines. It has been shown that immunity is enhanced by the addition of adjuvants to inactivated mycoplasma vaccines (Garba *et al.*, 1986; Garba *et al.*, 1987a and 1987b).

Previous works of Brown (1966), Shifrine and Beech (1968), Garba *et al.* (1986, 1987a, 1987b) indicate that heat or formalin inactivated vaccines were found advantageous to attenuated vaccines as far as their stability, sterility safety and potency are concerned. They have reported that the inactivated vaccine was found stable and sterile during four and 12 months at 37°C or +4°C, retained their immunogenicity and hence they concluded that, inactivated Gladysdale vaccines seemed more potent and safe than the attenuated T_1 vaccine.

Animals which received hyperimmune/immune serum from experimentally infected and recovered cattle 24 hours before subcutaneous challenge with virulent *MmmSC* inhibited the formation of "Willems reaction", but did not prevent the formation of lung lesion, indicating that possibly the antiserum had "sensitized" the lungs to subsequent infection with *MmmSC*. But these animals were found protected from incontact exposure to CBPP affected clinical cases for 8 to 9 days (Gourlay and Shifrine, 1965; Masiga and Read, 1972; Dyson and Smith, 1965, 1974 and 1975; Simam and Kagumba, 1989).

3. SERO-PREVALENCE STUDY

3.1 Introduction

Contagious bovine pleuropneumonia outbreaks have been reported in Awi and Western Gojjam zones since 1991 (MOA, 1997). No previous study on the prevalence and distribution of CBPP has been conducted and the present study is the first one on CBPP as far as North-West Ethiopia is concerned.

The specific objectives of this study were:
-to assess the apparent prevalence and distribution of CBPP in 11 selected districts of Awi and Western Gojjam zones,
-to propose appropriate measures that enable control and ultimately eradication of CBPP.

3.2 Materials and Methods

3.2.1 Description of the study area

The study was carried out in North-Western Ethiopia with Bahir-Dar town in the center of this area, located some 600 kms north-west of Addis Ababa. The survey area included 11 selected districts of the Awi and Western Gojjam zones (Ankesha, Banja, Dangila and Guangua from the Awi zone and Achefer, Bahir-Dar, Burie, Denbecha, Jabi-Tenan, Mecha, and Sekela from the Western Gojjam zone).

The area is bordered in the east by East Gojjam and South Gondar zones, in the west by the Benshagule-Gumz region, in the north by the Alefa-Takusa district of North Gondar and Lake Tana and in the south by the Blue Nile (Abay river) gorge adjacent to Oromia region.

Topographically, these areas are marked by hills, plains, steep slopes and gorges with a number of streams and rivers, the largest of which is the "Abay" river. The altitude ranges between 1650 and 2700 meter above sea level. Most areas have a mean temperature of about 25-30°C. The rainfall in most of these areas is typically bimodal with long rains extending from June to September and the short rain from March to April. The annual rainfall ranges between 1400 mm to 1600 mm.

Generally, the climate of the study area is classified as hot and humid and three agro-climatic zones are distinguished: Highland, medium altitude and lowland. Population living in the area consists of the Agew and Amhara ethnic groups.

3.2.2 Livestock production

The major livestock species in the study area include cattle, sheep, goats and equines. The cattle population of the study zones is estimated to reach 1.6 million heads, accounting for 18% of the region and 5% of the total national population. The predominant cattle breed is the indigenous Fogera zebu. Mixed crop-livestock agriculture is carried out under a subsistance agricultural management.

3.2.3 Sample size determination

For sample size determination the procedures recommended by Toma *et al.* (1996) were followed (Table 4). Calculations are based on the following basic informations and estimates:
1. The total number of villages listed for the 11 districts of the two zones was 350.
2. The minimum number of cattle was estimated to be 1000 per village on average.
3. 50% of the villages are affected by CBPP.
4. In the affected villages, 5-10% of the animals are infected.
5. CFT detects 75% of infected animals (Bashiruddin *et al.*, 1994).

Table 4 Tabulated values used to determine the sample size (Toma *et al.*, 1996)

I. Sample size to estimate prevalence at village level

Relative precision	20%		33%	
Infection rate	40%	50%	40%	50%
No of villages expect	102	75	46	32

II. Sample size to detect disease at village level

Minimal infection rate	5%	10%	5%	10%	5%	10%	5%	10%
Animals req per village	57	37	57	37	57	37	57	37

III. Correction for test properties

Test sensitivity	75%

IV. Final result

Total no of animals	7752	5032	5700	3700	3496	2269	2432	1597

Based on these information it was estimated that a minimum of 32 villages is required in which a total of 1,600 to 2,400 serum samples are to be collected. This estimation accommodates a 5% error.

3.2.4 Selection of villages

The study on CBPP is a national demand and one of the problem areas is Amhara region. The region and districts were selected based on discussion with the Amhara region veterinary service team. The existence of CBPP, financial, technical and all other supports from Amhara region MOA were some of the points considered to choose Amhara region. Villages in the respective districts were selected using a simple random sampling procedure. In cases of none response due to inaccessibility and other reasons, the nearest possible villages were considered. About five villages were initially selected from each district and three to five days were allocated for interviews and serum sample collection for each district.

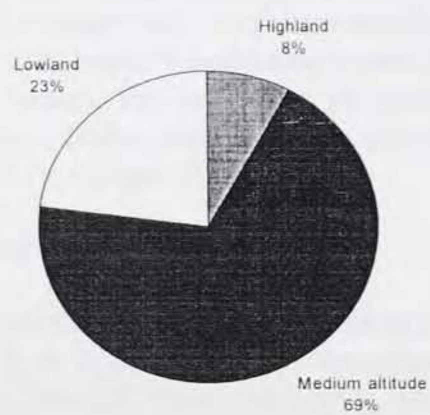

Figure 1. Agro-ecological description of the study area

Table 5. Description of districts based on factors of epidemiological interest

District	Agro-ecology	CBPP status	Production system	Year of CBPP vaccination
Ankasha	Lowland	Outbreak	Mixed +	1995
Banja	Lowland	Outbreak	Mixed +	1993
Dangila	Lowland	Outbreak	Mixed +	1995
Guangua	Lowland	Outbreak	Mixed +	1995
Achefer	M. Altitude	Free	Mixed *	1993
Bahir-Dar	M. Altitude	Free	Mixed *	1992
Burie W.	M. Altitude	Suspected	Mixed *	1995
Denbecha	M. Altitude	Suspected	Mixed *	1996
Jabi-Tenan	M. Altitude	Suspected	Mixed *	1996
Mecha	Highland	Free	Mixed *	1990
Sekela	Highland	Free	Mixed *	'1992

+ More inclined to livestock production, * Crop production is more dominant

Table 6. Various reasons and degree of cattle movement

District	Grazing	Marketing	Social	Trading
Dangila	++++	+++	++++	++++
Ankasha	+++	+++	+++	+++
Banja	++++	+++	++++	++++
Guangua	++++	++++	++++	++++
Burie	+++	++	++++	++
Sekela	+	++	++	+-
Jabi-Tenan	++	++	+++	+++
Denbecha	++	++	+++	+++
Mecha	++	++	++	++++
Bahir-Dar	++	++	+++	++++
Achefer	+++	+++	+++	++++

+ = Rarely, ++ = Sometimes, +++ = Frequently, ++++ = Very frequently

3.2.5 Questionnaire survey

During serum sample collection, a preliminary questionnaire survey was conducted on livestock owners at 38 selected villages and two governmental cattle breeding ranches. The purpose was to assess the type of livestock's production system, the prevalence and economic importance of major cattle diseases, the occurrence of bovine respiratory diseases and measures taken to deal with them. During serum sample collection the interviews were held with representatives or elders of the villages (Annex 5).

3.2.6. Serum sample collection

Cross-sectional serum sample collection using cluster sampling procedure has been conducted in the selected villages. About 5-10 ml of whole blood was collected by jugular vein puncture and allowed to clot in a slanted position. Blood was kept at room temperature overnight and the serum was carefully removed and transferred into test tubes. Turbid samples were centrifuged prior to serum collection. Serum tubes were labelled and transported to the laboratory on ice and were either tested immediately or kept deep frozen until tested. During sample collection, age, sex, breed and ear mark were recorded for every individual animal.

3.2.7 Serology

Samples for sero-prevalence study were tested using the standard complement fixation test. Any presentation of data related to sero-prevalence refers to CFT test results.

Complement fixation test (CFT)

The procedure of Alton *et al.* (1975) for testing against bovine brucellosis was closely followed in regard to materials and methods, with some alterations:

1). Complement (C) was preserved according to the method of Richardson (Huddart, 1963). For the test proper, 2 x 2 full haemolytic doses of C were used.
2). For antigen production, the *MmmSC* Pg1 strain from CIRAD-EMVT (Montpellier, France) has been cultured on Bacto-tryptose supplemented with yeast extract, horse serum, and thallium acetate plus penicillin. Sedimented and washed mycoplasmas were heat inactivated, homogenized and aliquoted. The freeze dried antigen was kept for a minimum period of six weeks at 4°C before use in the CFT. The working titre of the antigen was assessed by checkerboard titration against the strongly positive serum from an infected bovine.

Test proper for CFT

Prior to testing, samples were thawed and about 200 µl volume of each sample was pipetted into U-shape microplates. 25 µl undecomplemented test sera, diluted 1:10, were dispensed in duplicates into all rows of column three to 12 of the microplates. Positive control sera were serially diluted in all rows of column 2 in 25 µl amounts starting from 1:10 dilution. Column one was used for controls: A for positive control sera, B for negative control sera, C for Ag control, D to F for complement control and G-H hemolytic serum control.

25 µl of the working Ag (1:40) was added to all wells. 50 µl of titrated complement was then added to all wells and the content was agitated and either kept overnight at + 4°C or incubated for one hour at 37°C. Finally 50 µl of haemolytic system was added and incubated for 30 minutes. A positive reaction was indicated by the absence of hemolysis. The degree of sedimentation of unlysed erythrocytes was graded as +, ++, +++ and ++++. The decision criterion for positive test result is two ++ and above at 1:20 serum dilution.

Based on the above procedure, all samples were screened at 1:10 dilution and those positively reacting, two ++ and above at 1:10 dilution are subsequently retested in dilutions 1:10, 1:20 and 1:40 with individual control of anti-complementary activity (Annex 3).

3.2.8 Data analysis

Microsoft Excel served as data base. Descriptive statistics such as the mean, median, minimum, maximum and confidence interval of means were used to approximate the sero-prevalence of CBPP. Calculation of the Chi-square test and the Odds ratios served to investigate differences and associations of sero-prevalences of CBPP between different villages, districts, zones, agro-climates, years of last CBPP vaccination, age and sex categories of tested animals. Non parametric analysis of variance using the Kruskal-Wallis test and Box-and-Whisker plots were used to compare the medians.

Risk factors

Factors of epidemiological relevance were selected, such as origin (village, districts, zones), agro-ecology (lowland, medium altitude and highland), status of CBPP (outbreak areas, suspected areas and free areas), year of last CBPP vaccination, age and sex were considered as risk factors for comparison of outcome variables (test results). Animals having the same risk factors were assumed to have some degree of dependency in contrast to those without the factor of interest. For calculation, EpiInfo version 6.0 using the Mantel-Haenszel technique included in the program package and hand calculator were used.

Chi-square

To check whether there is an association between the factor of exposure and the outcome variable the chi-square was calculated according to Martin *et al.* (1987). Expected values of outcome variables was estimated as the proportion of the product of the row total and column total to the grand total using the standard 2 x 2 table. The chi-square was then calculated as the sum of the square of the difference between the observed and expected values in the groups with and without the factor of interest. Using one degree of freedom, the probability value was obtained from the chi-square table. The calculated value is compared to that of the table value.

$$X^2 = \sum(\text{observed-expected})^2/\text{expected}$$

Odds ratios (OR)

Odds ratios were used to quantify the strength of association between the groups which were exposed to the factor of interest from those which were not exposed. It is the ratio of the odds of disease occurring among animals exposed to the factor to the odds of disease occurring among animals not exposed to the factor. It was calculated by using the standard 2 x 2 table as follows:

$$OR = ad/bc$$

Outcome variable (serological result)

		Positive	Negative
Factor	Positive	a	b
	Negative	c	d

Interpretation of the OR: The OR = 1, indicates the factor has no effect, OR<1, the factor has "protective" effect, OR>1, the factor has a risk effect. In other words, if the CI of the OR include one the factor has no effect.

Confidence interval of a proportion (prevalence rate)

The confidence interval was estimated by addition and subtraction of two standard error from the mean. The standard error was determined according to Martin *et al.* (1987) using the prevalence and the respective sample size.

Standard error (SE) = (P (1-P)/n)^0.5

95% Confidence Interval of the mean (μ) = μ ± 1.96 SE

3.3. Results

3.3.1 Questionnaire survey

During serum sample collection for this study, a total of 39 farmers were interviewed. The farmers gave information on major cattle diseases and their ranks, local names for respiratory diseases of cattle, frequent causes of respiratory diseases of cattle, seasonality of respiratory diseases of cattle and on measures taken in cases of outbreaks.

The major cattle diseases named by farmers and ranked by them according to their economic importance include: Endoparasites, pneumonia, trypanosomiasis, anthrax and blackleg (Table 5).
Five local names were recorded for bovine respiratory diseases: Samba (21%), Sal (59%), Woziwuz (10%), Zihon-Wotetie (5%) and Aslig (5%) (n = 39) (Figure 2).

Suggested possible sources of cattle respiratory disease outbreaks were: Seasonal cattle movement (51%) and social factors involving cattle movement (3%); 46% of farmers did not mention specific causes (Figure 3). They considered CBPP as a new disease.

Seasonal occurrence of bovine respiratory diseases: 18% of farmers reported a rather frequent occurrence at the end of the rainy and beginning of the dry season, 23% in contrast, at the end of the dry and the beginning of the rainy season. 59% were of the opinion that the disease has no specific seasonal pattern (Figure 4).

The measures frequently taken in case of respiratory disease outbreaks were as follows: 69% have said to use treatment by non-professionals, 23% used vaccination and 8% of the farmers practiced traditional treatment (Figure 5).

Table 7. Major cattle diseases and their ranks (n = 39)

Rank	Endoparasite	Pneumonia	Trypanosomiasis	Anthrax	Blackleg
1	18	14	6	1	0
2	6	10	8	5	10
3	7	7	6	8	11
4	7	6	4	9	13
5	1	1	10	12	15
mean rank	2.2	2.2	2.7	3.36	4.6

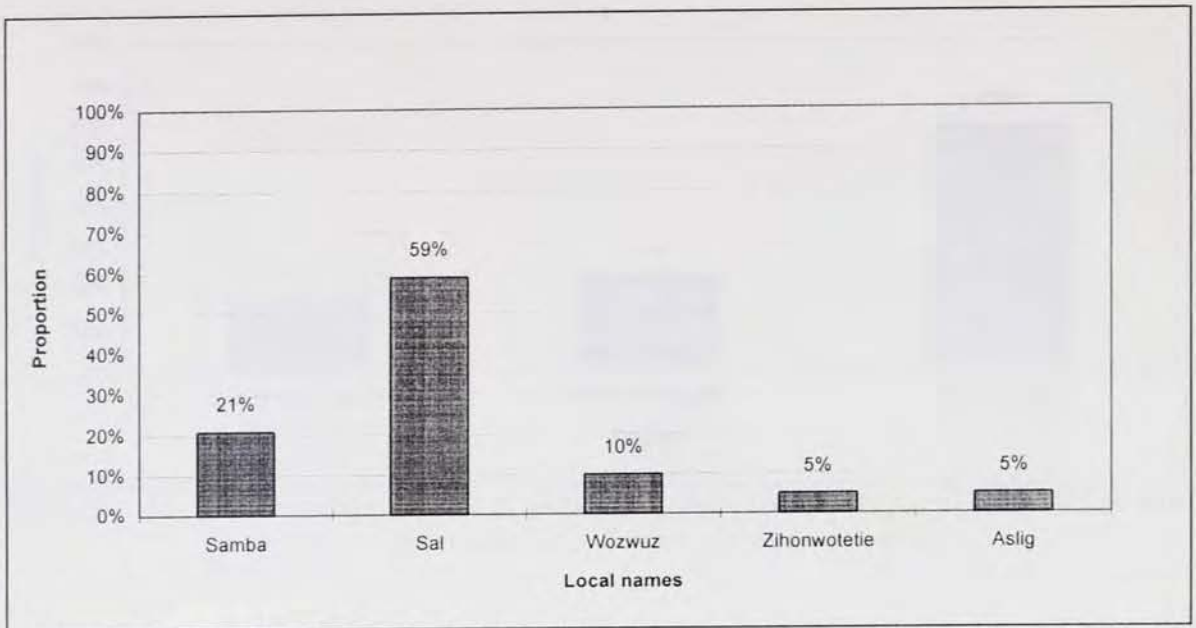

Figure 2. Local names for respiratory diseases of cattle given by livestock owners

Table 8. Vernacular names for respiratory diseases and their equivalence

Local names (Amharic)	Equivalent description
Samba	Lung
Sal	Chronic coughing
Woziwuz	disease with abdominal breathing
Zihon-Wotetie	Elephant disease
Aslig	Disease inducing chronic emaciation

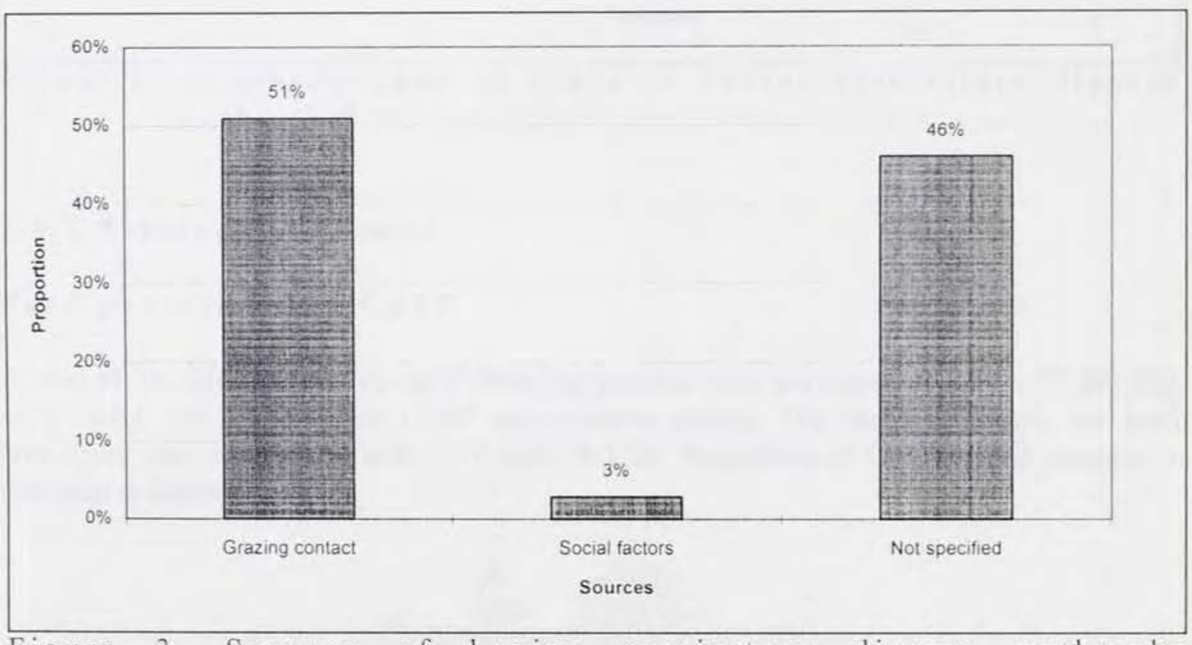

Figure 3. Sources of bovine respiratory disease outbreaks suggested by farmers

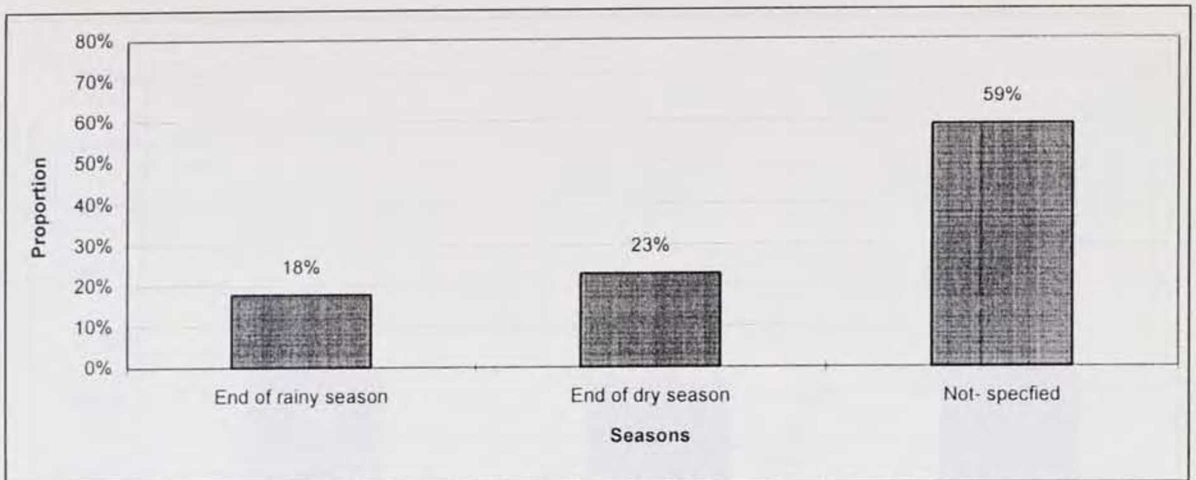

Figure 4. Seasonal occurrence of bovine respiratory disease outbreaks information from livestock owners

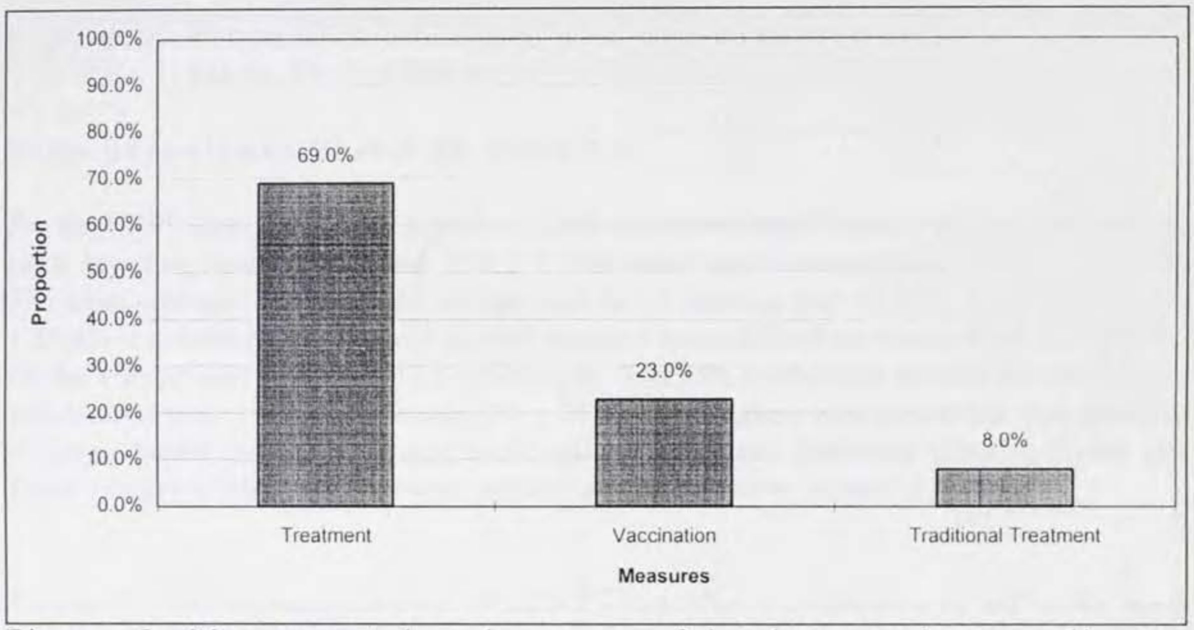

Figure 5. Measures taken in cases of bovine respiratory disease outbreaks

3.3.2 Serological results

Herd prevalence of CBPP

A total of 38 villages and two cattle breeding ranches were surveyed, of which 37 (92.5%) were found with at least one CBPP sero-positive animal. The decision criteria for herd prevalence was one reactor with CFT titre of 1:20. Proportion of CFT reacted samples is indicated in figure 6.

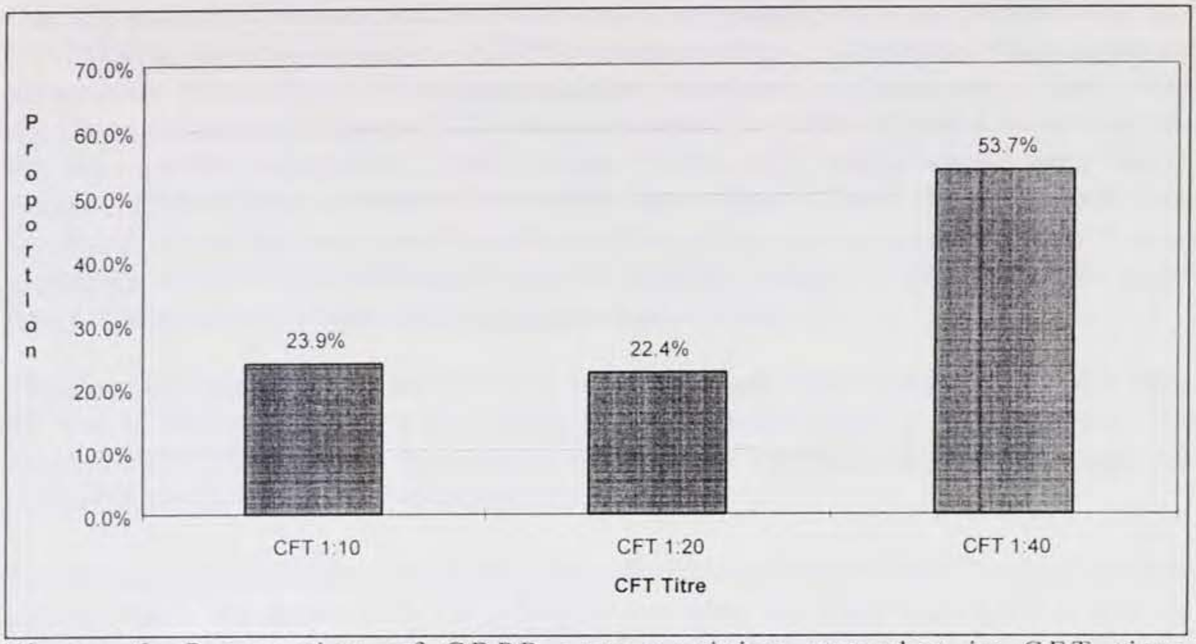

Figure 6. Proportion of CBPP sero-positive samples in CFT titre steps (n = 486)

Sero-prevalence based on districts

For the CBPP sero-prevalence, a total of 2,140 sera were tested from 38 villages and the two cattle breeding ranches. Of these, 370 (17.3%) were found sero-positive by CFT (Table 9). The mean sero-prevalence for the villages and the 11 districts was 19.3% $\pm$ 12.2% and 22.2% $\pm$ 24.6% respectively, the variance and the standard error (SE) of the means were 38.9 and 6.2 for the villages and 157.0 and 12.5 for districts. The 95% confidence interval for the villages and districts were 19.3 $\pm$ 12.2% and 22.2 $\pm$ 24.6%. The highest sero-prevalence was observed in Banja district (66.3%) followed by Dangila (41.7%) and Denbecha (33.3%) (Table 10). Three villages in Mecha district were without any sero-positive animal (Annex 6).

Table 9. Sero-prevalence of CBPP in the 11 districts of Awi and Western-Gojjam zones

Zone	District	Number of Villages	Sample (n)	CFT 1:20 positive	%CFT 1:20 positive	95% Confidence interval of the mean
Awi	Ankesha	3	180	45	25.0	(18.7, 31.3)
	Banja	2	80	53	66.3	(55.9, 76.7)
	Dangila	3	180	75	41.7	(34.5, 48.9)
	Guangua	5	300	29	9.7	(6.4, 13.1)
W-Gojjam	Achefer	4	280	31	11.1	(7.4, 14.8)
	B-Dar	6	340	40	11.8	(8.4, 15.2)
	Burie	2	160	28	17.5	(11.6, 23.4)
	Denbecha	3	60	20	33.3	(21.4, 45.2)
	J-Tenan	3	140	15	10.7	(5.6, 15.8)
	Mecha	6	260	19	7.3	(4.1, 10.5)
	Sekela	3	160	15	9.4	(4.9, 13.9)
	Total	40	2140	370	17.3	(15.7, 18.9)

One way analysis of variance indicates that there is a statistically highly significant difference ($p<0.0000$) of the sero-prevalence of CBPP between districts. To determine which means are significantly different from which others: Multiple comparison procedure using Fisher`s least significant difference technique (LSD) was conducted. The LSD of district means indicate that there are five homogeneous district groups: Mecha and Guangua (Group one), Sekela, Achefer, Finote-Selam and Bahir-Dar (Group two), Burie (Group three), Ankesha and Denbecha (Group four) and Dangila and Banja (Group five) were in ascending order of sero-prevalence. However, the assumptions that "the standard deviation of the means is the same" does not hold true and violates this analysis of variance (Table 10).

Therefore, information on the medians was found to be important. Non parametric test using the Kruskal-Wallis Test (one way analysis of variance) was found to be appropriate. The Kruskal-Wallis Test indicates that there is a statistically significant difference amongst the medians at the 95% confidence level ($p<0.05$) (Table 11).

To determine which medians are significantly different from which others, Box-and-Whisker plot was made. The median from Banja district (Awi zone) was found to be different from all other districs, followed by the median from Dangila district which is not significantly different from Ankesha and Denbecha but significantly different from other districts (Figure7).

Table 10. Summary of descriptive statistics for districts

District	Mean	Variance	Standard deviation	Standard error	Min	Max	Range
Ankesha	24.9	433.3	20.8	5.4	8.3	48.3	40.0
Banja	66.3	253.1	15.9	6.6	55.0	77.5	22.5
Dangila	41.7	19.2	4.4	5.4	36.7	45.0	8.5
Guangua	9.7	80.0	8.9	4.2	1.7	21.7	20.0
Achefer	10.8	16.1	4.0	4.7	6.7	16.3	9.6
Bahir-Dar	12.5	95.1	9.7	3.8	1.7	25.0	23.3
Burie	19.7	149.6	12.2	6.6	11.0	28.3	17.3
Denbecha	33.3	33.3	5.8	5.4	30.0	40.0	10.0
J-Tenan	11.7	33.3	5.8	5.4	5.0	15.0	10.0
Mecha	5.3	43.9	6.6	3.8	0.0	15	15.0
Sekela	9.7	16.9	4.1	5.4	5.0	12.5	7.5

Table 11. The Kruskal- Wallis Test shows average rank of mean sero-prevalence of CBPP in the 11 districts.

District	sample size	Average rank
Achefer	4	16.0
Mecha	6	8.9
Sekela	3	18.3
Ankesha	3	25.2
Bahir-Dar	6	17.3
Banja	2	38.5
Burie	2	23.0
Dangila	3	34.67
Denbecha	3	32.3
Guangua	5	14.3
Jabi-Tenan	3	17.0

Test statistic = 22.5641, p-value = 0.0124752

The p-value indicate that there is statistically significant difference between Districts on the sero-prevalence of CBPP. To know which District is different from which the Box-and-Whisker plot was sketched.

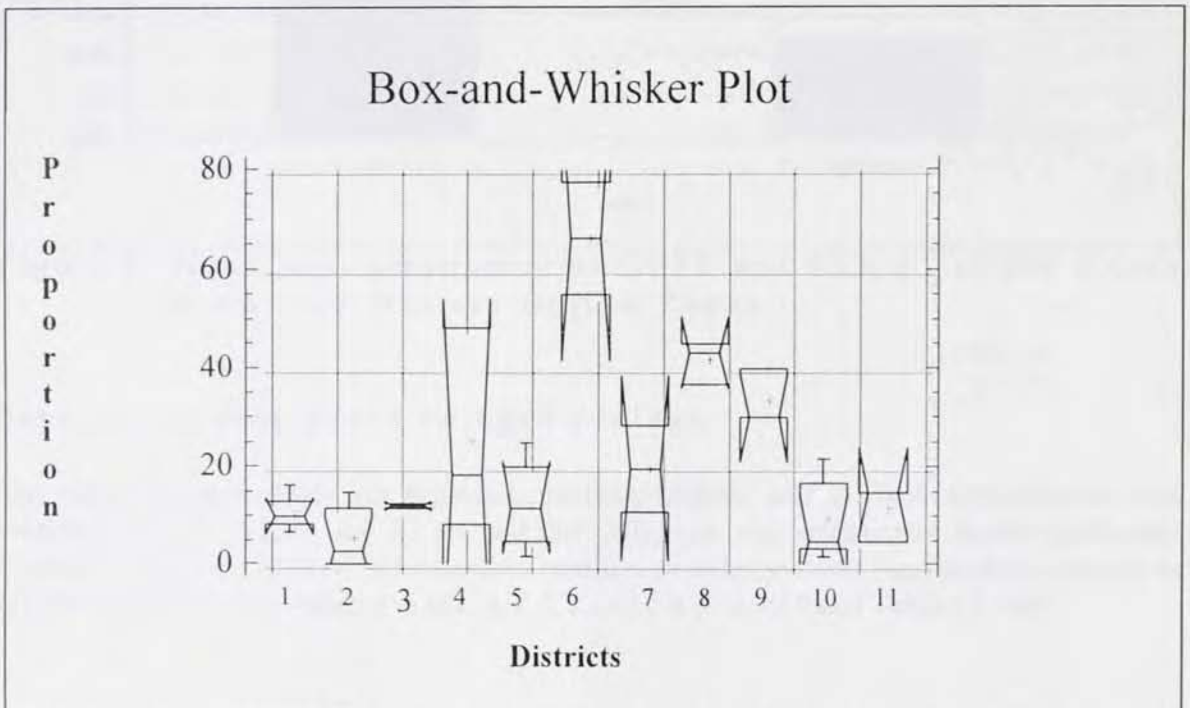

Figure 7. Box-and-Whisker plots showing mean sero-prevalence results of CBPP for the 11 districts, North west Ethiopia.

Key

1. Achefer
2. Mecha
3. Sekela
4. Ankesha
5. BahirDar
6. Banja
7. Burie
8. Dangila
9. Denbecha
10. Guangua,
11. Jabi-Tenan

The Box-and Whisker plots (Figure 7) indicate that Banja and Dangila (Both from Awi Zone) and Denbecha are significantly with high sero-prevalence of CBPP followed by Ankesha from Awi and Burie from Western Gojjam Zones. But the CI of the latters did overlap to other district means and hence was not significantly different from other Districts.

Sero-prevalence based on administrative zones

The mean sero-prevalence for Awi and Western Gojjam zones was found to be 27.3% and 12.0% and this observed sero-prevalence variation was statistically highly significant ($p<0.001$) with an OR of 2.8 (Figure 8, Table 13, 14).

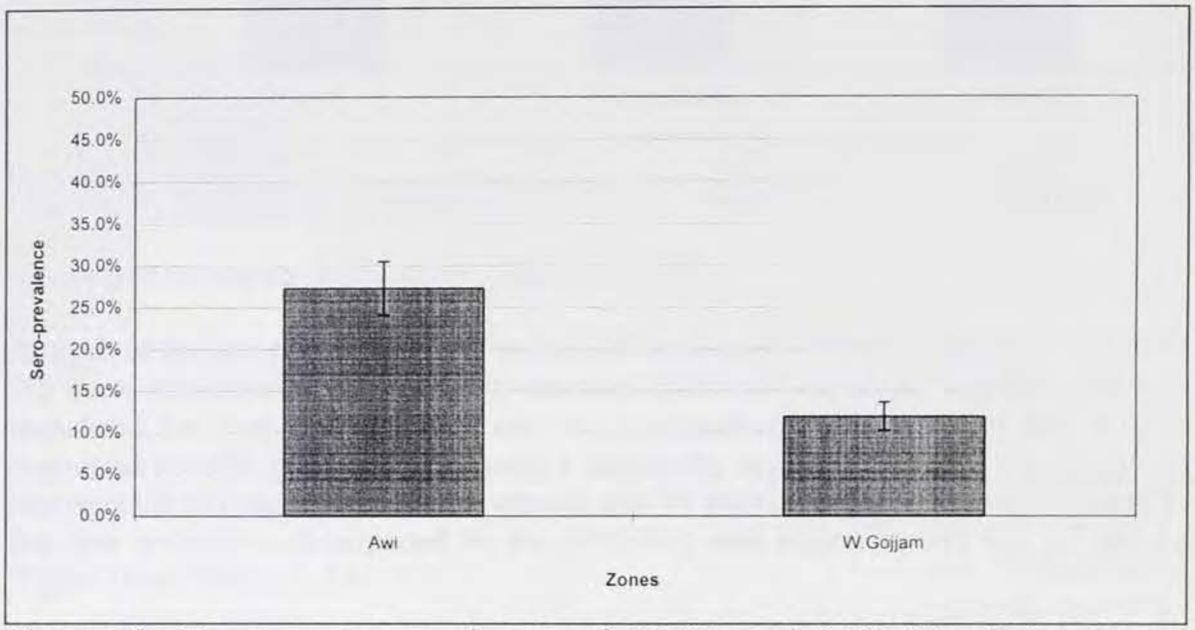

Figure 8. Mean sero-prevalence of CBPP and 95% CI of the means in Awi and Western Gojjam Zones

Sero-prevalence based on agro-ecology

The mean sero-prevalence for highland, medium altitude and lowland agro-climates was recorded as 8.1%, 13.7% and 27.3% and this difference was statistically found significant: lowland vs highland ($p<0.001$), lowland vs medium altitude ($p<0.001$) and medium altitude vs highland ($p<0.01$) with respective OR, 4.3, 2.4, and 1.8 (Figure 9 and Table 13, 14)

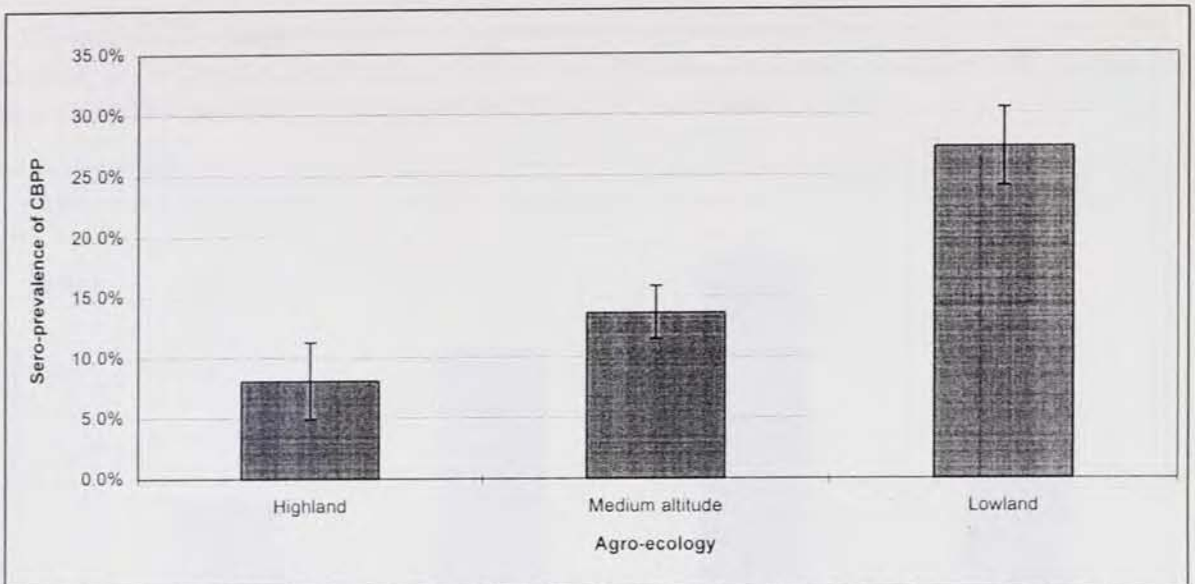

Figure 9. Sero-prevalence of CBPP in relation to agro-ecology

Sero-prevalence based on CBPP status

Analysis of the sero-prevalence based on the official disease outbreak information was made. The mean sero-prevalence for officially reported CBPP outbreak areas, suspected and areas considered free was 27.3%, 17.3% and 10.1% respectively. It was found that in areas considered to suffer from CBPP outbreaks a statistically significantly higher sero-prevalence was recorded as compared to others: outbreak area Vs suspected ($p<0.001$), outbreak area Vs free area ($p<0.001$) and suspected Vs free ($P<0.001$) with respective OR; 1.8, 2.7 and 1.9 (Figure 10 and Table 13, 14).

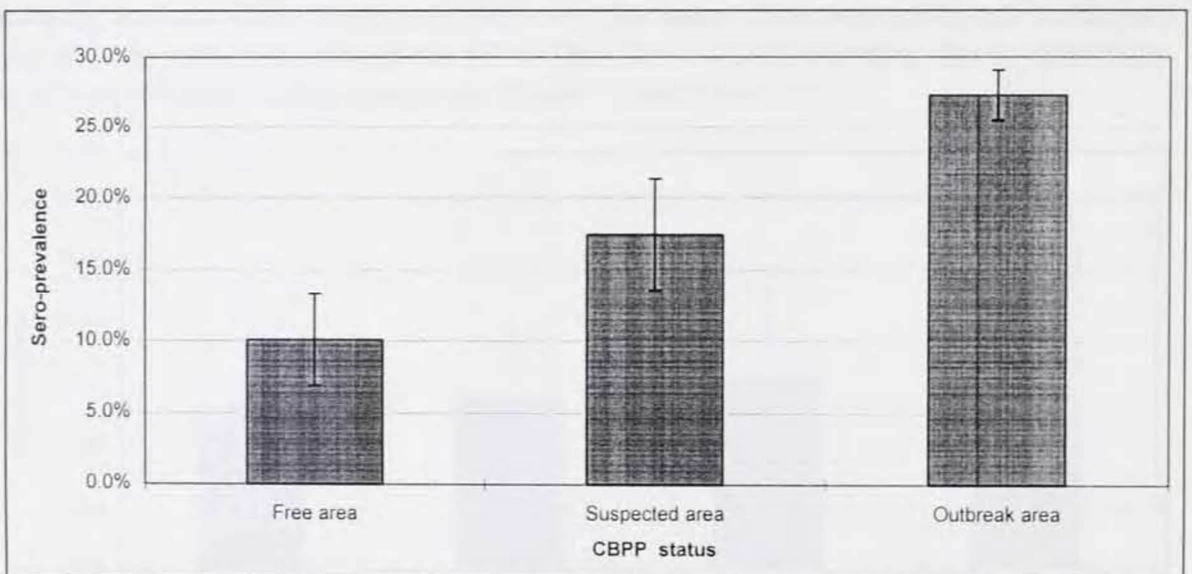

Figure 10. Sero-prevalence of CBPP in relation to CBPP status

Sero-prevalence based on year of CBPP vaccination

Analysis of sero-prevalence results based on year of last CBPP vaccination was recorded. The mean sero-prevalence for years 1990/91, 1992/93, 1994/95 and 1996+ was 7.3%, 16.2%, 21.6% and 17.5% respectively and no statistically significant difference for the years 1992 onwards, but there was a statistically significant difference between years after 1991 to that of years up to 1991 (p<0.001) and OR = 2.9 (Figure 11 and Table 13. 14).

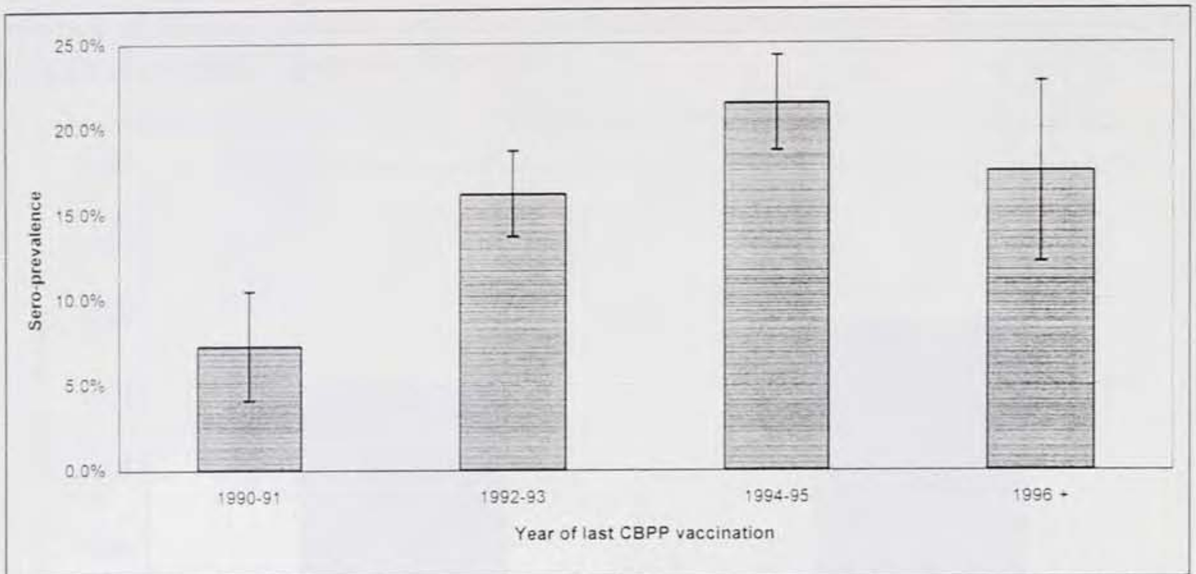

Figure 11. Sero-prevalence of CBPP in relation to year of last CBPP vaccination

Sero-prevalence in relation to age

The mean sero-prevalence for four age groups; <1, 1-<2, 2-3 and >3 years was. 13.8%, 19.0%, 16.9% and 18.1% respectively. This difference seems statistically significant (p<0.05). However, analyses of the confidence intervals of the means show overlapping and confidence interval of the odds ratios include one (CI of The OR = 1.0-1.9), indicating that no statistically significant difference among age groups (Figure 12 and Table 13, 14).

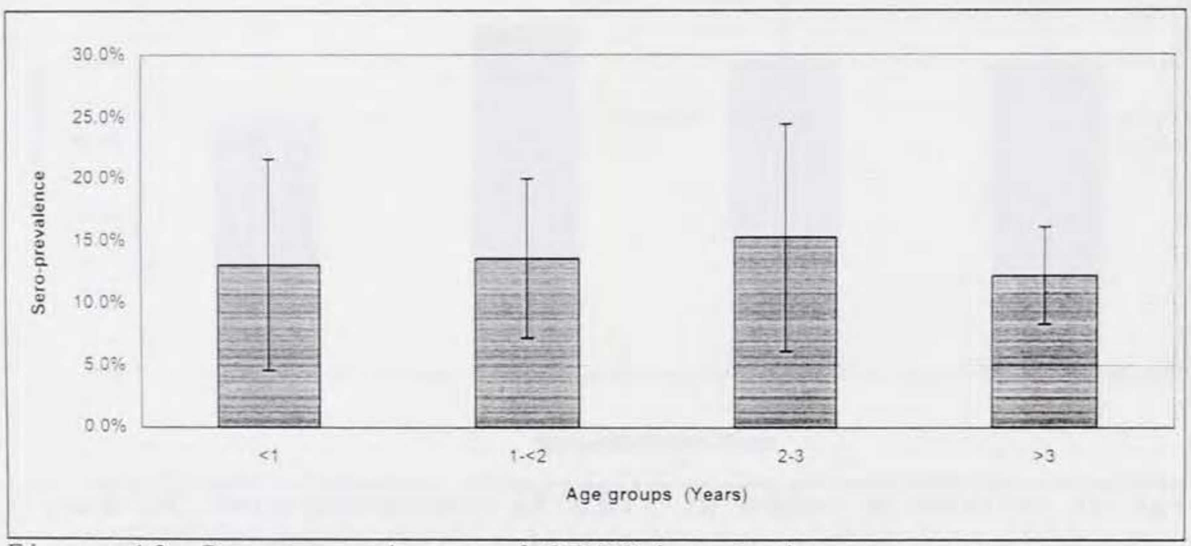

Figure 12. Sero-prevalence of CBPP in relation to age groups

Sero-prevalence in relation to sex

The mean sero-prevalence for males and females recorded was 19.7% and 15.6% and this difference was found to be statistically significant ($p<0.05$) with OR = (1.1-1.7). Further analyses of the data indicated a higher sero-prevalence in males of one year of age and older (Figure 13, 14 and 15, Table 12, 13 and 14).

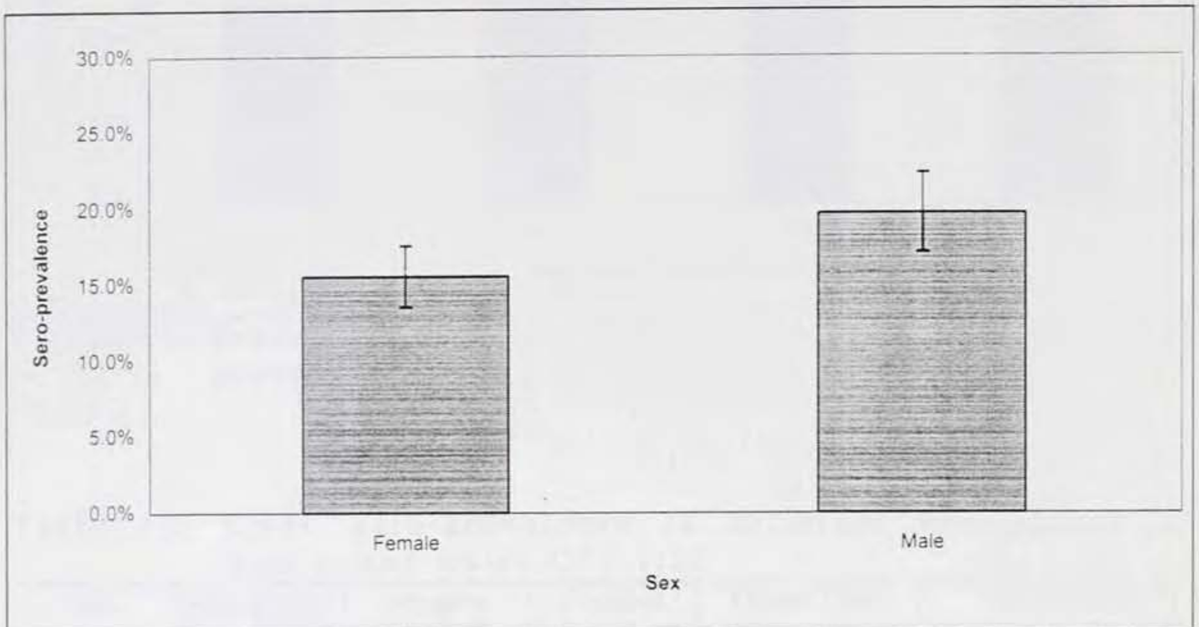

Figure 13. Sero-prevalence of CBPP and 95% CI of the means in relation to sex

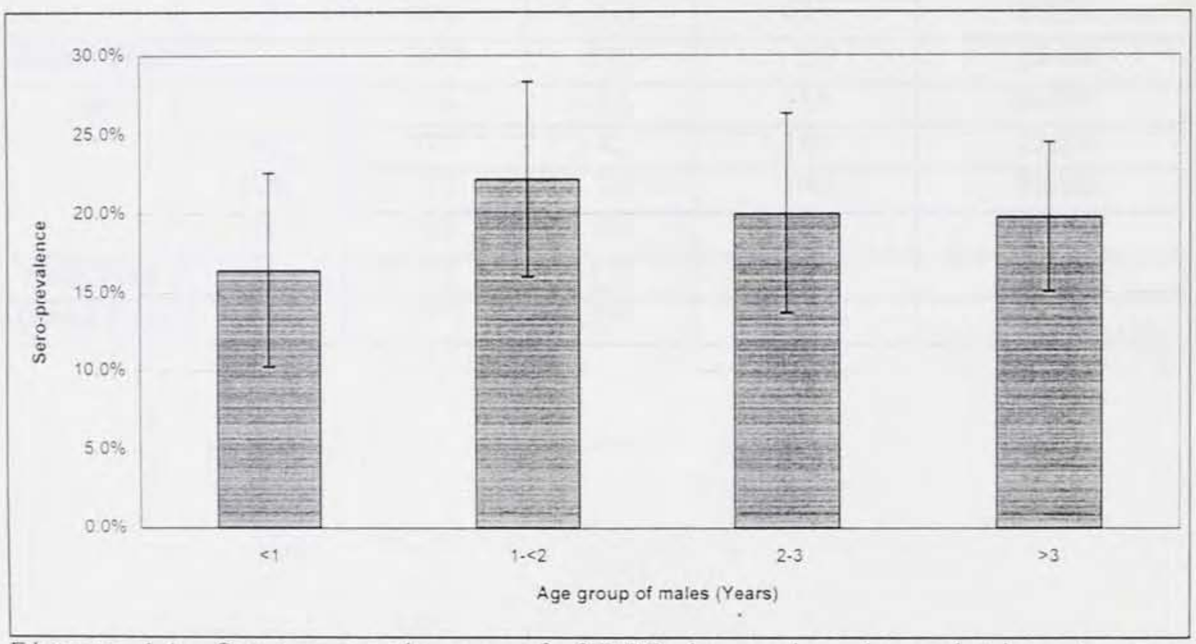

Figure 14. Sero-prevalence of CBPP in males in relation to age groups

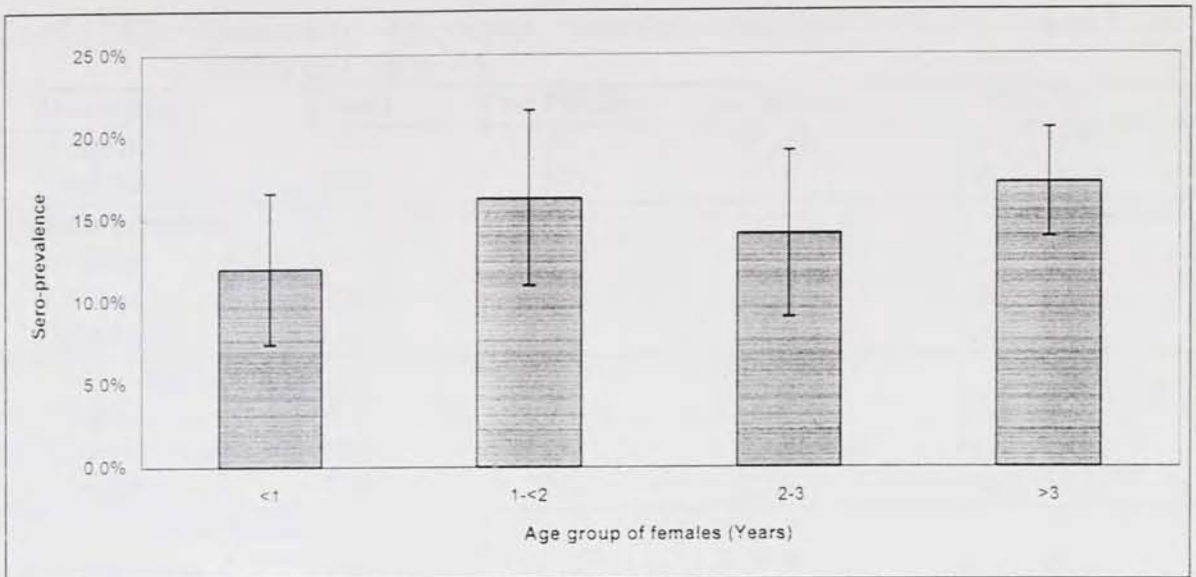

Figure 15. Sero-prevalence of CBPP in females in relation to age groups

Table 12. CBPP sero-prevalence in different age groups of females and males CFT 1:20

Sex	Age groups	Negative	Positive	Grand Total	% positive
female	<1	192	26	218	12.0%
	1-<2	185	36	221	16.3%
	2-3	183	30	213	14.1%
	>3	498	103	601	17.2%
female Total		1058	195	1253	15.6%
male	<1	138	27	165	16.4%
	1-<2	147	42	189	22.2%
	2-3	152	38	190	20.0%
	>3	275	68	343	19.8%
male Total		712	175	887	19.7%
Grand Total		1770	370	2140	17.3%

Table 13. Summary of mean sero-prevalence results based on different factors

Description	Tested	Test Positive	% Positive	95% CI
Total herds	40	37	92.50	(84.3, 100)
Total serum	2140	370	17.3	(15.7, 18.9)
Administrative zone				
Awi	740	202	27.3	(24.1, 30.5)
W.Gojjam	1400	168	12.0	(10.3, 13.7)
Agro-climate				
Highland	420	34	8.1	(5.5, 10.7)
Medium altitude	980	134	13.7	(11.5, 15.9)
Lowland	740	202	27.3	(24.1, 30.5)
CBPP status				
Outbreak area	740	202	27.3	(24.1, 30.5)
Suspected area	360	63	17.5	(9.6, 21.4)
Free area	1040	105	10.1	(8.3, 11.9)
Year of last CBPP vaccination				
1990-91	260	19	7.3	(4.1, 10.5)
1992-93	860	139	16.2	(13.7, 18.7)
1994-95	820	177	21.5	(18.7, 24.3)
1996+	200	35	17.5	(12.2, 22.8)
Age groups				
<1	383	53	13.8	(10.3, 17.3)
1-<2	410	78	19.0	(15.2, 22.8)
2-3	403	68	16.9	(13.2, 20.6)
>3	944	171	18.1	(15.6, 20.6)
Sex				
Female	1253	195	15.6	(13.6, 17.6)
Male	887	175	19.7	(17.1, 22.3)

Table 14. Summary of X^2 and OR values based on the different variables tested

Description	X^2	Df	P-value	OR	Confidence interval OR
Awi Vs W.Gojjam	79.2	1	<0.001	2.8	2.2 - 3.4
Agro-climates					
Lowland Vs Highland	60.9	1	<0.001	4.3	3.0 - 6.1
Lowland Vs Medium altitude	49.8	1	<0.001	2.4	1.9 - 3.0
Medium altitude Vs Highland	8.7	1	<0.01	1.8	1.2 - 2.7
Outbreak Vs suspected	12.7	1	<0.001	1.8	1.3 - 2.4
Outbreak Vs Free	89.6	1	<0.001	2.7	2.6 - 4.3
Suspected Vs Free	13.9	1	<0.001	1.9	1.4 - 2.6
Year vaccination					
After 91 Vs before 91	20.6	1	<0.001	2.9	1.8 - 4.6
≥ 1 year Vs < 1 year	3.9	1	<0.05	1.4	1.0 - 1.9*
Male Vs Female	6.3	1	<0.05	1.3	1.1 - 1.7

* = No significant variation between age groups

4. VACCINATION TRIAL

4.1 Introduction

Vaccination against CBPP has been used as sole means of controlling and ultimately eradicating the disease in many African countries. The T1-44 freeze dried monovalent vaccine is largely in use in many regions of Ethiopia and it is the only recommended vaccine by the OIE (Tulasne *et al.*, 1996).

However, no sufficient information exists on the sero-conversion of this vaccine among a vaccinated population. Available information on the duration of serologically detectable antibodies after vaccination varies from about 30 days (Masiga and Domenech, 1995), 10 weeks (Chima and Pam, 1985), three months (LeGoff and Thiacourt, 1995), four months (Daleel, 1972) to 23 months (Onoviran and Taylor-Robinson, 1979).

Therefore, the specific objectives of investigation were:

- to study the sero-conversion of the T_1-44 freeze dried monovalent vaccine,
- to assess the level and duration of vaccinal antibodies,
- to compare C-ELISA and CFT results on samples collected at various stages of the post-vaccination period.

4.2 Materials and Methods

4.2.1 Description of the study area:

This study was conducted at the Debre Zeit research farm. The farm is located about 2-3 kms north of the Faculty of Veterinary Medicine.

4.2.2 The animals

A total of 77 cross-breed cattle were used which are kept for research purposes at the farm. The animals are grazing on an enclosed area and are fed additionally concentrates. About 90% of the animals were females. There were four age groups: calves less than one year, weaned groups aged 1-<2 years, breeding heifers aged 2-3 years, cows and bulls over three years. The number of animals from each age group were; 9, 15, 13 and 40 in ascending order (Table 15).

Animals were grouped into a vaccination and a control group (50 vaccinated and 27 unvaccinated controls). The number of animals to be in each group was decided by common sense. However, the selection and assignment of individual animals was age dependent and this was done at random. About one third of the respective age group was taken for control by using simple random procedure. One ml of the vaccine was inoculated subcutaneously on either side of the neck. Serum samples were collected at days 0, 7, 14, 21, 28, 35, 42, 49, 56 and 63. All samples were tested with both the CFT and the C-ELISA tests.

Table 15. Age distribution of animals used for vaccination trial

	Age groups (Years)				
Group	< 1	1-< 2	2-3	>3	Total
vaccinated	6	11	5	28	50
Control	3	4	8	12	27
Total	9	15	13	40	77

4.2.3 Vaccine

The vaccine used was the T_1-44 freeze dried monovalent vaccine. batch number PL4-97. production titre $10^{7.6}$ cfu.

4.3 Results

Serological response of cattle vaccinated against CBPP

The serological response of cattle vaccinated against CBPP and detected by CFT and C-ELISA are reported. Unvaccinated control animals remained sero-negative in both tests. C-ELISA and CFT. There were some animals that had not been sero-converted or serologically undetected; four animals were by CFT 1:10, ten animals were by CFT 1:20 and 18 animals were by C-ELISA out of the 50 animals vaccinated and tested (Annex 4). Table 16 and 17 provide a breakdown of the actual trial protocol.

Table 16. Number of sero-converted animals detected by CFT 1:10 and C-ELISA

Days	0	7	14	21	28	35	42	49	56	63
No Animals	50	50	50	50	50	50	50	50	50	50
CFT-1 +	0	5	9	26	45	20	1	3	1	0
CFT-2 +	0	5	6	16	19	7	0	0	0	0
C-ELISA-1 +	0	0	15	31	0	0	0	0	0	0
C-ELISA-2 +	0	0	15	17	0	0	0	0	0	0

But considering the proportion of sero-detected animals in a particular day of samples. C-ELISA was found to be more sensitive than CFT 1:20, 64% with C-ELISA Vs 40% with CFT 1:20 (Table 17).

Table 17. Number of sero-converted animals detected by CFT 1:20 and C-ELISA

Days	0	7	14	21	28	35	42	49	56	63
No Animals	50	50	50	50	50	50	50	50	50	50
CFT-1 +	0	5	9	20	8	20	1	2	0	0
CFT-2 +	0	5	6	14	6	7	0	2	0	0
C-ELISA-1 +	0	0	15	31	0	0	0	0	0	0
CFT-2 +	0	0	15	17	0	0	0	0	0	0

By C-ELISA, antibodies were detected only on days 14 and 21. In contrast, CFT detected antibodies as from day 7 onwards, with peak values at day 28 with subsequent decline of values thereafter (Figure 16, 17 and Annex 4).

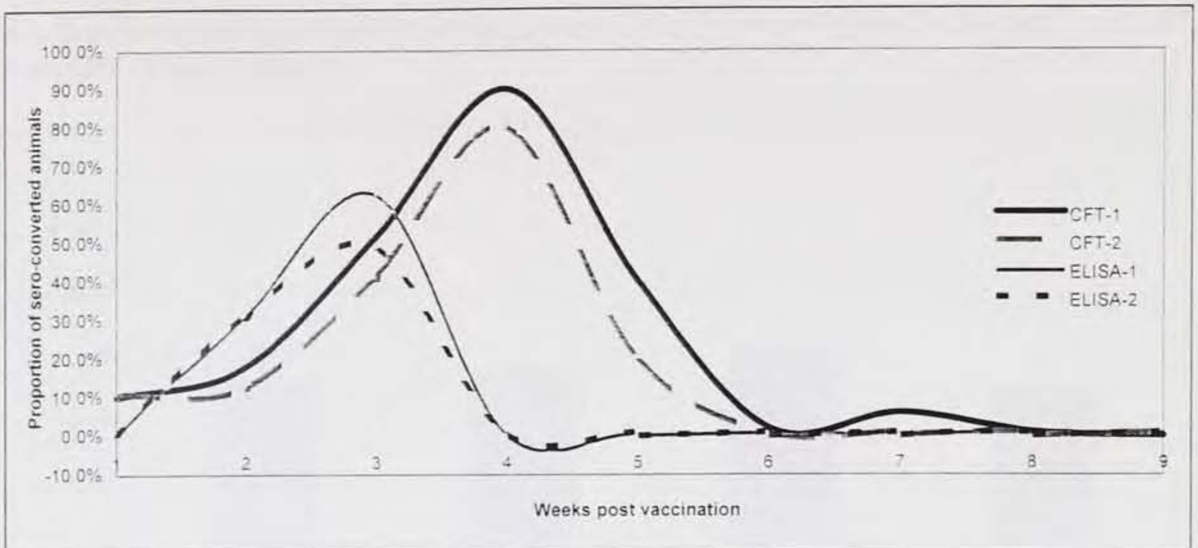

Figure 16. Prevalence and incidence of sero-converted animals CFT 1:10 and C-ELISA.

CFT-1 and C-ELISA-1 indicate the proportion of sero-positive animals, which includes all animals found sero-positive at the day of testing when compared to the total number of tested animals. CFT-2 and C-ELISA-2 indicate newly detected sero-positive animals.

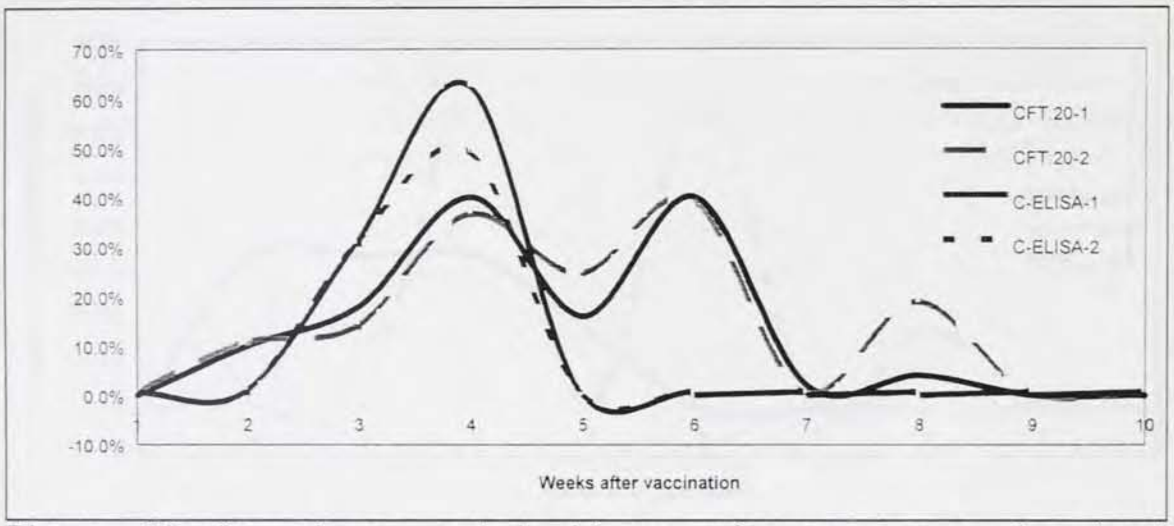

Figure 17 Prevalence and incidence of sero-converted animals PV, CFT 1:20 Vs C-ELISA.

CFT-1 and C-ELISA-1 indicates the proportion of sero-positive animals, which includes all animals found sero-positive at the day of testing when compared to the total number of tested animals. CFT-2 and C-ELISA-2 indicates newly detected sero-positive animals.

Vaccination response in relation to age

Assessment of sero-conversion in relation to age indicated that all age groups have shown similar responses to vaccination. There was a similar serological response of the various age groups to T_1-44 freeze dried monovalent vaccine. There was a relatively higher response in middle age groups (1-<2 and 2-3 years), but the confidence interval of the four age groups did overlap, showing no statistically significant difference of vaccination response in relation to age (Figure 17). Age response in relation to time was assessed and an interesting observation was that, there was a double fold in age groups 1-<2, 2-3 and >3 years in the four[th] and six[th] weeks PV (Figure 18 and 19).

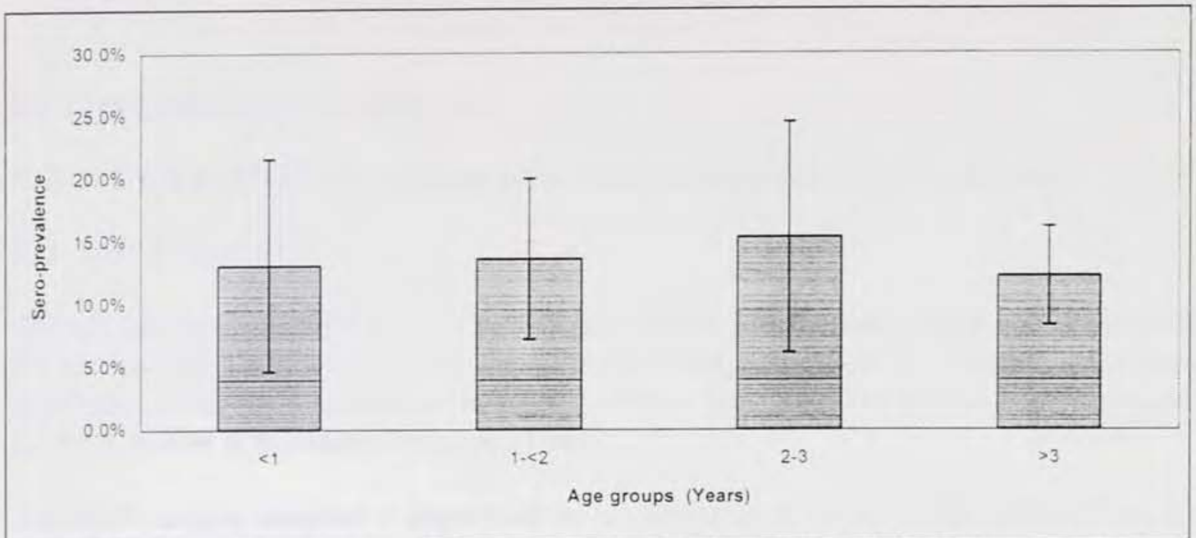

Figure 18. Proportion of sero-converted cattle in relation to age and the 95% CI of the means

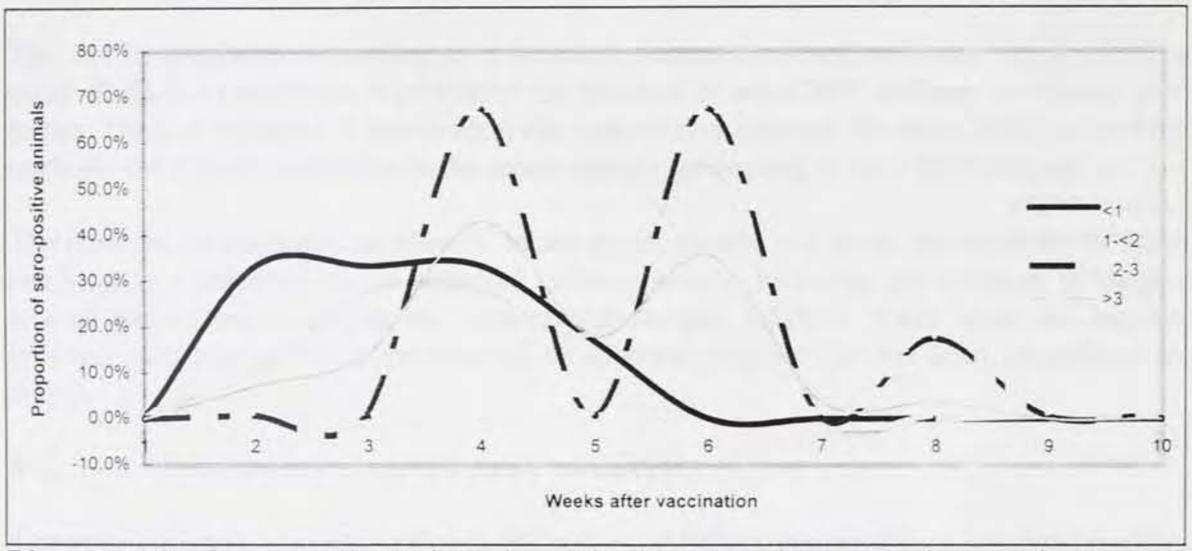

Figure 19. Sero-convertion in relation to time in the various age groups (during 10 weeks), CFT 1:20

5. COMPARISON OF CFT AND C-ELISA TEST RESULTS

5.1 Introduction

The diagnosis of CBPP depends mainly on serological tests of which only the CFT has been accepted by the OIE for international cattle trade. Recently, a competitive enzyme linked immunosorbent assay based on monoclonal antibodies has been proposed by CIRAD-EMVT. The test is under evaluation in five African veterinary laboratories, one of which is the National Veterinary Institute (NVI), Debre Zeit, Ethiopia.

The specific objective of this study was: To compare the two serological tests (CFT and C-ELISA) for the diagnosis of CBPP, using bovine serum samples of different origins in Ethiopia.

5.2 Materials and Methods

5.2.1 Competitive Enzyme Linked Immuno-sorbent Assay

5.2.1.1 General

The test has been prepared as a kit by CIRAD-EMVT, Montpellier, France and the Animal Production and Health Section of the joint FAO/IAEA division on nuclear and related techniques in food and agriculture in support of their programs. The kit has been developed for the detection of serum antibodies to *MmmSC*.

The CBPP antigen supplied is prepared from a wild strain of *MmmSC*. The *MmmSC* culture has been lysed through an osmotic shock and a detergent. The mouse monoclonal antibody supplied is directed against the membrane antigen of *MmmSC*. The anti-species conjugate supplied is polyclonal rabbit anti-mouse immunoglobulin conjugated to horse radish peroxidase (HRPO).

The test is conducted according to a standard competitive enzyme-linked immunosorbent assay (C-ELISA) technique to determine the presence of anti-CBPP antibody in a serum to be tested. The test principle is based upon the competition between the anti-CBPP monoclonal antibody (MAb) and antibodies in the serum sample for binding to the CBPP antigen.

The presence of antibodies to *MmmSC* in the serum sample will block the reactivity of MAb, resulting in a reduction in the expected colour reaction, following the addition of enzyme labeled anti-mouse conjugate and substrate/chromogen solution. Wash steps are required between each step to ensure the removal of unbound reagents (for the assay procedures see Annex 1).

5.2.1.2 Interpretation of test results

Test sera with mean percent inhibition (PI) values of 50% or greater were considered positive. Test sera with PI values less than 50% were considered negative.

5.2.1.3 Samples used for test comparison

In order to assess the diagnostic efficacy of CFT and C-ELISA, samples collected for a sero-prevalence study, others collected from the Metekel and Andassa cattle breeding ranches and samples collected for vaccination trial studies were analysed.

Extensive management system

Vaccinated cattle from two districts, namely Jabi-Tenan and Denbecha were sampled at about four months after vaccination. The vaccine was applied by the respective district animal health professionals as a prophylactic measure in November, 1996: CBPP outbreaks had been experienced in the corner of these districts.

The vaccine strain used was the T_1-SR freeze dried attenuated monovalent vaccine $10^{7.7}$ cfu per dose. batch number PL17-94. the route of vaccination was subcutaneous on either side of the neck. A total of about 200 serum samples were collected and examined. All the animals were local indigenous zebu of both sexes and all ages.

Metekel ranch

A total of 60 animals (30 pure Fogera and 30 Frisian-Fogera cross breeds) were sampled starting from 55 days after vaccination and sampling continued at days 84. 105 and 140 PV. The vaccine was applied by animal health professionals working in the ranch. it was known that all animals have received one ml of the T_1-SR attenuated freeze dried monovalent vaccine having had a titre of $10^{8.04}$ cfu per dose. batch number PL19-94.

Andassa ranch

A total of 60 animals (30 pure Fogera and 30 Frisian-Fogera cross-breeds) were used. 12 animals (six from each breed were used as unvaccinated controls). Prevaccination serum samples were collected a day before vaccination and post vaccination samples about 50 days PV. The vaccine was applied by animal health professionals working in the ranch and the vaccine details were the same as in Metekel ranch.

5.3 Data analysis

Test comparisons (CFT and C-ELISA): Calculation of confidence intervals. level of measure of concordance and kappa values were performed on samples of different localities. ranches and one research institute. Comparison on sensitivity (Se) and specificity (Sp) to detect vaccinal response was made. For calculation. WinEpiscope 1.0 included in the program package was used.

5.4 Results

5.4.1 Comparison based on confidence interval

Analysis of the the actual serologically detected proportion of the tested samples indicate that CFT detects relatively more positive animals than C-ELISA. But. the confidence intervals of the means of the CFT and C-ELISA tests overlapped in 85% of the villages and 73% of the districts (Table 18 and 18. Figure 20 and Annex 6).

Table 18. Proportion of overlapped results. CFT vs C-ELISA

Source	No of means	Overlapping CFT 1:10	Overlapping CFT 1:20
Village	40	28 (70%)	34 (85%)
District	11	5 (46%)	8 (73%)

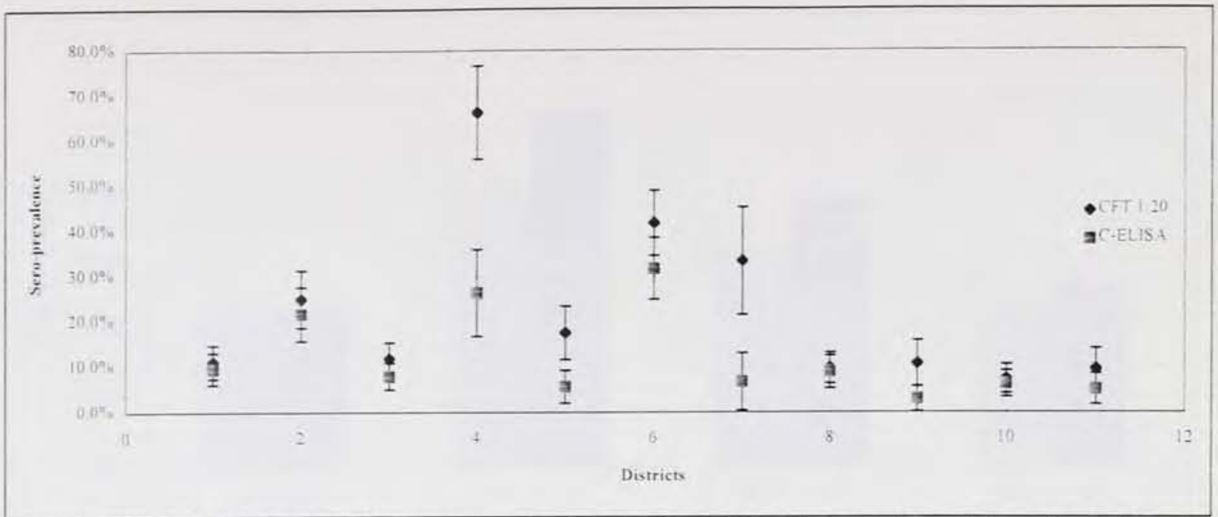

Figure 20. Mean sero-prevalence of CBPP in the 11 districts. Overlapping of CI of the means. CFT and C-ELISA test results.

When the cut off value for CFT inccreased to 1:20 and 1:40, the confidence interval of the means related to districts, sex, age, year of last CBPP vaccination and CBPP status, the closer was the mean sero-prevalence results to the C-ELISA and much of the confidence intervals have shown overlapping. Comparison of C-ELISA to the 1:10, 1:20 and 1:40 titres of CFT in relation to districts, sex, age, status of CBPP and year of last CBPP vaccination is indicated on (Figure 21, 22, 23, 24 and 25).

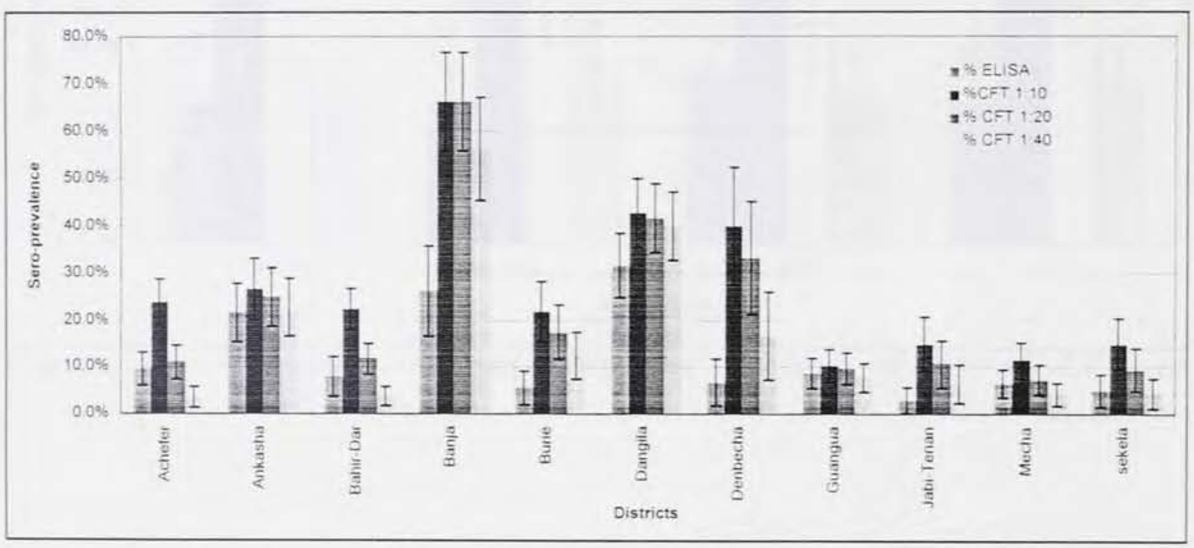

Figure 21. C-ELISA compared to the various titres of CFT in relation to districts

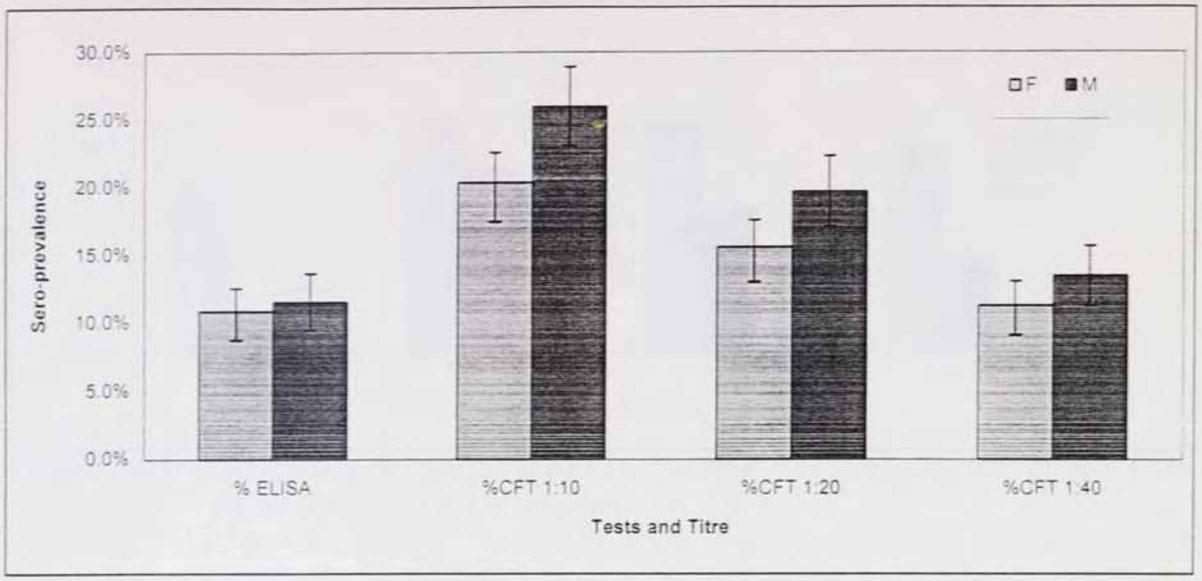

Figure 22. C-ELISA compared to the various titres of CFT in relation to sex

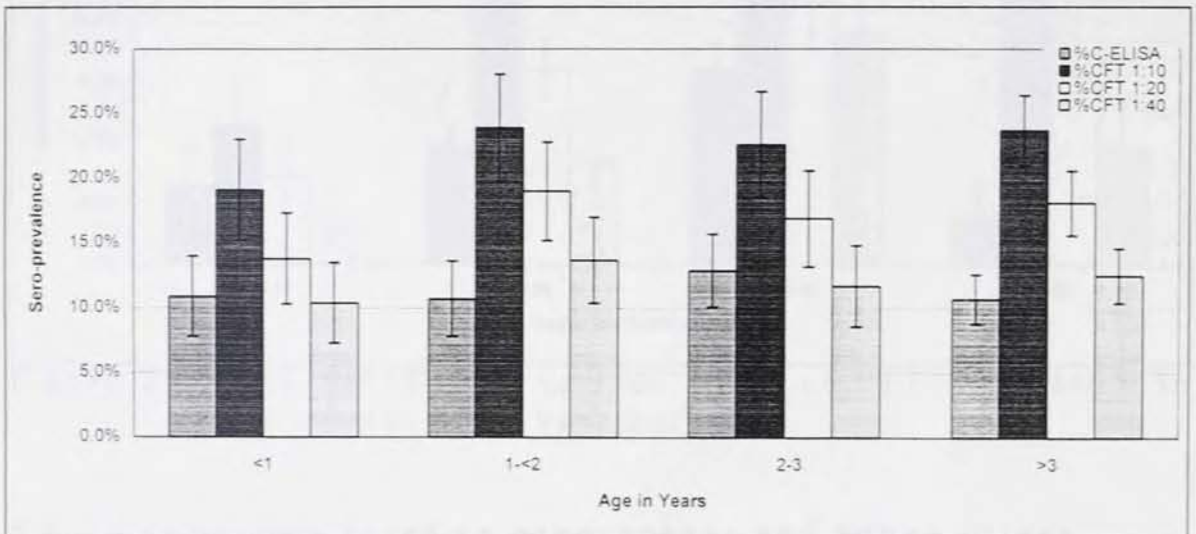

Figure 23. C-ELISA compared to the various titres of CFT in relation to age

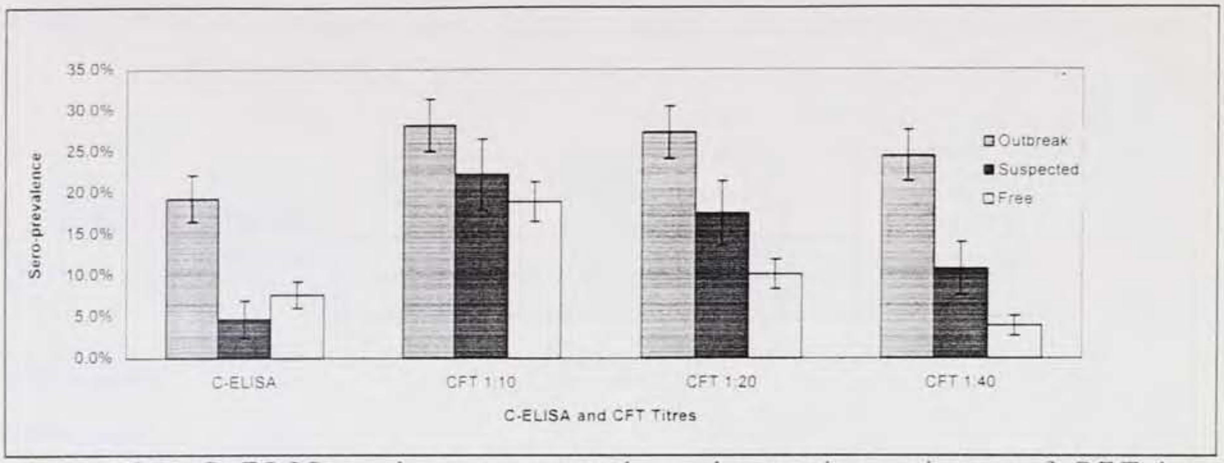

Figure 24. C-ELISA when compared to the various titres of CFT in relation to CBPP status

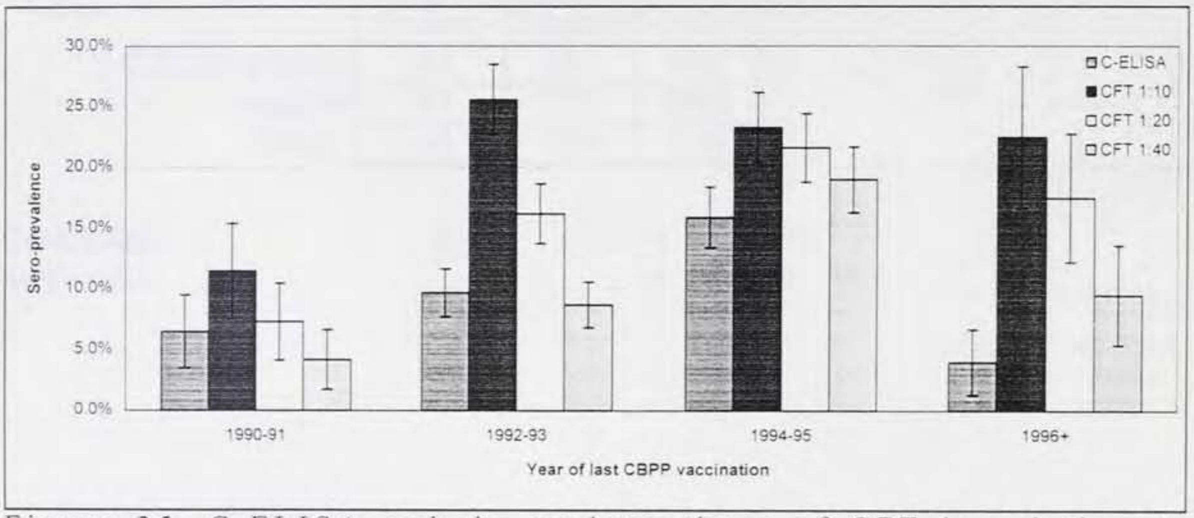

Figure 25. C-ELISA and the various titres of CFT in relation to year of last CBPP vaccination

5.4.2 Comparison based on concordance and kappa values

A total of 3150 samples were tested by CFT and C-ELISA. It was observed that the concordance ranges from 42 to 100% and kappa value from 0 to 0.78. The mean concordance and kappa values for different origin samples were found to be 85.3% and 0.30 respectively. There were discrepancies between the two tests: A total of 163 samples that were identified as negative by CFT were positive by C-ELISA Vs 301 samples identified as negative by C-ELISA were positve by CFT (Table 20 and 21). Concordance and kappa values calculated for different origin samples shown in (Table 22).

Table 19. Concordance and kappa values on samples used for sero-prevalence study

		CFT		
		positive	negative	Total
C-ELISA	positive	113	126	239
	negative	257	1644	1901
	Total	370	1770	2140

Concordance = 82.1%

kappa value = 0.27

Table 20. Concordance and kappa values on overall samples

	CFT 1:20		
	Positive	Negative	Total
C-ELISA positve	145	163	308
negative	301	2541	2842
Total	446	2704	3150

Concordance = 85.3%

kappa value = 0.30

Table 21. Concordance and kappa values on samples from various origin

District	Samples	C-ELISA	CFT 1:20	Concordance	kappa value	Agreement
Achefer	280	27	31	81.4%	0.00	no
Ankesha	180	39	45	75.6%	0.32	poor
Bahir-Dar	340	27	40	87.4%	0.29	poor
Banja	80	21	53	42.5%	0.00	no
Burie	160	9	28	85.6%	0.32	poor
Dangila	180	57	75	61.1%	0.17	poor
Denbecha	60	4	20	70.0%	0.16	poor
Guangua	300	26	29	96.3%	0.78	good
Jabi-Tenan	140	4	15	74.3%	0.15	poor
Mecha	260	17	19	90.0%	0.22	poor
Sekela	160	8	15	89.4%	0.21	poor
Sub-Total	2140	239	370	82.1%	0.27	poor
Andassa 50	60	9	7	93.3%	0.71	good
Metekel 84	60	6	1	88.3%	0.03	poor
Metekel 105	60	7	1	90.0%	0.23	poor
Metekel 140	60	0	2	96.7%	0.00	no
Sub-Total	240	22	11	92.1%	0.24	poor
Debre-Zeit 0	77	0	0			
Debre-Zeit 7	77	0	5	93.5%	0.00	no
Debre-Zeit 14	77	15	9	84.4%	0.42	moderate
Debre-Zeit 21	77	32	20	81.8%	0.60	acceptable
Debre-Zeit 28	77	0	8	89.6%	0.00	no
Debre-Zeit 35	77	0	20	74.0%	0.00	no
Debre-Zeit 42	77	0	1	98.7%	0.00	no
Debre-Zeit 49	77	0	2	97.4%	0.00	no
Debre-Zeit 56	77	0	0			
Debre-Zeit 63	77	0	0			
Sub-Total	770	47	65	92.0%	0.40	moderate
Grand Total	3150	308	446	85.3%	0.30	poor

5.4.3 Sensitivity and specificity to detect vaccination response

The sensitivity and specificity of C-ELISA to detect vaccination response was investigated on samples from vaccinated and unvaccinated animals. CFT was used as gold standard. The sensitivity (Se) and specificity (Sp) is indicated under (Table 22). Of the vaccinated 50 cattle, 40 were found sero-converted by CFT of which 32 were found sero-positive by C-ELISA, while all the unvaccinated control were sero-negative by both CFT and C-ELISA.

Table 22. Sensitivity and Specificity of C-ELISA using CFT as gold standard

		Vaccination status		
		vaccinated	control	Total
CFT	positive	40	0	40
	negative	10	27	37
	Total	50	27	77

		CFT		
		Positive	Negative	Total
C-ELISA	positive	32	0	32
	negative	10	27	45
	Total	40	27	67

C-ELISA:
Sensitivity = a/(a+c) = 32/(32 +10) = 80%.
Specificity = d/(d+b) = 27/(27+0) = 100%

6. DISCUSSION

According to this study including questionnaires and serological results, bovine respiratory disease which mainly refers to CBPP is a prime concern in Awi and Western Gojjam zones. The disease with various local names like Sal, Samba, Wozwuz, Zihon-Wotetie and Aslig is associated with rapid spread to districts hypothetically considered free.

The local names given for bovine respiratory disease from the different localities of the study area are Amharic descriptions: Sal, refers to coughing; Samba means lung; Wozwuz refers to a disease with abdominal breathing; Zihon-Wotetie means a disease of elephant referring to the origin and frequent occurrence in the lowland area. Aslig refers to a disease which induces chronic emaciation. These indigenous descriptions of bovine respiratory disease syndrome have a certain degree of resemblance to that of the clinical and pathological description of CBPP by Bygrave *et al.* (1967), Egwu *et al.* (1996) and Masiga *et al.* (1996). This indicates that livestock owners in the study area have been challenged most probably with the hazard of CBPP.

Further information from stock owners about respiratory disease syndrome in relation to seasons resulted in divergent answers: About 18% reported the majority of cases to turn up at the end of the dry and beginning of the rainy seasons and 23% reported the opposite. A seasonal occurrence in reference to the study area might be due to the following considerations:

- During the end of the dry and beginning of the rainy season, a number of stress factors turn up including ploughing for draught oxen, shortage of forage, climatic changes from warm dry to cold wet conditions. In this respect, Dennis (1986) has reported that the change in temperature and relative humidity brings about a change in survival of pathogens and lowering of resistance of the host in relation to respiratory diseases. A change in the rate of multiplication and virulence of the disease causing organisms and reduction of mucosal resistance of the host was demonstrated.

- During the end of the dry and beginning of the rainy season and vice versa bovine herds used to shift from one grazing area to another as a result of which the contact rate to other herds are higher. This herd contact during grazing and watering has been considered as an ideal situation for the transmission and occurrence of outbreaks for diseases such as CBPP (Seifert, 1996; Masiga *et al.*, 1996)

According to the serological results from CFT, the mean sero-prevalence for the 11 selected districts and cattle breeding ranches (17.3%), are suggestive of the occurrence for CBPP as most areas have been reported as unvaccinated. This sero-prevalence finding disagrees to that of the report of Zessin *et al.* (1985), where 8% CBPP prevalence was reported in neighbouring Sudan.

The difference in sero-prevalence recorded for Awi zone and Western Gojjam, 27.3% Vs 12.0% was found to be statistically significant ($P<0.001$). This higher sero-prevalence in Awi zone might be due to the following reasons:

- Awi zone is geographically located adjacent to the Benshangule-Gumz region, where CBPP is expected to be endemic. The Benshangule-Gumz region is in turn adjacent to the Sudan a country where CBPP has been reported to exist for many years (Abdalla, 1969; Abdulla, 1969; Masiga *et al.*, 1996). Therefore, the risk and pressure of CBPP infection to Awi herds was expected to be higher.
- There is a wide range of grazing land as a result of gorges and mountainous landscape and the dominant lowland agro-climate in Awi zone as opposed to the plain and extensively cultivated land in Western Gojjam, and hence livestock movement from and to the Awi zone is relatively frequent. Larger herd sizes support spreading of CBPP like in Awi zone in opposition to arable land, where primarily oxen are used for ploughing in Western Gojjam.

The statistically significant sero-prevalence difference between districts hold the same explanation given to the two zones. Except Guangua, all districts in Awi zone are in the higher rank of CBPP sero-prevalence, the lower sero-prevalence in Guangua might be due to the presence of concurrent infection with trypanosomiasis. The district Guangua is known as endemic for CBPP as well as trypanosomoiasis. The immunosuppression effect of trypansomiasis has been reported by Provost *et al.* (1987).

On the other hand, from Western Gojjam Denbecha district, unlike the others was among the high sero-prevalence ranking group. This might be due to its geographical location adjacent to one of CBPP endemic district of Awi zone, i. e. Ankesha district.

Considering the overall sero-prevalence of 17.3% in the absence of vaccination seems to be high. The finding of 10.1% sero-prevalence in districts hypothetically considered free was interesting and it is highly suggestive for a poor disease reporting system and the mishandling of CBPP. The higher sero-prevalence might be attributed to a number of epidemiological factors such as:

- Absence of control measures such as vaccination. CBPP vaccine had been given with Rinderpest. However, the PARC program has been changed and vaccination against CBPP has been ceased since 1992/93 (personal communication to PARC Ethiopia).
- Veterinary drugs including antibiotics are traded in the open market as commodities and as a result of lack of basic vaccination service against CBPP. Due to the pressure of the

CBPP problem, owners were forced to use whatever antibiotics available. This practice might have increased the proportion of CBPP carrier animals (Bölske *et al.*, 1995; Nicholas and Bashiruddin, 1995; Blancou, 1996; Egwu *et al.*, 1996).

- The traditional livestock husbandry system in communal grazing land and watering points has been considered a conducive factor for the transmission of diseases like CBPP. Masiga and Domenech (1995) and Masiga *et al.* (1996) have underlined this husbandry system in tropical and sub-tropical African countries as one of a major constraint for the control and eradication of CBPP. The sero-prevalence results based on the different agro-ecology in this study with 8.1% in the highland, 13.7% in the medium altitude and 27.3% in the lowland, clearly supports this explanation, as herd size and the degree of communal grazing and watering contact increases starting from the highland towards the lowland agro-ecology.

Although not significant, a higher sero-prevalence in animals of one year and above was recorded. Similar findings were reported by Zessin *et al.* (1985) Provost *et al.* (1987). Nicholas and Bashiruddin (1995) have reported a higher sero-prevalence related to age increase and the explanation given was maturation of the immune system in older animals. On the other hand, Masiga and Windsor (1972) have reported that animals younger than three years of age were more susceptible than those over three years and the explanation given for this was associated with completion of body tissue development in older animals.

According to this study, a higher sero-prevalence was recorded in males than females. This variation might be due to the sexual behavior of males as they prefer smelling at the vulva of females which predispose them for "urinary tract to nose" mode of infection. Masiga *et al.* (1972) have reported urinary tract to nose transmission as a new mode of infection in CBPP and indicated that the sexual behavior of bovine animals as a risk factor for this mode of infection during micturation with splashing of the urine. Males by virtue of their sexual behavior are frequently wondering and smelling whenever new animals are introduced into the herd and this might predispose them to a higher rate of infection than females.

Regarding the vaccination trial of this study, 80% sero-conversion was detected by CFT and 64% by C-ELISA. The duration of detectable antibodies was found to be up to 49 days PV with CFT and up to three weeks post vaccination by C-ELISA. Opinions on the duration of PV antibodies detected with CFT varies. Chima and Pam (1985) have reported about 10 weeks. Masiga *et al.* (1972) have reported that nearly all cross-bred cattle and about 75% of zebu have shown antibodies with CFT starting from the first week post vaccination and detectable up to 30 days. Onoviran and Taylor-Robinson (1979) on the other hand have reported detection of post-vaccinal antibodies for at least 23 months using ELISA. It was found that CFT detects reactors at an earlier date PV and persists for longer periods in opposition to C-ELISA by which antibodies have been detected for only three weeks. This discrepancy is difficult to explain, but is probably based on the high specificity of monoclonal antibodies in C-ELISA.

The detection of vaccinal antibodies up to 49 days PV using CFT, with a peak number of sero-converted animals at day 28 is generally in agreement to that of the report of Staak (1974) in Tanzania. It was reported that sero-converted animals were detected up to 69 days PV and the peak proportion of sero-converted animals detected were between days 17 and 23 days post vaccination.

In this vaccination trial study, an interesting observation between CFT and C-ELISA was that the C-ELISA detects relatively a higher proportion (64%) as sero-positive in a single batch of samples at day 21 while CFT detects about 40% in a single batch of samples, that was at days 21 and 35. This might be an indication of the sensitivity potential of C-ELISA. But, considering the overall proportion of sero-positive animals during the experimental period (63 days), CFT detected 80% and C-ELISA 64% of the vaccinated animals. This variation is due to CFT which detects vaccinal antibodies for an extended duration, while C-ELISA detects this proportion in a single batch of sample at day 21. This might be considered as a diagnostic advantage of the C-ELISA as it detects vaccinal antibodies for shorter periods.

The fall of vaccinal antibodies after vaccination has to be put into account when testing cattle for investigation of antibodies due to natural infection. This supports that the sero-positive animals found in this study are most likely due to natural infection. The highest specificity obtained in this vaccination trial study for CFT and C-ELISA (neither of these tests detected the control animals as positive) indicate that the risk of false positive reactors is minimal.

Further comparison of CFT and C-ELISA indicates that there were two peaks of serologically positive animals in the fourth and sixth week with CFT in opposition to one peak with C-ELISA in the third week. This variation might be due to CFT which detects both IgM and IgG (Pearson and Lloyd, 1972), while C-ELISA detects mainly IgG.

According to the comparison between CFT 1:10 and C-ELISA on serum samples from the prevalence study it was noted that in all villages and districts CFT has detected many more positive animals than C-ELISA. However, when the minimum positive value for CFT was raised to 1:20 (OIE, 1996) the results for CFT and C-ELISA became closer and the confidence interval of 85% of the villages and about 73% of the districts showed overlapping, indicating that the variation was due to chance. Similar test comparison studies have been reported in two groups of Italian cattle and indicated that Western blot detects about 60%, CFT about 50% of infected animals in opposition to 45% discovered by ELISA. In contrast to these reports, Onoviran and Taylor-Robinson (1979) have described ELISA as the most sensitive test. They have reported that it was possible to detect reactions over 23 months post vaccination.
Comparing the results from C-ELISA with those from CFT 1:20 and CFT 1:40 in relation to districts, year of last CBPP vaccination, CBPP status, age and sex indicate that results from both systems gained a higher degree of identity and much of the confidence intervals overlapped. The C-ELISA is tested presently in several countries and consequently not yet established for routine diagnostic work. Like for other C-ELISAs, the main emphasis is laid upon the threshold PI during this phase, which was presently given as PI 50% for the CBPP C-ELISA. This can give an explanation for more positive reactors detected by CFT but not for positive C-ELISA reactors which reacted negatively to CFT.

It has to be realized that the CBPP situation in the areas tested is of endemic nature. In such a situation, CFT detects only 74% of infected animals (Bashiruddin *et al.*, 1994). This could mean that the C-ELISA is capable to detect infected animals which are not detected by CFT, thereby qualifying C-ELISA as a valuable test for serological diagnosis of CBPP. It is obvious that additional work is required in this field.

Comparison of the observed proportional agreement and the agreement beyond chance (kappa value) indicate that there was a wide range of results to the various samples. The observed proportional agreement was found to be in the range of 42 to 100% with a mean agreement of

85.3%, and the kappa value was found in the range of 0 to 0.78 with mean value 0.30. This indicates the need for further study on the test comparison.

Considering that this study was conducted under time pressure, it was hardly possible to assess clinical and sub-clinical cases for the isolation and identification of the causative mycoplasma organism. Furthermore, it was very difficult to know the CBPP status of the animals used for the comparison of CFT and C-ELISA.

Although outbreaks of CBPP had been reported in many parts of the country, its epidemiology has not yet been thoroughly investigated. The findings of this preliminary study, although not exhaustive, are indicative that CBPP is of greater importance in cattle production than anticipated before, especially in North-West Ethiopia.

Factors reported such as cattle movement, traditional husbandry are favouring the spread of the disease and the extensive use of antibiotics and traditional medicines makes the problem more complex and serious. Measures to control the spread of the disease used elsewhere, such as test and slaughter with compensatory payment, quarantine and cattle movement control can not be applied in African countries, in particular in Ethiopia where there are various constraints associated with socio-cultural factors, poor economy, geography, lack of diagnostic facilities etc. (Masiga *et al.*, 1996).

6.2 Conclusion and recommendations

Sero-prevalence study:

- CBPP is found to be a major constraint in North-West Ethiopia.
- The distribution of the disease was found to be larger than anticipated before.
- Awi Zone is considered as the home base for CBPP.
- Herd size and management which are associated to geographical locations and climate are identified as risk factors.

Vaccination trial study:

- 80% Mean sero-conversion after the first vaccination indicate that T_1-44 is immunogenic.
- The duration and diagnostic importance of Abs due to T_1-44 is 1-8 weeks.

Test comparison:

- 80% Sensitivity and 100% Specificity of C-ELISA was found to be encouraging.
- The agreement between CFT and C-ELISA evaluated as poor to moderate.

Therefore, the following recommendations are suggested:

- Since CBPP is rapidly spreading, nation wide epidemiological study is necessary in order to ascertain the prevalence and distribution of the disease.
- Selective action plans and programs should be designed to increase herd immunity.
- Improvement of the infrastructure and facilities of the veterinary services.
- Establishment of centralized disease reporting system, so that CBPP will be regarded as a national problem.

- Education and information dissemination to the farmers to increase the level of awareness.
- Although treatment with antibiotics used by farmers might be an immediate measure to alleviate the problem, the long-term effect of antibiotics in relation to CBPP should clearly be discussed among professionals, livestock owners and responsible authorities and hence alternative measures should then be encouraged and implemented.
- Further study on the test comparison on these and other alternative tests is recommended.
- Vaccination trial studies under the extensive management system needs to be considered.
- CBPP is not hold back by borders, it spreads by crossing any locality and geographical areas as long as carrier animals are moving and as long as there is a susceptible population. Therefore, border harmonization of vaccination campaigns between regions and countries should be set up. An inter African tackling of CBPP will then be promoted.

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Annex1 Assay procedure for C-ELISA

1. Reagents, recipes for buffers and preservatives for C-ELISA

Antigen Preparation for C-ELISA

About 20 ml of broth culture of *MmmSC* centrifuged at 20.000 xg for 30 min. at + 4°C and the supernatant was discarded. The sediment was washed three times with PBS. The protein content was determined and adjusted to 2 mg/ml of suspension. Added were 1% sodium dodecylsulphate equal volume with the antisine (calculated for the determined protein concentration). Keep at 60°C for 30 min. in a water bath, centrifuge at 20.000 g for 20min. at +4°C and collect the supernatant. Store the final suspension at -20°C. Titrate to working dilution in blocking buffer solution (1:100, 1:1000 or 1:10.000) before use.

CBPP antigen stock

Reconstitute the freeze dried contents of a vial with 0.2 ml of sterile water supplied with the kit and mix gently until completely dissolved.

Anti-CBPP monoclonal antibody stock

Reconstitute the freeze dried contents of a vial with 1ml of the sterile water supplied with the kit and mix gently until completely dissolved.

Anti-species conjugate stock

The rabbit anti-mouse immunoglobulin (HRPO) conjugate stock should be further sub-divided into 500 µl aliquots in 1ml cryopreservation vials, label and stored at +4°C.

Control serum stock

Reconstitute the freeze dried contents of a vial of each control serum with 1ml of sterile water supplied with the kit, labled and stored at -20°C.

Blocking agent

Reconstitute the freeze dried contents of a vial blocking antigen (normal horse serum) with 2ml of sterile water, label and store at -20°C.

Substrate buffer

0.05M phosphate citrate buffer, pH 5 ± 0.20: Dissolve the powder in 250 ml of locally produced distilled/deionized water, label and store at + 4°C.

Chromogen stock solution

Add 12 ml locally produced distilled/deionized water to the bottle with the chromogen powder. Store no longer than one month

Substrate stock

3% (w/v) H_2O_2 (882mM). place one hydrogen peroxide tablet in the brown bottle supplied and dissolve with 10 ml locally produced distilled/deionized water. label and store at + 4°C.

PBS stock solution

0.1M phosphate buffered saline. pH 7.4 ± 0.20. Dissolve the contents of one packet per one litre of locally produced distilled/deionized water. label and store at + 4°C no longer than one month.

Coating buffer

0.01 M phosphate buffered saline. pH 7.4 ± -0.20. Dilute the PBS stock solution 1:10 in locally produced distilled/deionized water. label and store at + 4°C for no longer than two weeks. If not being used immediately. store in aliquots of 500 ml at -20°C.

Blocking buffer

0.01 M phosphate buffered saline. pH 7.4 ± - 0.20 plus 0.05% (v/v) Tween 20 plus 0.5% (v/v) normal horse serum .On the day of testing add 0.5% (v/v) normal horse serum (NHS) to the 0.01 M PBS plus 0.05% (v/v) Tween 20.

Wash buffer

0.002 M phosphate buffered saline. pH 7.4 ± -0.20 plus 0.05% (v/v) Tween 20. Prepare one litre of 0.01 M phosphate buffered saline. pH 7.4 ± -0.20. add 2.50 ml Tween 20 and mix well. Transfer to a wash fluid container with a tap to which tubing may be attached and further dilute with addition of four litres of distilled/deionized water. label and store at room temperature no longer than two weeks.

Stopping solution

4% of sodium dodecyl sulphate. dissolve the contents of one packet in 250 ml of locally produced distilled/deionized water. label and store at room temperature.

The test proper:

Coating of microplates

Dispense 50 µl volumes of the working dilution of CBPP antigen (diluted in PBS 1:2.000) into all 96 wells of the flat bottom microplates. Tap the sides of the microplates to ensure that the antigen is evenly distributed over the bottom of each well. Cover the microplates and either incubate overnight at + 4°C or incubate for two hours at 37°C. Discharge the contents of all the antigen coated microplates into a sink or another reservoir. Wash all wells with wash buffer and do three complete wash cycles.

Addition of test sera, control sera and monoclonal antibody

Dispense 45 µl volumes of blocking buffer to all 96 wells of the microplates and add 5 µl volumes of test and control sera to the appropriate wells. This gives an initial serum dilution of 1:10. Add 5 µl of blocking buffer to the monoclonal control wells and 55 µl of blocking buffer to the control conjugate wells. Immediately prepare a working dilution of the monoclonal antibody in blocking buffer for all the plates (6 ml of working dilution per plate). Then add 50 µl volumes of the working dilution of the monoclonal antibody to all wells of the microplates except the conjugate control (Cc) wells following the plate layout. This will result in the final serum dilution of 1:20. The microplates are covered or sealed and placed on an orbital shaker placed in a + 37°C incubator and are incubated for one hour with continuous shaking. Return the test sera and the remainder of the control and monoclonal antibody stocks to + 4°C.

Addition of conjugate

After one hour of serum incubation, remove the microplates from the incubator and wash three to four times with wash buffer. Immediately after washing, 50 µl volumes of the working dilution of conjugate are added to all wells of the microplates. The sides of the microplates are tapped to ensure that the conjugate working dilution is evenly distributed over the bottom of each well. Cover or seal the microplate and incubate for one hour at + 37°C with continuous shaking.

Addition of substrate/chromogen and stopping solutions

Immediately before the end of the conjugate incubations, prepare a working dilution of the substrate/chromogen solution in a volume sufficient for the number of microplates. Use a clean microplate (not coated with antigen) as the 'blanking plate' for the photometric reading. After one hour of conjugate incubation, wash the microplates three to four times with wash buffer and immediately after washing, add 50 µl volumes of the substrate/chromogen solution to all wells of the microplates, starting with the first column of the 'blanking plate' followed by all 96 wells of the microplates in the test run. Incubate for 25 minutes at + 37°C with continuous shaking.

When the optical density of the monoclonal antibody control (cm) wells reaches 0.7 (approximately after 20-30 minutes of substrate/chromogen incubation), add immediately 50µl volumes of the stopping solution (SDS) to the wells of the microplates, starting with the first column of the 'blanking plate followed by all 96 wells of the microplate in the test run. Briefly shake the microplates using the orbital shaker to ensure thorough mixing. All wells should now contain 50 µl of substrate/chromogen solution plus 50 µl of stopping solution.

Measurement of substrate development

Place the 'blanking plate' in the holder of the microplate reader for blanking. Place the test plate in the holder of the blanked reader for reading test results. Repeat for every microplate.

Calculation and acceptance of control data

The reading are expressed in **OD** values and converted into **PI** values for the monoclonal antibody control (cm) and the reading for the four other controls (Cc, C++, C+ and C-)

expressed was in PI values are used to determine whether or not the test competition background has been performed within acceptable limits of variability and therefore, whether or not the test sera data may be accepted for any given microplate.

Data expression

Microplate readings are used in two types of data analysis: Percent inhibition (PI) values which are used for quality assurance (QA) acceptance and percent inhibition (PI) values which are used for acceptance of replicate values for test sera and diagnostic interpretation. These (PI) values are calculated as follows.

$$PI = 100 - \frac{\text{(Replicate OD of each control X 100)}}{\text{mean OD of Cm - OD conjugate (Cc)}}$$

Acceptance of individual test sera data

The diagnostic threshold for this assay has been set at 50% inhibition (50 PI) of the monoclonal antibody control (cm). To accept or reject individual test sera, both replicate PI values of a test serum must fall either above or below 50 PI. Test sera were re-tested if their replicated PI values lay on either side of 50 PI.

2. ELISA DATA RECORDING SHEET

PLATE LAYOUT FOR CBPP C-ELISA

controls serum samples in duplicate

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cc	Cc	1	5	9	13	17	21	25	29	33	37
B	C ++	C ++	1	5	9	13	17	21	25	29	33	37
C	C ++	C ++	2									
D	C +	C +	2									
E	C +	C +	3									
F	Cm	Cm	3									
G	Cm	Cm	4									40
H	C -	C -	4									40

Notes:Cc: Conjugate control
C++: strong positive serum control
C+: Moderate positive serum control
Cm: Monclonal antibody control
C-: Negative serum control

Control data

					OD		
	OD 1	OD 2	OD 3	OD 4	UCL	LC L	Median OD
Cm					1.60	0.70	

									PI	
	OD 1	OD 2	OD 3	OD 4	PI1	PI2	PI3	PI4	UCL	LCL
Cm										
C++									+90	+80
C+									+59	+49
C-									+30	+10
Cc									+105	+95

Sample data

ID					Mean	ID					Mean
	OD 1	OD 2	PI 1	PI 2	PI		OD 1	OD 2	PI 1	PI 2	PI
1						21					
2						22					
3						23					
4						24					
5						25					
6						26					
7						27					
8						28					
9						29					
10						30					
11						31					
12						32					
13						33					
14						34					
15						35					
16						36					
17						37					
18						38					
19						39					
20						40					

Table 1A. Cm control data

Replicate PI values(4)		Status
In	Out	
4	0	Accept
3	1	Accept
2	2	Reject
1	3	Reject
0	4	Reject
Replicate PI values(3)		Status
In	Out	
3	0	Accept
2	1	Reject
1	2	Reject
0	3	Reject

									PI	
	OD 1	OD 2	OD 3	OD 4	PI1	PI2	PI3	PI4	UCL	LCL
Cm										
C++									+90	+80
C+									+59	+49
C-									+30	+10
Cc									+105	+95

Sample data

ID					Mean	ID					Mean
	OD 1	OD 2	PI 1	PI 2	PI		OD 1	OD 2	PI 1	PI 2	PI
1						21					
2						22					
3						23					
4						24					
5						25					
6						26					
7						27					
8						28					
9						29					
10						30					
11						31					
12						32					
13						33					
14						34					
15						35					
16						36					
17						37					
18						38					
19						39					
20						40					

Table 1A. Cm control data

Replicate PI values(4) In	Out	Status
4	0	Accept
3	1	Accept
2	2	Reject
1	3	Reject
0	4	Reject
Replicate PI values(3) In	**Out**	**Status**
3	0	Accept
2	1	Reject
1	2	Reject
0	3	Reject

Tantibodiesle 1B. Cc and C- control data

Replicate PI values		Status
In	Out	
2	0	Accept
1	1	Reject
0	2	Reject

Tantibodiesle 1C. C++ and C+ control data

Replicate PI values		Status
In	Out	
4	0	Accept
3	1	Accept
2	2	Reject
1	3	Reject
0	4	Reject

In within UCL and LCL range
Out out of the range

Annex 2 Reagents, diluents and preservatives for CFT

Complement

A Complement is a mixed serum from healthy male guinea pigs, 0.8 ml serum, 0.1 ml Richardson sol. B and 0.1 ml Richardson sol. A. Stored at + 4°C for six months.

Evaluation of Complement

Set up three rows of nine tubes each. Prepare 1:40 C dilution. 0.5 ml of preserved complement " + " 3.5 ml deionized water " + " 12 ml diluent (working solution of VBD)
Table 4 Complement evaluation continue as shown below

Tube	C 1:40	VBD diluent	VBD diluent	C titre
1	0.1 ml	0.4 ml	1.5 ml	1:200
2	0.15 ml	0.35 ml	1.5 ml	1:133
3	0.20 ml	0.3 ml	1.5 ml	1:100
4	0.25 ml	0.25 ml	1.5 ml	1:80
5	0.3 ml	0.20 ml	1.5 ml	1: 67
6	0.35 ml	0.15 ml	1.5 ml	1: 57
7	0.40 ml	0.1 ml	1.5 ml	1: 50
8	0.45 ml	0.05 ml	1.5 ml	1.44
9	0.5 ml	-	1.5 ml	1:40.

Incubate at 37°C in a water bath for one hour and in the mean time prepare 2% SRBC and equal amount of Amboceptor at working dilution, both to be kept separately. After one hour of incubation 0.5ml amboceptor and 0.5ml of 2% sheep red blood cells (SRBC) added to each tube. The tubes are properly mixed and incubate in a water bath at 37°C for 30 minutes.The test is read by recording the minimum haemolytic dose (MHD) of complement which is represented by the first tube showing complete hemolysis. The next tube contains the full haemolytic dose (FHD).

Amboceptor (Anti-sheep hemolysin)

Amboceptor is a serum containing lytic antibodies against SRBC, commonly prepared in rabbits. This reagent is available from CIRAD-EMVT, France.

Sheep Red Blood cells (SRBC)

About 75 ml of free flowing blood is drawn from the jugular vein directly into a bottle containing 125 ml of Alsever's solution. A dash of crystalline penicillin is added, stored at +4°C. The blood can be used for about two weeks. Blood for CFT should be at least one day old.

Ag and Control sera:

Both positive and negative control sera supplied with the kit by CIRAD-EMVT.

Diluents:

Veronal buffered diluent (VBD), five fold concentrated VBD

Nacl	83.00 gm
Na. 5.5 diethylbarbiturate	10.19 gm
Distilled (deionized) water	800 ml

Add slowly 34.6 ml of 1N HCl and afterwards 5 ml of '

Preparation of VBD stock Solution:

$Mgcl_2 . 6H_2O$	20. 3 gm
$Cacl_2 . 2H_2O$	4.4 gm

add slowly 100ml deionized water

200 ml of the concentrated VBD should be diluted with 800ml of deionized water (working solution).The PH of a 1:5 diluted stock VBD should be between 7.4 and 7.5. Stock VBD is stored in 100 and 200 ml aliquots at -20°C .

Supplemented Normal Saline:

Five fold concentrated solution

Nacl	90.00 gm.'
VBD stock solution	5.00 ml
Distilled H_2O ad	2000.00 ml

Dissolve NaCl. mix well, check and adjust to pH 7.4

Preservatives:

Alsevers Solution:

Dextrose (glucose)	18.66 gm
NaCl	4.18 gm
Na - citrate	8.00 gm
Distilled water ad -	1000.00 ml

Richardson solutions:

Richardson solution A	Richardson Solution B	
Boric acid 1.86 gm	Borax	1.14 gm
Borax 4.58 gm	Na azide	1.62 gm
Sorbitol 22.94 gm	Satur.	NaCl ad 200 ml
Satur. NaCl ad 200 ml		

Preparation of SRBC for haemolytic system:

About 10 ml of SRBC in Alsever's solution is centrifuged at 2500 RPM for 5 minutes. The supernatant is discarded and replaced by working solution of VBD. The red blood cells are resuspended in the diluent completely. This procedure is repeated 4 times; before discarding the supernatant after the last washing, the PCV is measured. The PCV can be read directly; Otherwise, an identical tube is placed next to the blood containing tube and filled up to the level of the blood by a measured amount of diluent. SRBC 2% suspension is prepared. This 2% SRBC becomes individually standardised by the C-evaluation.

Amboceptor titration

Pre-dilution of amboceptor in jumping dilutions: Prepare 1:500 amboceptor and dilute serially upto 1:8000,prepare 1:750 and dilute serially upto 1:1200

1:500 1:1000 1:2000 1:4000 1:8000

1:750 1:1500 1:3000 1:6000 1:1200

Transfer from these tubes 0.5 ml into a set of tubes, always start with the 1:1200 dilution (doing from lower concentration towards higher concentration allows to use only one pipette to this procedure. Add 1.0 ml of diluent to each of the test tubes. Add 0.5 ml of 2% SRBC to each of the test tubes, shake well. Leave on bench for 10 minutes. Add 1.0ml of complement at working dilution or if complement has not been evaluated use a 1:40 dilution. Read and record the last tube showing MHD.The working dilution of amboceptor is four times the MHD. If MHD was present at 1:4000, the working dilution is 1:1000

Annex-3: Duration of antibodies individual animals when tested with C-ELISA and CFT

Duration of post vaccination antibody in individual animals when tested with C-ELISA.

Days of sampling after vaccination

Animal ID	0 7 14 21 28 35 42 49 56 63
D1-2	0-----------------------0
D1-3	0-----------------------0
D1-5	0-----------------------0
D1-12	0-----------------------0
D1-13	0-----------------------0
D1-24	0-----------------------0
D1-27	0-----------------------0
D1-31	0-----------------------0
D1-32	0-----------------------0
D1-33	0-----------------------0
D1-34	0-----------------------0
D1-35	0-----------------------0
D1-38	0-----------------------0
D1-43	0------------0
D1-46	0-----------------------0
D1-1	0--------0
D1-6	0--------0
D1-7	0--------0
D1-8	0--------0
D1-9	0--------0
D1-10	0--------0
D1-11	0--------0
D1-14	0-------- 0
D1-16	0---------0
D1-17	0---------0
D1-18	0---------0
D1-20	0---------0
D1-28	0---------0
D1-30	0----------0
D1-31	0----------0
D1-40	0----------0
D1-48	0----------0

Prevalence 0.0% 0.0% 30.0% 62.0% 0.0% 0.0% 0 0 0 0 0

Incidence 0.0% 0.0% 30.0% 48.6% 0.0% 0.0% 0 0 0

2. Duration of antibody in individual animals when tested with CFT 1:10

Days sample tested

ID	0 7 14 21 28 35 42 49 56 63
D1-32	0-----------------------------------0
D1-35	0---0
D1-37	0----------------0 0----------0
D1-48	0-----------------------------------0
d1-49	0------0 0----------------0
D1-26	0-----------------------------0
D1-27	0-----------------------------0
D1-38	0-----------------------------0
D1-43	0---------0 0-------------------0
D1-46	0---0
D1-47	0---------------------------------0
D1-6	0---------------------------------0
D1-7	0----------------------0
D1-8	0----------------------0
D1-9	0----------------------0
D1-10	0--------------------------------0
D1-13	0-----------------------0
D1-16	0-----------------------0
D1-17	0---------------0
D1-18	0-----------------------0
D1-20	0-----------------------0
D1-23	0--------------------------------0
D1-24	0-----------------------0
D1-28	0 --------------------------------0
D1-30	0---------------------------------0
D1-31	0----------------------0
D1-33	0---------------------------------0
D1-36	0-----------------------0
D1-1	0----------0
D1-2	0----------0
D1-3	0--------------------0
D1-4	0-----------0
D1-5	0--------------------0
D1-12	0--------------------0
D1-14	0----------0
D1-15	0----------0
D1-21	0--------------------0
D1-22	0--------------------0
D1-25	0-----------0
D1-29	0-----------0
D1-34	0-----------0
D1-39	0----------------------0
D1-41	0-----------------------------------0
D1-44	0----------------------0
D1-45	0-----------0
D1-50	0 ----------0
D1-19	0----------0

Prevalence 0.0% 10.0% 20.0% 54.0% 90.0% 40.0% 2% 6% 2% 0%
Incidence 0.0% 10.0% 16.6% 42.1% 81.8% 25.0% 0 0 0 0

3. Duration of antibody in individual animals when tested with CFT 1:20

Days sample tested

ID	0 7 14 21 28 35 42 49 56 63
DI-32	0------------------0
DI-35	0------------------------- 0--------0
DI-37	0-------- 0-----0
DI-48	0-------0
dI-49	0------0
DI-26	0----------0
DI-27	0----------0
DI-38	0----------0
DI-43	0---------- -----------0
DI-46	0----------0 ------------0
DI-47	0---------- -----------0
DI-6	0------0
DI-8	0-------0
DI-9	0-------- ------0
DI-13	0--------
DI-16	0--------
DI-17	0--------
DI-18	0--------
DI-20	0--------
DI-23	0-------- --------0
DI-24	0--------
DI-28	0 ------- ---------0
DI-30	0------- ---------0
DI-31	0--------
DI-33	0-------- ---------0
DI-1	0--------
DI-3	0---------0
DI-4	0---------
DI-5	0----------0
DI-12	0-----------0
DI-14	0-----------0
DI-15	0-----------
DI-21	0-----------
DI-22	0----------- ------------0
DI-25	0-----------
DI-39	0-----------
DI-41	0-----------
DI-44	0-----------
DI-16	------------0
DI-19	-----------0

Prevalence 0.0% 10.0% 18.0% 40.0% 16.0% 40.0% 2.0% 4.0% 0.0% 0.0%
Incidence 0.0% 10.0% 13.3% 35.9% 24.0% 28.9% 0.0% 18.2% 0.0% 0.0%

4. Level of competition of antibodies in samples from vaccinated and unvaccinated cattle to the monoclonal antibodies.

ID	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day42	Day49	Day56	Day63
D1-1	35%	31%	39%	52%	17%	21%	33%	37%	48%	35%
D1-2	25%	36%	53%	51%	27%	24%	32%	32%	44%	36%
D1-3	46%	37%	57%	51%	46%	33%	38%	35%	45%	29%
D1-4	1%	16%	29%	8%	5%	1%	2%	10%	18%	13%
D1-5	45%	31%	57%	50%	30%	27%	34%	31%	47%	38%
D1-6	30%	32%	46%	63%	37%	21%	33%	35%	45%	32%
D1-7	34%	26%	37%	64%	20%	11%	32%	19%	37%	31%
D1-8	48%	32%	45%	62%	26%	30%	38%	31%	41%	32%
D1-9	47%	33%	47%	54%	28%	33%	40%	39%	45%	29%
D1-10	44%	38%	46%	61%	28%	36%	34%	32%	40%	28%
D1-11	31%	38%	43%	55%	28%	29%	31%	35%	44%	35%
D1-12	24%	30%	63%	51%	39%	28%	36%	32%	42%	34%
D1-13	39%	34%	50%	56%	24%	14%	26%	22%	41%	28%
D1-14	40%	31%	39%	52%	23%	46%	25%	22%	37%	34%
D1-15	30%	36%	47%	41%	26%	23%	31%	26%	37%	32%
D1-16	40%	41%	45%	56%	27%	22%	40%	30%	42%	38%
D1-17	39%	34%	38%	59%	23%	11%	23%	24%	42%	35%
D1-18	42%	37%	46%	62%	33%	34%	43%	42%	47%	37%
D1-19	39%	28%	48%	45%	35%	26%	35%	28%	41%	34%
D1-20	36%	33%	46%	57%	19%	33%	32%	28%	37%	29%
D1-21	43%	29%	43%	39%	16%	39%	11%	25%	34%	44%
D1-22	31%	34%	49%	32%	43%	48%	36%	35%	44%	30%
D1-23	35%	34%	56%	50%	26%	48%	30%	25%	43%	33%
D1-24	41%	33%	58%	55%	18%	26%	24%	21%	20%	20%
D1-25	44%	40%	47%	34%	35%	30%	34%	34%	45%	38%
D1-26	35%	38%	44%	48%	24%	24%	38%	29%	38%	42%
D1-27	41%	40%	58%	57%	44%	35%	44%	43%	49%	34%
D1-28	29%	28%	46%	54%	26%	36%	29%	27%	36%	16%
D1-29	42%	35%	44%	28%	14%	42%	27%	25%	30%	32%
D1-30	35%	29%	44%	60%	40%	39%	32%	26%	31%	15%
D1-31	45%	37%	48%	61%	36%	35%	39%	40%	45%	46%
D1-32	45%	31%	57%	57%	32%	33%	34%	38%	36%	30%
D1-33	25%	40%	66%	55%	29%	25%	36%	28%	40%	22%
D1-34	41%	37%	53%	55%	27%	38%	26%	33%	43%	41%
D1-35	47%	48%	58%	54%	45%	27%	43%	41%	46%	48%
D1-36	41%	26%	42%	57%	27%	18%	35%	30%	35%	35%
D1-37	44%	36%	44%	45%	26%	19%	37%	26%	33%	37%
D1-38	44%	48%	53%	57%	39%	38%	42%	41%	40%	43%
D1-39	45%	29%	46%	38%	29%	32%	41%	38%	38%	33%
D1-40	31%	33%	48%	52%	19%	31%	35%	12%	30%	25%
D1-41	37%	36%	43%	37%	10%	41%	13%	33%	31%	33%

D1-42	40%	31%	49%	17%	17%	38%	11%	40%	39%	41%
D1-43	35%	36%	64%	30%	19%	42%	21%	34%	39%	34%
D1-44	43%	42%	44%	28%	10%	37%	6%	28%	35%	32%
D1-45	45%	37%	46%	29%	26%	36%	12%	35%	39%	43%
D1-46	35%	28%	68%	55%	25%	41%	18%	40%	34%	40%
D1-47	46%	36%	39%	33%	16%	44%	25%	34%	33%	29%
D1-48	47%	35%	38%	53%	5%	26%	5%	29%	34%	24%
D1-49	42%	23%	29%	11%	26%	40%	6%	38%	35%	36%
D1-50	42%	26%	49%	32%	16%	40%	3%	34%	32%	30%
D2-1	47%	40%	39%	35%	20%	38%	17%	40%	37%	38%
D2-2	33%	32%	32%	10%	16%	34%	10%	25%	31%	31%
D2-3	41%	43%	47%	19%	23%	32%	20%	34%	38%	35%
D2-4	45%	44%	43%	39%	19%	34%	15%	30%	37%	36%
D2-5	39%	35%	37%	24%	19%	23%	7%	26%	26%	30%
D2-6	42%	36%	32%	30%	10%	17%	1%	23%	26%	22%
D2-7	39%	33%	26%	40%	17%	28%	15%	29%	31%	32%
D2-8	41%	38%	41%	36%	12%	29%	6%	33%	35%	31%
D2-9	46%	40%	46%	21%	19%	40%	6%	40%	38%	3%
D2-10	44%	34%	37%	25%	11%	31%	5%	28%	23%	27%
D2-11	37%	4%	36%	31%	17%	31%	11%	31%	32%	36%
D2-12	26%	4%	29%	12%	13%	26%	7%	28%	31%	30%
D2-13	35%	14%	39%	24%	22%	33%	16%	34%	36%	32%
D2-14	30%	11%	46%	34%	14%	31%	13%	30%	35%	28%
D2-15	37%	18%	42%	24%	16%	28%	11%	31%	31%	33%
D2-16	40%	22%	41%	33%	23%	40%	15%	41%	41%	35%
D2-17	48%	18%	34%	38%	24%	38%	27%	45%	40%	40%
D2-18	39%	17%	45%	36%	17%	42%	15%	36%	39%	37%
D2-19	30%	17%	34%	5%	13%	32%	8%	27%	23%	25%
D2-20	42%	14%	39%	29%	28%	45%	13%	37%	34%	32%
D2-21	35%	6%	46%	21%	30%	32%	20%	41%	36%	40%
D2-22	38%	4%	49%	35%	36%	44%	20%	34%	38%	40%
D2-23	27%	12%	36%	15%	31%	40%	14%	36%	40%	32%
D2-24	37%	18%	46%	32%	32%	47%	18%	45%	42%	43%
D2-25	33%	14%	46%	24%	25%	40%	13%	32%	35%	34%
D2-26	35%	14%	32%	24%	25%	47%	18%	40%	45%	43%
D2-27	26%	14%	36%	38%	22%	42%	21%	31%	37%	32%

D1, 1-50 = Vaccinated group
D2, 1-27 = Control group

Annex 4: Questionaire for CBPP sero-survey

Questionnaire for CBPP sero-survey

Cluster No._____Date collected ____________

1. Owner Name: _______Sex __________Age______Ethnicity_____

Addressee: Region Zone District Village Altitude Latitude Longitude

Amhara_____ _____ ______ _____ ______ _______ ________

2. Livestocks owned: Cattle Sheep Goats others
3. Age and sex distribution of cattle owned / herd(s): <1, 1-<2, 2-3, >3
4. Breed of cattle owned: Local: Arsi / Fogera / Horro / Kereyu / Boran / others

   Cross:------/----------/--------/-----------/----------/-------
5. Objective of Cattle keeping: Dairy Draught Both Feedlot Other
6. Production system: Settled Nomadic Semi-nomadic Trader
7. Feeding: Communal grazing Enclosed grazing Grazing with supplmentation
8. Cattle movement: Yes/No: If yes Grazing and watering Trade Cultural exchange
9. What major livestock/Cattle diseases/with priority do you know ?

   First________Second______Third______Fourth______Fifth_____
10. Did you have any experience of respiratory problems in your cattle herds ?Yes / NO

    If Yes, since when? since Six months/since one year/since two years/others
11. Local names for respiratory diseases of cattle(CBPP)_________________________
12. Is the disease present in your herd / neighbouring herd / districts/zones currently ? y/n
13. Which age groups were more affected ? < 1, 1-<2, 2-3, > 3
14. What possible reasons for the disease out break?. Watering and grazing contact with other herds, New stock: gift, purchased, others
15. What measures did you take to overcome the outbreak? Treatment/Vaccination /segregation / sale / slaughter / No measure / others ? when ?__________
16. Was there any traditional treatment used? Y / N , what is that ?____
17. Is the above traditional procedure effective / Clinically efficient to cure ? Yes /No
18. When was the last CBPP vaccination in the village?_____District?______Estimated vaccination coverage?_______%
19. Is there any specific season that the problem of CBPP more frequent? Y/N, no opinion/I do not know. If yes which season?__________From_________To__________

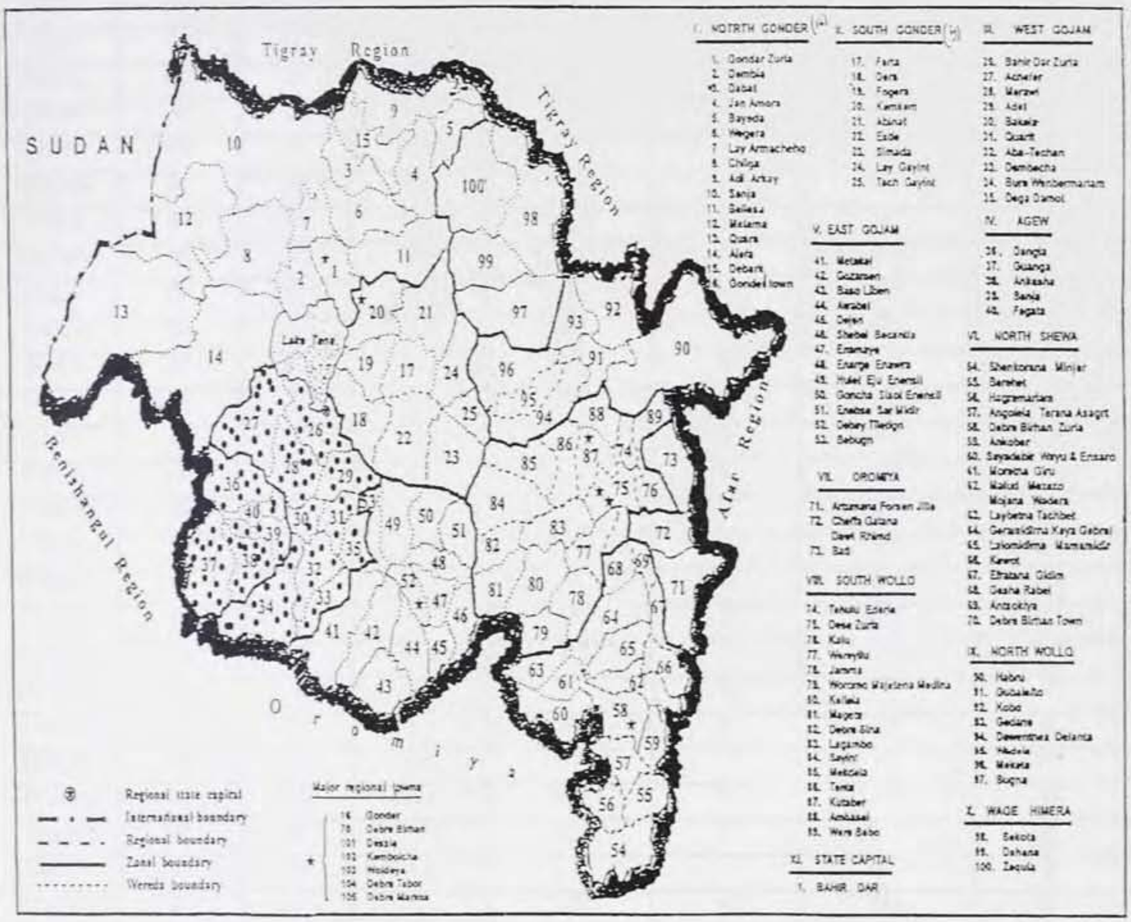
CBPP Sero-Survey Individual Animal Record

Tube No	Sex		Age			Ear Y/N	Lab. Resu	Tube No	Sex		Age			Ear Y/N
		< 1	>1-2	>2-3	>3					< 1	>1-2	> 2-3	> 3	
1								21						
2								22						
3								23						
4								24						
5								25						
6								26						
7								27						
8								28						
9								29						
10								30						
11								31						
12								32						
13								33						
14								34						
15								35						
16								36						
17								37						
18								38						
19								39						
20								40						

Annex 5: MAP OF THE STUDY AREA

MAP OF ETHIOPIA

ADMINISTRATIVE DIVISION OF THE AMHARA NATIONAL REGIONAL STATE



Annex: 6: Mean sero-prevalence of CBPP and 95% CI at village level, CFT 1:20 Vs C-ELISA

villages	Samples	CFT 1:20 +	%CFT 1:20 +	1.96 x SE*	ELISA+	%ELISA+	1.96 x SE*	Overlapping
Afere F	60	4	6.7	6.3	9	*15.0*	9	o
Ahuri	80	13	16.3	8.1	7	*8.8*	6	o
Kunzila	80	8	10.0	6.6	9	*11.3*	7	o
Wonberia	60	6	10.0	7.6	2	*3.3*	4.5	o
Boko	60	5	8.3	6.9	1	*1.7*	3	o
S Bitita	60	29	48.3	12.6	25	*41.7*	12	o
Shumata	60	11	18.3	9.8	13	*21.7*	10	o
Andasa	60	3	5.0	5.5	7	*11.7*	8.1	o
Mendel	60	3	5.0	5.5	9	*15.0*	9	o
S Tamit	60	1	1.7	3.3	4	*6.7*	6	o
Wonjeta	60	11	18.3	9.8	9	*15.0*	9	o
Zelema	60	12	20.0	10.1	9	*15.0*	9	o
Zegie	40	10	25.0	13.4	0	*0.0*	0.0	*
Anguay	40	31	77.5	12.9	11	*27.5*	11.3	*
Askuna	40	22	55.0	15.4	10	*25.0*	13.4	o
Sebadar	100	11	11.0	6.1	2	*2.0*	2.7	*
Markuma	60	17	28.3	11.4	7	*11.7*	8.1	o
Badani	60	27	45.0	12.6	28	*46.7*	12.6	o
Kachana	60	22	36.7	12.2	11	*18.3*	9.8	o
Singuri	60	26	43.3	12.5	18	*30.0*	11.6	o
Addis A	20	6	30.0	20.1	1	*5.0*	9.6	o
Wad E	20	6	30.0	20.1	0	*0.0*	0.0	*
Sholit	20	8	40.0	21.5	3	*15.0*	15.7	o
Sigadi	60	1	1.7	2.5	0	*0.0*	0.0	o
Bizre K	60	2	3.3	4.5	1	*1.7*	3.3	o
Metekel	60	3	5.0	5.5	5	*8.3*	7.0	o
Yimali	60	10	16.7	9.4	9	*15.0*	9.0	o
Woldu H	60	13	21.7	10.4	11	*18.3*	9.8	o
FinoteS	60	3	5.0	5.5	3	*5.0*	5.5	o
Arbatu E	40	6	15.0	11.1	1	*2.5*	4.8	o
Hodansh	40	6	15.0	10.1	0	*0.0*	0	*
Ambo M	40	0	0.0	0	0	*0.0*	0	o
Bachima	60	9	15.0	9.0	1	*1.7*	3.3	*
Enamirt	20	0.0	0.0	0.0	0	*0.0*	0	o
Enguti	60	7	11.7	8.1	9	*15.0*	9	o
Tagel B	20	0	0.0	0.0	0	*0.0*	0	o
Tagel W	60	3	5.0	5.5	6	*10.0*	8	o
Linguayta	60	7	11.7	8.1	3	*5.0*	6	o
Gishe A	40	5	12.5	10.2	4	*10.0*	9	o
Kortaita	60	3	5.0	5.5	1	*1.7*	3.3	o

0 = Overlapping = 85%, * = Not overlapping = 15%

CURRICULUM VITAE

I. Civil status:

Name:	Gashaw Takele Teferi
Date of birth:	May 27, 1962,
Place of birth	Chuahit, North Gondar, Ethiopia
Sex:	Male
Nationality:	Ethiopian
Marital status:	Single
Address:	C/o Serebe Derso,
	P. O. Box 80162, Addis Ababa, Ethiopia, Tel. 251-1-11 32 54

II. Educational Background

1971-1974	Chuahit elementary School
1974-1976	Gogora Junior Secondary School
1976-1980	Gorgora Senior Secondary School
1980-1986	Addis Ababa University, Faculty of Veterinary Medicine

III. Qualification: Doctor of Veterinary Medicine

IV. Language skills: Amharic: Listening, speaking and writing
English: Listening, speaking and writting
A little bit of Deutsch

V. Professional experience:

1986-1987	District veterinary field officer, Bale region, MOA, Ethiopia.
1987-1988	Gobe cattle breeding and improvement ranch, MOA.
1988-1995	Department head of microbiology, junior research officer research officer, regional team leader for sero-surveillance
1996 to date	Postgraduate student for MSc degree in Tropical Veterinary Epidemiology in the Freie Universität of Berlin and Addis Ababa University.

VI. Scholarly publications

1 Preliminary survey of mange mites on Black head Ogaden sheep, goats and swine in Harrarghe administrative region (Eastern Ethiopia). Thesis presented to the Faculty of Veterinary Medicine, Addis Ababa University, as a partial fulfilment of the degree of Doctor of Veterinary Medicine, June 1986, Debre Zeit, Ethiopia.

2 Isolation of *Moraxella bovis* and efficacy of propolis alcohol mixture in the treatment of infectious bovine keratoconjunctivitis. Institute of agricultural research, fourth national livestock improvement conference proceedings, 13-15 November, 1991, Addis Ababa, Ethiopia; No 4, 298-303.

3 Reproductive problems in indigenous cattle at the ministry of agriculture farms in central Ethiopia. Trop. Agric. (Trinidad) vol. 69 No 3 July 1992, 247-249.

4 Prevalence of sub-clinical mastitis at Assela livestock farm, Ethiopia. (compiled but unpublished).

VII. Professional Referees:

1 Prof. Dr. C. Staak

Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin
P.O Box: 33 00 13, D-14191 Berlin, Germany.
Tel: (..49) (30) 84 12 20 53, Telefax: (..49) (30) 84 12 47 41

2 Dr. F. Roger

Centre de cooperation internationale en recherche agronomique le development/department d'elevage et de medecine veterinaire (CIRAD-EMVT): Tel. Fax: 00 334 67 59 37 98

3 Dr. Getachew Abebe

Addis Ababa University Faculty of Veterinary Medicine, former Dean of the Faculty and currently, Associate Dean for Research and Graduate studies.
P.O.BOX 34, Tel 251-1-33 80 62

4 Dr. Wondwosen Asfaw

Ministry of Agriculture, Animal Health Service Team Leader, Addis Ababa
Tel: 251-1 51-03-05

Declaration

I the under signed, declare that the thesis is my original work and has not been presented for a degree in any university.

Name Gasham Takele

Signature [signature]

Date of submision 07/01/98

This thesis has been submitted for examination with our approval as university advisors.

[signature] Prof. Dr. Staak

Epidemiological survey of CBPP in AWI &
Western GOJJAM zones of Amhara Region &
Comparison of CFT & C-Elisa for the Diagnosi
of CBPP

Gashaw Takele