SERO-EPIDEMIOLOGICAL SURVEY ON
CONTAGIOUS BOVINE PLEUROPNEUMONIA USING
SEROLOGY AND PARTICIPATORY EPIDEMIOLOGY
METHODS IN UNITY STATE - SOUTHERN SUDAN

By
Mohamed Babiker Abdelrahim Babiker
B.V.Sc. (2001) University of Khartoum

A thesis
Submitted to the University of Khartoum
In fulfillment of the requirements for the
Degree of Master of Veterinary Science

Supervisors
Professor. Mohamed Elnasri Hamza
Doctor. Mohamed Elamin Hamid

Co-Supervisor
Professor. A. Mutaal A.O. Shallali

Department of Preventive Medicine and Public Health
Faculty of Veterinary Medicine
University of Khartoum
2005
## LIST OF CONTENTS

- LIST OF CONTENTS: i
- LIST OF TABLES: vi
- LIST OF FIGURES: vii
- LIST OF ABBREVIATIONS: viii
- DEDICATION: ix
- PREFACE: x
- ACKNOWLEDGMENT: xi
- ABSTRACT: xii
- ARABIC ABSTRACT: xiii
- INTRODUCTION: 1

## CHAPTER ONE

### LITERATURE REVIEW

1.1. Definition 3
1.2. History of the disease 3
1.3. A etiology 4
   1.3.1. Classification of the causative agent 5
   1.3.2. The Agent 5
      1.3.2.1. Morphology 5
      1.3.2.2. Pathogenicity 6
1.4. Epidemiology 6
   1.4.1. Host range 7
   1.4.2. Transmission 8
   1.4.3. Incubation period 8
   1.4.4. Morbidity and Mortality 9
   1.4.5. Carriers 9
   1.4.6. Resistance 9
   1.4.7. Outbreaks 10
1.5. Symptoms and Signs of the disease 10
   1.5.1. Per acute form 10
   1.5.2. Acute form 11
   1.5.3. Sub acute form 11
   1.5.4. Chronic form 12
1.6. Pathology 12
   1.6.1. Lesions 12
1.6.1.1. Lungs and Pleura 12
1.6.1.2. Lymph nodes 13
1.6.1.3. Joints and Bursa 13
1.6.1.4. Kidneys 13
1.6.1.5. Heart 13

1.7. Diagnosis 14
1.7.1. Clinical diagnosis 14
1.7.2. Isolation of the organism 14
1.7.3. Post mortem findings 15
1.7.4. Biochemical tests 16
1.7.5. Serological tests 18
  1.7.5.1. Slide Agglutination Test 18
  1.7.5.2. Tube Agglutination Test 18
  1.7.5.3. Precipitation Test 18
  1.7.5.4. Complement Fixation Test (CFT) 19
  1.7.5.5. Enzyme Linked Immuno Sorbent Assy (ELISA) 19
  1.7.5.6. Immuno Blotting Test (IBT) 21
1.7.6. Antigen Detection System 21
  1.7.6.1. Monoclonal Antibody-Based sandwich ELISA 21
  1.7.6.2. Immuno Cyto-Chemistry (ICC) 22
1.7.7. Molecular Diagnosis 22
  1.7.7.1. Polymerase Chain Reaction (PCR) 22

1.8. Disease Control & Eradication 24
1.8.1. Stamping out 24
1.8.2. Testing and Elimination of Reactors 24
1.8.3. Vaccination 25
1.8.4. Chemotherapy 25

1.9. Disease in the Sudan 27

CHAPTER TWO 28
GENERAL MATERIALS AND METHODS 28
2. Materials & Methods 28
  2.1. Study area 28
  2.2. Clinical Data 31
  2.3. Sample for Bacteriological and Serological Examination 31
    2.3.1. Tissue Samples 31
    2.3.2. Serum Samples 32
      2.3.2.1. Serum Samples Data 32
        2.3.2.1.1. Location of Samples 32
        2.3.2.1.2. Sex of Sampled Animals 32
2.3.2.1.3. Breeds of Sampled Cattle 34
2.3.2.1.4. Season of Sample Collection 34
2.3.2.1.5. Vaccination Status of Samples 34

2.4. Culture Media 35
   2.4.1. Liquid Media 35
      2.4.1.1. Gourley’s Media 35
      2.4.1.2. Brain Heart Infusion Broth 35
      2.4.1.3. Brucella Broth 36
   2.4.2. Solid Media 36
      2.4.2.1. Gourley’s Agar Media 36
      2.4.2.2. Brain Heart Infusion Agar 36
      2.4.2.3. Brucella Agar Media 36
   2.4.3. Basic Cultivation Technique for Mycoplasma 36

2.5. Buffers 37
   2.5.1. Veronal Buffer 37
   2.5.2. CFT Diluent Tablets 37
   2.5.3. Coating Buffers 37
   2.5.4. Phosphate Buffer Saline 38
   2.5.5. Diluting Buffers 38
   2.5.6. Substrate Buffer 38
   2.5.7. Washing Buffer 38

2.6. Solutions 39
   2.6.1. Alsever’s Solution 39

2.7. Reagents 39
   2.7.1. Antigens 39
      2.7.1.1. Whole Mycoplasma Cell Antigen 39
   2.7.2. Test Sera 40
   2.7.3. Preparation of Blood Cell 40

2.8. Biochemical Test 40
   2.8.1. Digitonin Test 40
   2.8.2. Growth Inhibition Test 40

2.9. Serological Tests: 40
   2.9.1. Lindley Plate Complement Fixation Test 41
      2.9.1.1. Materials 42
         2.9.1.1.1. Titration of the Complement 42
         2.9.1.1.2. Sheep Red Blood Cell Suspension 42
         2.9.1.1.3. Hemolytic Amboceptor 43
         2.9.1.1.4. Sensitization of Red Blood Cells 43
         2.9.1.1.5. Negative Bovine Serum 43
         2.9.1.1.6. Antigen 43
CHAPTER THREE

RESULTS

3. Results

3.1. Clinical Data

3.2. Sample Collection Year

3.3. Isolation of the Agent

3.3.1. Culture of the Samples

3.4. Identification of the Agent

3.4.1. Biochemical Test

3.4.1.1. Digitonin Test

3.4.1.2. Growth Inhibition Test (GIT)

3.5. Serological Tests

3.5.1. Complement Fixation Test

3.5.1.1. CFT Result

3.5.1.2. Sample Collection Year vs. CFT Cross Tabulation

3.5.1.3. Location vs. CFT Result Cross Tabulation

3.5.1.4. Sex vs. CFT Result Cross Tabulation

3.5.1.5. Breed vs. CFT Result Cross Tabulation

3.5.1.6. Season vs. CFT Result Cross Tabulation

3.5.1.7. Vaccination vs. CFT Result Cross Tabulation

3.5.2. Competitive Enzyme Linked Immuno Sorbent Assay (C-ELISA)

3.5.2.1. C-ELISA Results

3.5.2.2. Sample Collection Year vs. c-ELISA Cross Tab
3.5.2.3. Location of Samples vs. c-ELISA Cross Tab  63
3.5.2.4. Sex vs. c-ELISA Cross Tabulation  64
3.5.2.5. Breed vs. c-ELISA Cross Tabulation  64
3.5.2.6. Season vs. c-ELISA Cross Tabulation  64
3.5.2.7. Vaccination vs. c-ELISA Cross Tabulation  64
3.5.3. CFT vs. c-ELISA Results Comparison  72
  3.5.3.1. Result  72
  3.5.3.2. Year  72
  3.5.3.3. Location  72
  3.5.3.4. Sex  72
  3.5.3.5. Breed  72
  3.5.3.6. Season  73
  3.5.3.7. Vaccination Status  73
3.6. Participatory Epidemiology  73
  3.6.1. Direct Observation  73
  3.6.2. Interviews  73
  3.6.3. Mapping  74
  3.6.4. Seasonal Calendar  74
  3.6.5. Ranking, Scoring and Proportional Piling  74

CHAPTER FOUR
DISCUSSION
4. Discussion  77

REFERENCES  85
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Frequencies and Percentages of Collected Serum Samples from</td>
<td>33</td>
</tr>
<tr>
<td>Different locations.</td>
<td></td>
</tr>
<tr>
<td>2. Year of Sample collection vs. CFT Result Cross Tabulation.</td>
<td>57</td>
</tr>
<tr>
<td>3. Location of Samples vs. CFT Result Cross Tabulation.</td>
<td>59</td>
</tr>
<tr>
<td>4. Sex of Sampled Animals vs. CFT Result Cross Tabulation.</td>
<td>60</td>
</tr>
<tr>
<td>5. Types of Sampled Cattle Breed vs. CFT Result Cross Tabulation.</td>
<td>61</td>
</tr>
<tr>
<td>6. Season of Samples Collection vs. CFT Result Cross Tabulation.</td>
<td>62</td>
</tr>
<tr>
<td>7. Vaccination Status vs. CFT Result Cross Tabulation.</td>
<td>65</td>
</tr>
<tr>
<td>8. Year of Sample Collection vs. C-ELISA Result Cross Tabulation.</td>
<td>66</td>
</tr>
<tr>
<td>9. Location of Samples vs. C-ELISA Result Cross Tabulation.</td>
<td>67</td>
</tr>
<tr>
<td>10. Sex of Sampled Animals vs. C-ELISA Result Cross Tabulation.</td>
<td>68</td>
</tr>
<tr>
<td>11. Types of Cattle Breed vs. C-ELISA Result Cross Tabulation.</td>
<td>69</td>
</tr>
<tr>
<td>12. Season of Samples Collection vs. c-ELISA Result Cross Tabulate.</td>
<td>70</td>
</tr>
<tr>
<td>13. Vaccination vs. vs. C-ELISA Result Cross Tabulation.</td>
<td>71</td>
</tr>
<tr>
<td>14. CFT Results vs. C-ELISA Results Percentages by Locations.</td>
<td>75</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Map of Major Ethnic Groups and Cattle Movement Paths.</td>
<td>30</td>
</tr>
<tr>
<td>2. Bar Chart illustrates the Highly Infectious Cattle Diseases</td>
<td>52</td>
</tr>
<tr>
<td>in Unity State as obtained from the Clinical Data.</td>
<td></td>
</tr>
<tr>
<td>3. Bar Chart demonstrates the Highly Infectious Cattle Diseases</td>
<td>53</td>
</tr>
<tr>
<td>in Different Seasons from Clinical Data.</td>
<td></td>
</tr>
<tr>
<td>4. Pie Chart demonstrating the Highly Infectious Cattle diseases</td>
<td>54</td>
</tr>
<tr>
<td>Percentages from the Clinical Data.</td>
<td></td>
</tr>
<tr>
<td>5. Complement Fixation Test on Micro Plate image.</td>
<td>56</td>
</tr>
<tr>
<td>6. Bar Chart illustrating Ranked Bovine Diseases by Nuer groups.</td>
<td>76</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

B.Q : Black Quarter.
C.F.U : Colony Forming Units.
C’ : Complement.
C° : Celsius.
CBPP : Contagious Bovine Pleuropneumonia.
CCPP : Contagious Caprine Pleuropnemonia.
C-ELISA : Competitive Enzyme Linked Immuno Sorbent Assay.
CFT : Complement Fixation Test.
CVRL : Central Veterinary Research Laboratories.
ELISA : Enzyme Linked Immuno Sorbent Assay.
H.S : Hemorrhagic Septicemia.
IBT : Immuno Blotting Test.
ICC : Immuno Cyto Chemistry.
LC : Large Colony.
M.H.D : Minimum Hemolytic Dose.
Mmm : Mycoplasma mycoides mycoides.
MOD : Mean Optical Density
PCR : Polymerase Chain Reaction.
RBCs : Red Blood Cells.
RPM : Rounds per Minute.
SC : Small Colony.
WN-POC : White Nile Petroleum Operating Company
CD : Community Development
DEDICATION

To My Father, Mother and Brothers
To My Best Friends and Colleagues
Wish You All the Best
PREFACE

This work was carried out in the Department of Preventive Medicine and Veterinary Public Health, Faculty of Veterinary Medicine, University of Khartoum under supervision of Professor Mohamed El Nasri Hamza and the Department of Mycoplasma, Central Veterinary Research Laboratories (Soba) under supervision of Professor. A. Mutaal Shallali.
ACKNOWLEDGMENT

I wish to show my appreciation to Professor Mohamed El Nasri Hamza for his direct supervision and leadership.

Truthful thanks are due to Professor A. Mutaal Shallali for his close supervision and permission to carry out this research work in the Department of Mycoplasma.

My gratitude is due to Dr. Mohamed El Amin Hamid for early supervision and guidance.

I am indebted to my colleagues Dr. Hussameldin O. El Nasri and Dr. Mamdooh Sabar for their full support and encouragement.

My thanks are also due to Dr. Ameera Mahjoub and Dr. Sawsan Abbas for their assistance.

My thanks are due to Mr. Mustafa Ahmed, Senior Technician and the team of Mycoplasma Dept, Central Veterinary Research Laboratories (Soba).

I would like to show my recognition to Dr. Johnson Boll Met and Dr. John Bleiu from the Ministry of Animal Resources and Fisheries, Unity State for their useful cooperation and support in the field.

I would like also to express my great regards to the Community Development Department of White Nile Petroleum Operating Company (WN-POC) for allowing me to carry out this research work and affording limitless support.
SUMMARY

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

This study was carried out to assess the prevalence, distribution and importance of CBPP using serology and participatory epidemiology methods so as to provide useful information which support the surveillance and control strategies of the disease in Unity State.

Eight bovine lung tissue samples were been cultured to isolate the *Mmm* organism and total of 600 serum samples were collected from different locations in Unity State and have been tested by using two serological tests; Complement Fixation Test (CFT) and Competitive Enzyme Linked Immuno Sorbent Assay (c-ELISA).

The results showed that the rate of positive reactors was 26% by CFT and 16.5% by c-ELISA.

The CFT was found to be more successful in the diagnosis of CBPP but was found to be more complicated to be applied at the field as well.

The Competitive ELISA was found to be useful and straight forward but showed high range of doubtful results in comparison with CFT.

The Participatory Epidemiology Approaches result among Nuer and Messeria groups indicated that those communities have a useful knowledge about the disease which supports the disease surveillance and control.
ملخص الأطروحة

مرض ذات الرئة الساري في الأبقار يمثل أحد المهامدة الخطيرة لقطاع الزراعي في ولاية الوحدة، جنوب السودان. هدفت هذه الدراسة لمعرفة معدل انتشار وتوزيع وأهمية مرض ذات الرئة الساري في الأبقار عن طريق الاختبارات المصلية وطرق علم الأوبيئة بمشاركة المجتمعات الريفية لكي نروم بالإبلاغ بالمعلومات اللازمة التي تدعم التقصي المرضي واستراتيجيات السيطرة على المرض في ولاية الوحدة، جنوب السودان.

وفي هذا البحث تم استزراع ثمان عينات من نسيج الرئة لعزل المسبب المرضي كما تم جمع 600 عينة مصل من مناطق مختلفة داخل ولاية الوحدة وتم اختبارها باستعمال إثنان من الاختبارات المصلية؛ اختبار تثبيت المتمم (CFT) وأختبار الأليزا التنافسي (c-ELISA)، أوضح النتائج بأن نسبة التفاعلات الإيجابية كانت (26%) باختبار تثبيت المتمم و(16.5%) من قبل اختبار الأليزا التنافسي.

ولقد دلت النتائج على أن اختبار تثبيت المتمم أكثر موثوقية في تشخيص مرض ذات الرئة الساري في الأبقار ولكن لوحظ أن الاختبار أشد تعقيدا بحيث يصعب التنفيذ على مستوى الحقل. حقق اختبار الأليزا التنافسي (c-ELISA) نتائج مقبولة ومفيدة ومبسطة لكن لوحظ أن هناك مدى عالية من النتائج الغير مؤكدة مقارنة بالنتائج المتصورة من اختبار تثبيت المتمم.

dلت النتائج المستخلصة من مجموعات النوبي والمسيري عبر علم الأوبيئة التشاركي بأن لهذه المجموعات الريانية حرصية معرفية بيطرية قيمة ومفيدة في مجال المراقبة والسيطرة على المرض.
INTRODUCTION

CBPP is caused by *Mycoplasma mycoides* subspecies *mycoides SC* (Small Colony, variant) (*Mmm SC*). The disease is present in West, Central, East and parts of Southern Africa but not North Africa. From a historical perspective, CBPP was a disease of Europe and Asia. A comprehensive historical account of the spread of CBPP in view of the economic significance of the disease in Europe and Africa in the 19th century has been provided by Windsor (1977). With the near eradication of Rinderpest in Africa (except the Somali ecosystem), CBPP has become the most significant epidemic disease of cattle in Africa with 22 countries reporting outbreaks of the disease in 2003.

CBPP has been a major cause of cattle mortality and production losses in many parts of Africa. Being an OIE List A disease and with implications for rapid spread between herds and across international borders, CBPP has engaged the attention and resources of the FAO for many years in attempts to curb the spread of the disease and limit its devastating economic effects, especially at the village or community level. CBPP is an economically important, contagious respiratory disease of cattle that affects domestic ruminants of the genus *Bos*, mainly *Bos taurus* and *Bos indicus*. CBPP has become a serious obstacle to livestock development in sub-Saharan Africa and Asia. It is characterized by a morbidity rate of 75%–90%, a mortality rate from 50% to 90%, and a case-fatality rate of 50%.

In the Sudan the disease has been enzootic since the beginning of this century and is now presented in all states except the Eastern and Northern States (Anon, 1969).
The similarity of CBPP with other forms of pneumonia (Hudson, 1971) makes it difficult for veterinarian in the field to reach a conclusive diagnosis on clinical symptoms only (Abdulla, 1975). These problems will adversely affect the quick control measures to be undertaken in case of CBPP outbreaks. Further more no single serological test is 100% accurate in diagnosis the disease (Shifirine and Gourlay, 1967). Even the complement fixing test gave 6% false positive reactors (Gourlay, 1965).

The aim of this study was to accomplish a potential database to assess the prevalence, distribution and importance of Contagious Bovine Pleuropneumonia (CBPP) in Unity State, Southern Sudan. The study also aimed to evaluate and compare the current CBPP serological tests (CFT and c-ELISA) in both sensitivity and viability at the field levels. The study also aimed to practice participatory epidemiology techniques for qualitative epidemiological intelligence in the disease surveillance. The study intended to participate in the surveillance and control strategies for CBPP by establishing Early Warning System indicators.
1.1. **Definition:**

Contagious Bovine Pleuropneumonia (CBPP) is a devastating disease of cattle and water buffaloes caused by *Mycoplasma mycoides subsp. mycoides (M.myciodes)* (Turner, 1959 and Hudson, 1971). Naturally CBPP is an essential disease of cattle and buffalos (Turner; 1954, Lloyd; 1970 and Hudson; 1971).

1.2. **History of the disease:**

CBPP appears to have existed in the ancient world according to early classical writings (Provost *et al.*, 1987). It was referred to as *Polmonera* by Gallo.

At the end of 18th century (CBPP) could be distinguished from other respiratory diseases as a distinct disease caused by *M. mycoides* Small Colony (*MmmSC*). Evidence based on clinical, epidemiological and pathological information suggested a long history of the disease, Hudson (1965) reported that the disease originated in Europe and was disseminated widely by transboundary trade in livestock. Foster (1934) stated that the disease first appeared in Switzerland and Germany from where it spread to different European countries probably during the Napoleon wars.

According to Currason; (1967) the disease was first observed in Italy and France in the middle of the sixteenth century. The disease was reported in the United States of America in 1843 (Jasper, 1967). The disease was carried by sea to Australia through infected cattle from Europe in 1858 (Turner, 1959).
Later the disease was introduced in Africa; South Africa became infected in 1854 from either Britain (Hutyra et al., 1938) or Netherlands (Provost et al., 1987).

Due to strict control measures undertaken at the end of the 19th century and at the beginning of the 20th century the disease was eradicated from most of the European countries England (1898), Russia (1940) and from the United States of America (1852) (Jasper, 1967). Later some cases of the disease were reported in Poland in (1936) and Spain (1959) as mentioned by Viettoz (1969). The later cases of the disease in Europe were registered in France in (1973), Anon; (OIE Report, 1974).

However in recent years there have been outbreaks of the disease in Spain, Portugal, and Italy (Nicholas, and Bashiruddin. 1995). It is thought to exist in the Middle East and Asia (Nicholas, and Bashiruddin; 1995). The disease is still present in most African countries south of the Sahara, Angola, India and China (Hudson, 1971).

1.3. Etiology:

CBPP is caused by the organism now classified as (M.mycoides) strain PG-1 by Edward and Freundt (1956). Later this strain was classified into small and large colony types on the basis of growth and biochemical characteristics (Cottew and Yeast, 1978). The bovine strains belong to the small colony type, they do not digest casein, do not liquefy coagulated serum and survive for a shorter time at 45° C than the caprine large colony type.

1.3.1. Classification of the causative Agent:

Mmm Small Colony type, the causative agent of CBPP belongs to:
Class; Mollicutes, Order; Mycoplasmatales, Family; Mycoplasmataceae and Genus; Mycoplasma (Edward and Freundt., 1969)

*Mmm*SC is a member of the *M. mycoides* cluster which includes: *M. mycoides* large colony type (*Mmm*LC), *M. capricolum* subsp. *capricolum* (*Mcc*), *M. capricolum* subsp. *capriumeoniae* (*Mccp*), *M. mycoides* subsp. *capri* (*Mm. capri*) and *Mycoplasma* sp. bovine group 7.

There is only one serotype of *Mmm*SC. The six *Mycoplasmas* share the same serological and genetic characteristics and this causes taxonomic and diagnostic problems (Cottew, Beard, Damassa et al. (1987).

### 1.3.2. The Agents:

*Mmm*SC strains cause (CBPP) in cattle and buffalo (Edward and Freundt, 1956) and (Provost, Perreau, Breard, Le Golf, Martel, and Cottew 1987).

#### 1.3.2.1. Morphology:

The organism was described as pleomorphic occurring as round, ovoid, ring and filamentous forms in liquid media. On solid medium the colonies have dark centers and light periphery which give them characteristic fried-egg appearance. *M. mycoides* has no cell wall (Kandler and Zehander, 1957; Plackett, 1959). Instead it is surrounded by three-layered cytoplasmic membrane (Domermuth et al., 1964 and Anderson, 1969). This organism stains well with Giemsa (Bordet, 1910), Methylene blue (Dienes, 1945) and other Aniline stains (Turner, 1959). In young cultures it tends to appear as branching filaments and in old-cultures as small coccal bodies. It requires cholesterol (added serum) for its growth. The
organism is fragile, sensitive to desiccation and disinfectants and survives poorly outside the host (Turner, 1959).

1.3.2.2. Pathogenicity:

The “cluster” contains six important mycoplasmas affecting ruminants: Two of them are pathogenic to cattle; *MmmSC* causes CBPP and *Mycoplasma sp. bovine group 7* (BG-7) which may cause bovine arthritis and bovine mastitis (Cottew, 1960). The other four; two subspecies within *Mycoplasma mycoides* species and two subspecies within *Mycoplasma capricolum* species are responsible for goat respiratory and other diseases (Cottew, 1960).

1.4. Epidemiology:

Contagious bovine pleuropneumonia is naturally a disease of cattle and buffaloes (Turner; 1954, Llyoid; 1970 and Hudson; 1971). There is a considerable variation in the degree of symptoms seen in cattle affected with CBPP ranging from per acute through acute, sub clinical and chronic forms. Respiratory distress and coughing evident on stimulation of resting animals are the main signs of CBPP (Scudamore, 1976). Experimental reproduction of the disease is difficult but best achieved by bronchial intubations of low passage of *M.mycoides.mycoides* (Gourlay and Howard, 1982, Martel et al., 1983).

In experimental infections; Regalla *et al.* (1994) reported disease symptoms appearing in cattle 40 days after contact with infected animals; these symptoms lasted for 20 days. Turner and Campbell (1937) reported a range of 29-58 days and Provost *et al.*, (1987) stated 20-40 days.
1.4.1. Host Range:

Cattle of all ages are susceptible to CBPP and animals less than three years old are more susceptible. CBPP under natural condition occurred in cattle of species *Bos* and allied animals including buffalos, yak, bison and even reindeer (Hutyra, 1938); these authors also reported that goats and sheep were susceptible under experimental conditions. Small ruminants, in particular goats, have also been shown to harbor the *MmmSC* (Hudson et al., 1971). Brando (1995) isolated *MmmSC* from the milk of sheep with mastitis, as well as from goats with pneumonia in Portugal outside the endemic region of CBPP. Dick (1937) in Australia found sheep very susceptible to subcutaneous inoculation, some even dying as a result of extensive reaction. Yak and bison have been infected in zoos, and infection has been reported in water buffalo (*Bubalus bubalis*).

Experimental work in Australia showed that buffalo could be infected by artificial means but didn’t spread CBPP to in-contact buffaloes (Newton, 1992). Smith (1967) showed that after inoculation of mice, complete elimination was often delayed and mycoplasmaemia was present in many mice for considerable period.

Martins-Mendes (1958) isolated *M.mycoides* from male and female ticks of the species *Boophilus decoloratus* and *Amblyomma pomposum* that had fed on cattle suffering from natural infection with CBPP and from experimental subcutaneous reactions. European breeds seem to be more susceptible than African breeds, wild bovids and camels are both resistant to infection (OIE-1997).


1.4.2. Transmission:

Spread of the disease from infected to susceptible animals is mainly through inhalation of infected droplets which may be carried 10-20 or more meters by the air currents (Turner, 1959). Factors which influence the rate of spread of infection are closeness of contact, intensity of infection and the level of individual susceptibility (Turner, 1954).

Direct contact is the principal mode of transmission. However alternative routes like wind-borne and indirect transmission cannot be excluded (Masiga et al., (1996); Regalla et al., (1996)). Other modes of infection were also reported; According to Masiga et al., (1972); Scundamore (1976) and Elmahi (1980); *M. mycooides* was isolated from the urine of infected animals and this contaminates the pasture and so causes indirect spread of the disease. Typical pulmonary lesions of pleuropneumonia were reported in 5 out of 6 cattle fed fodder infected with virulent culture of *M. mycooides* (Windsor and Masiga, 1977). The organism was re isolated from all infected animals. *Mm* can pass through the placenta from the infected dams to the offspring (Stone, 1969).

1.4.3. Incubation period:

Time from natural exposure to overt signs of disease is variable but generally quite long. It has been shown that animals placed in CBPP infected herd may begin showing signs of disease 20 to 123 days later (Provost et., al; 1988). The incubation period of the natural disease may range from 5 to 207 days although Turner and Campbell (1937) reported a range of 29-58 days and Provost et al., (1987); stated 20-40 days. In experimental bronchial intubations infection, Regalla et al., (1996) reported the appearance of signs and serological response with 40 days after contact with intubated animals.
1.4.4. Morbidity and Mortality:
In Africa mortality rates are typically 10-70% in epizootics characterized by low morbidity and low or non-existent mortality with the majority of infected cattle showing chronic lesions; (Masiga et al., 1996; Regalla; 1996), this is characteristic of endemic disease. This difference maybe due to the fact that European cattle are healthier in general, better in fed, subjected to less physical stress and often permanently housed throughout the year (Nicholas and Palmer., 1994) and probably experienced strains of lower virulence than in Africa (Abdo et al., 1998). In Italy during the early 1990s CBPP forms with mild or without clinical signs were frequently observed in cattle with lesions of CBPP at slaughter. In infected herds mortality was around 2-3% (Guadagnini et al., 1991). In typical outbreaks about 40% of the exposed cattle contracted the disease and mortality among diseased cattle can range from 10% to 70%.

1.4.5. Carriers Role:
Some recovered cattle may harbour sequestra in their lungs and become a carrier. If stressed the walls of sequestra break down and the animal may become an active shedder and a source of infection. Carrier animals are dangerous because they frequently introduce the disease into previously uninfected areas. In fact most new outbreaks had been traced and found that carrier animals or healthy looking animals were the source of infection.

1.4.6. Resistance:
In Africa up to one third of cases that recover from acute disease become potential carriers. This figure is probably higher in Europe where there is far more widespread use of antimicrobials (Nicholas and Palmer., 1994).
The use of antibiotics and anti-inflammatory drugs may help to mask clinical signs and to accelerate the formation of chronic lesions.

1.4.7. Outbreaks:

Outbreaks usually begin by introducing an infected animal into a naive herd. It is widely believed that recovered animals harboring infectious organisms within a pulmonary sequestrum may become active shedders when stressed. Although this may be a factor in some outbreaks, it has not been substantiated experimentally (Windsor and Masiga, 1977).

1.5. Symptoms and Signs of the disease:

The early stages of the disease are indistinguishable from any severe pneumonia with pleurisy (Scudamore, 1976). The disease may be clinical or subclinical, the clinical form may be acute, sub acute or chronic (Turner, 1959). Sub-clinical and chronic cases of CBPP constitute more than 50% of the animals involved in the epizootic (Bygrave et al., 1968). In calves about one year old or less the clinical picture of the disease is primarily characterized by arthritis and the chest may be free from any lesions (Moulton et al., 1965), Turner and Trethewie, (1961); Simmons and Johnson, (1963); Harbi and Salih, (1979) and Elmahi, (1980).

1.5.1. Per acute form:

The clinical signs are observed in per acute form are much accelerated. The pathological signs are usually characteristic with marked pleural adhesion accompanied by exudative pericarditis and myocarditis (Provost et al., 1987). Affected animals may die within a week exhibiting classical respiratory signs.
1.5.2. Acute form:

The clinical signs in the typical acute case can be summarized as follows: A rise in body temperature to $41^\circ$ C, shallow and rapid respiration in the early stage of the disease, which soon becomes abdominal with painful grunting and dilation of the nostrils. The neck is extended and the animals stand facing the wind. There is an excess mucous in the nostrils and frothy saliva around the mouth and occasional soft coughs are heard (Bygrave et al., 1968 and Hudson, 1971). Animals show; Dullness, Anorexia, Irregular rumination with moderate fever (up to $40^\circ$-$42^\circ$C) and may show signs of respiratory distress. Complications in calves may include valvular endocarditis and myocarditis (Martel et al., 1983).

While classical respiratory signs may be evident in calves, articular localization of the causative agent with attendant arthritis usually predominates. The clinical picture of infection in many calves is manifested by arthritis (Turner, 1959); the chest may be free from lung lesion even though the causative agent is introduced in the pulmonary system (Harbi and Salih, 1979, and El Mahi, 1980).

1.5.3. Sub acute form:

In sub acute cases lesions are localized in small parts of the lung, the position can not be easily located by percussion and auscultation. The only symptom is a rare cough. Sometimes new foci of infection are created and acute symptoms set in (Henning, 1956). In the sub acute form signs may be limited to a slight cough only noticeable when the animal is exerted or exercised.
1.5.4. Chronic form:

In chronic cases the only symptom is a cough which usually appears when the animal rises or when it suddenly passes from a warm stable to a cold one or when it drinks cold water (Henning, 1956).

1.6. Pathology:

1.6.1. Lesions:

1.6.1.1. Lungs and Pleura:

The pathological lesions of the CBPP are confined to the thoracic cavity and the lungs, and lesions are usually unilateral. The diaphragmatic lobe was observed to be more commonly affected than the cranial lobe (Nunes and Pestica et al., 1988; 1990). The thoracic cavity of affected animals may contain many liters of clear yellowish brown fluid containing some pieces of fibrin (Ter-Laak, 1992). This pleural fluid is the ideal diagnostic material from which the causative *mycoplasma* can be isolated from or on which PCR can be carried out with DNA purification (Nicholas and Bashiruddin, 1995). Caseous fibrinous deposits are observed on the parietal visceral surfaces of the lungs (Provost et al., 1987). The interlobular septa of the affected lungs show distension with amber colored fluid surrounding the distended lymphatics. This fluid separates the lung lobules which vary in color with red, grey and yellow hepatization being evident indicating different stages of inflammatory lesions (Hudson, 1971). Consolidation of the lungs with typical marbled appearance sometimes accompanied by adhesions of the parietal and visceral surfaces of the pleura. Sequestrum consisting of necrotic lung parenchyma surrounded by a fibrous capsule which varies in size between 1 and 10 cm in diameter is formed in chronic or advanced cases (Martel et al., 1983; Trichard et al., 1989; Santini et al.,
The sequestrum may constitute a source of infection to cattle when ruptured, particularly where the sequestrum is drained by bronchus which forms an outlet for dissemination of infected aerosol droplets (Provost et al., 1987), such mechanism will account for outbreaks of disease in closed herds (Windsor and Masiga; 1977).

1.6.1.2. Lymphnodes:
The mediastinal and bronchial lymph nodes are swollen and juicy. On section the inner surface is either normal (Yellowish) congested or granular (Bygrave et al., 1968).

1.6.1.3. Joints and Bursa:
Lesions in calves are often restricted to the joint and bursa (Moulton et al, 1956; Turner and Trethewie, 1961; Simmon and Johnson, 1963; Harbi and Salih, 1979 and El Mahi, 1980). Turner and Trethewie (1961) described the lesions in the joints and bursae as non purulent serofibrinous polyarthritis characterized by hyperemia and effusion. In addition to respiratory disease affected calves may present exudative peritonitis, arthritis, bursitis and fibrinous arthritis of the carpal and tarsal joints (Provost et al., 1987).

1.6.1.4. Kidneys:
In kidney, sharply demarcated renal infarcts are clearly visible when the capsule is striped (Hudson, 1971 and El Mahi, 1980).

1.6.1.5. Heart:
In calves heart lesions were described as vegetative vulvitis, endocarditis and myocarditis (Turner and Trethewie, 1961).
1.7. Diagnosis:

1.7.1. Clinical diagnosis:

Diagnosis of all forms of the disease on clinical ground alone is very difficult since the symptoms simulate those of other forms of pneumonia (Turner, 1959 and Hudson, 1971).

1.7.2. Isolation of the organism:

*Mmm*SC grows well in both anaerobic and aerobic environments at a pH of 7.6-7.8. It usually grows well in sealed liquid broth cultures especially if the broth level is few inches deep to allow for oxygen or air gradient. Gentle aeration increases the growth rate and the yield of *Mmm*SC (Rodwell and Mitchell, 1979). In actively growing cultures *Mmm* is filamentous which the result of genetic division preceding cytoplasmic division. At the end of growth however short beaded filaments predominate and ultimately only coccoid bodies are seen (Razin, 1978).

The addition of serum to the medium is essential for growth (Bennett, 1932). Many media have been described which enable the growth of *Mmm*SC for example; Bennett, 1932; Hudson, 1971; Freundt, 1983; Nicholas and Barker, 1998; Rice *et al*., 2000.

Conventional growth media with the addition of inhibitors such as ampicillin, bacitracin, penicillin-G, polymyxin-B and thalium acetate to inhibit the growth of cell walled bacteria may be particularly useful as it inhibits *Acholeplasma* as well (Abu-Amero *et al*., 1996).

The growth of arginine-hydrolysing mycoplasmas may be inhibited by citrulline, lysine and ornithine (Ozcan *et al*., 1999).
In acute cases the causative agent can be isolated from the blood and nasal swabs as follow: -

Drawn 10 ml of blood from jugular vein under sterile conditions is added to 90 ml of liquid medium containing horse serum, penicillin and thalium acetate and incubated aerobically at 37° C for few days (Hudson, 1971). When turbidity is evident sub culture is made on solid medium and incubated aerobically at 37° C and examined daily for the appearance of mycoplasma colonies.

If the animal has died; pleural fluid or affected lung can be collected aseptically for cultural examination in selective media (Gourlay, 1973). If putrefaction has commenced sterile filter paper can be soaked with the pleural fluid or allowed to absorb fluid from lung lesion (Turner, 1959).

Since *Mycoplasma* rises higher by capillary than do most contaminating bacteria (Elbert and Peretz, 1932).

In chronic cases cultural examination from the sequesterum in Mycoplasma medium usually yields *M. mycoides* for differential diagnosis a culture of *Actinobacillus* is necessary (Hudson, 1971).

### 1.7.3. Post-mortem findings:

If the animals has died or was slaughtered the necropsy findings are suggestive but differentiation should be made from acute pulmonary pasteurellosis. A smear stained with Methylene blue or Giemsa may reveal bipolar *Pasteurella* organism (Hudson, 1971).

In chronic cases differentiation should be made between the sequestrum and *Echinococcus* cyst. A precipitin test or isolation of the organism will distinguish between the two organisms (Turner, 1959 and Hudson, 1971).
1.7.4. Biochemical Tests:

The sensitivity to digitonin indicates the requirement for sterol, members of the genus *Mycoplasma* are digitonin sensitive and this test is performed after the initial isolation of suspicious *mycoplasmas*. A series of biochemical tests standardized by (Aluotto et al., 1970) should follow and *Mmm*SC strains may be identified by the fermentation of glucose, the reduction of tetrazolium aerobically and anerobically and the digestion of casein; they do not hydrolyse arginin, liquify coagulated serum, produce film and spots and have no phosphatase activity.

Final identification of *mycoplasmas* is usually achieved by growth inhibition (GI) and or immunofluorescence (IF) tests which are carried out on agar with specific antiserum. The tests are relatively specific; they can identify the two subspecies of *M.m.mycoides* but can not distinguish between SC and LC.

Recently a nitro-blue tetrazolium (NBT) reduction technique has been described for the detection of substrate metabolism by washed cell suspensions and may be suitable for use in routine laboratories to differentiate *Mmm* LC, SC and *Mm* subsp. *Capri* strains (Miles, 1938).

Bashiruddin et al., (1999) and Bashiruddin and Windsor., (1998) have developed a medium in which *Mmm* colonies were colored red due to tetrazolium reduction (Reigate, UK). Using clinical materials and isolated *Mycoplasmas* to inoculate plates, *M.m.mycoides* LC and *Capri* colonies were dark red after 3 days whereas *Mmm* SC colonies much lighter colored and only became dark red after approximately 7 days. This medium may be useful in the primary isolation of *Mmm* SC from clinical material enabling immediate identification of colonies for subsequent testing by standard and molecular methods (Bashiruddin et al., 1999c; Windsor, 1977).
The results of substrate oxidation studies have also led to the development of rapid tests for the utilization of maltose and glycerol by members of the *Mycoplasma mycoides* cluster. The inability to use maltose differentiates *Mmm*SC strains (32/32 strains negative) from other *Mycoplasma mycoides* strains (32/35 strains +ve). A test was developed based on hydrolysis of a colorless chromogenic alpha-glucoside (Maltase) substrate p-nitrophenyl-a-D-glucopyranoside (p-NBG) to give a brightly colored yellow product (p-nitro phenol). It can be carried out using cells washed and resuspended in buffer or by simply adding p-NBG (100 Mm) to colonies on agar plates; colonies of maltose-utilizing strains become colored in about 40 min (Rice *et al.*, 2000).

Glycerol utilizing ability leads to the production of hydrogen peroxide which may be detected in colorimetric assays (Houshaymi *et al.*, 2000). These tests confirmed that European *M.m.mycoides* SC strains did not oxidize glycerol (i.e. there was no hydrogen peroxide produced) and the high rates of hydrogen peroxide production were observed for non European *Mmm* SC strains, subsp. *Mycoides* LC and subsp. *Capri* strains. In qualitative assay using colonies grown on serum agar (72/96h), plates were flooded with a reagent prepared by mixing glycerol, 3’3’ di-aminobenzidine and peroxidase. Plates were then incubated at 37°C and observed for red coloration of colonies. The time for development of color was 45-135 minutes for European *Mmm* SC strain but 1-20 minutes for other strains of *Mmm* (Houshaymi *et al.*, 2000). Requirement of serum and sensitivity to digitonin reversion both are essential for identification.
1.7.5. Serological Diagnosis:

1.7.5.1. Slide Agglutination Test:

The slide agglutination test using serum (Priestly, 1951) or blood (Newing and Field, 1953) is sensitive in early stages of the disease and suitable for establishing a preliminary diagnosis when large numbers of cattle are involved and for selecting these cattle to be bled for CFT (Turner and Etheridge, 1963). This test is less suitable for detecting chronic cases (Parker, 1960 and Alder and Etheridge, 1964), so it is recommended to be used as a herd test rather in individual animal.

1.7.5.2. Tube Agglutination Test:

The tube agglutination test using a formalin-killed suspension of the organism was found unsatisfactory for diagnosis of some of the severe cases and all the carrier animals (Campbell, 1938).

1.7.5.3. Precipitation Test:

The precipitation test whether in tubes (Turner, 1962a) or agar plates (White, 1958) has proved to be of great value in the examination of suspected sequestra and other lesions (lungs and pleural fluids). This test may be used to detect both antibody in sera and antigen in blood, pleural exudates, body tissue and urine (Gourlay, 1964, 1965). The same author found this test as accurate as the CFT in detecting clinical signs but less accurate in detecting carriers.
1.7.5.4. Complement Fixation Test (CFT):

The most reliable test for detecting infected animals particularly those with sequestra is CFT (Campbel and Turner, 1953; Lindley, 1960; Huddart, 1963 and Pearson and Mc Pherson, 1966). The advantage of the CFT is the low number of false positive and negative reactions (Hudson, 1971). The CFT is the approved OIE test although specific lacks of sensitivity. With a positive result being any reaction at 1/10 or higher, the CFT is also not robust. In addition it requires highly trained staff to perform it accurately and consistently (Regalla, 1995). In a thorough examination of the CFT in which over 33,000 sera from healthy herds were tested during 1991 to 1994 in Italy, Bellini et al., (1998) had reported that the CFT was 98% specific, while its sensitivity based on nearly 600 cattle with specific lesions from 11 infected herds was only 64%. Isolation of the causative mycoplasma from affected animals was even more insensitive, 54%. It follows that by using the CFT as a screening test, some CBPP affected cattle, in the early or later stages of infection are likely to be missed.

1.7.5.5. Enzyme Linked Immuno Sorbant Assay (ELISA):

Recently the enzyme linked immuno sorbant assay (ELISA) was use to detect antibodies against MmmSC (Onoviran and Taylor Robinson, 1979). The ELISA was found to be more sensitive than the CFT, Slide agglutination serum test and agar gel test. It could detect Mycoplasma mycoides antibodies in sera of cattle at least 19 months of infection and 25 months after vaccination. Indirect ELISA has been described for CBPP for nearly 20 years using crude antigens (Onoviran and Taylor-Robinson, 1979; Le Goff, 1986; Poumarat et al., 1989; Nicholas et al., 1996). All appear to be sensitive but may lack sensitivity.
A blocking ELISA with monoclonal antibody which although raised against \textit{MmmSC} nonetheless recognized all members of the cluster was compared with the CFT for detecting antibodies in cattle during the Italian CBPP eradication campaign (Mia \textit{et al}., 1993). There was 96% agreement between the tests over 300 positive and negative sera. Nevertheless a small number of sera from apparently healthy herds was positive in CFT but gave negative results in the ELISA and in both pathological and cultural examinations.

(Brocchi \textit{et al}., 1993) reported monoclonal antibodies which recognized a 70 kDa protein from the PG1 strain of \textit{MmmSC} but did not react with the type strains of other members of the \textit{M.mycoides} cluster. Competitive ELISA using the monoclonal antibodies were more sensitive than the CFT for detecting antibodies in affected herds and were generally more specific when used in negative herds.

A c-ELISA has been developed (Le Goff and Thiacourt, 1998) and validation tests have been performed in Africa (Thiacourt \textit{et al}., 1999). This test uses a mouse monoclonal antibody IgG1 that recognizes an epitope localized on protein with an apparent molecular weight of 80 kDa. Availability of the test seems to be a problem. While this test has considerable advantages in term of ease of testing and standardization of results, the c-ELISA has sensitivity levels similar to the CFT. Clearly more sensitive techniques such as ELISA using better antigens enabling mass screening are necessary blocked by highly specific confirmatory tests such as immuno-blotting tests.

A Tween 20 treated \textit{MmmSC} cell fraction shows increased specificity in the indirect ELISA compared to conventional whole cell antigens whilst maintaining sensitivity (Ayling \textit{et al}., 1999b). Tween 20 ELISA was
compared to the CFT in an examination of 170 sera from Portuguese herds affected by CBPP; there was 88% agreement between the ELISA and IBT; 81% agreement between the CFT and ELISA and 79% agreement between the CFT and the Immunoblotting test (IBT). Furthermore in a study with 88 cattle with CBPP lesions, the ELISA detected antibodies in 92%, the IBT 91% and the CFT only 82% (Ayling et al., 1999).

1.7.5.6. **Immunoblotting test (IBT):**

Immunoblotting tests (IBT) was described by (Goncalves et al., 1998) in which the simultaneous presence of five antigens (110, 98, 95, 62/60 and 48) was highly characteristic of sera from infected cattle. These tests were compared to the CFT in an examination of sera from Portuguese herds affected by CBPP (170 cattle). There was 79% agreement between CFT and IBT. In a study of 88 cattle with CBPP lesions IBT detected 80 and CFT detected 72 (Ayling et al., 1999b).

(Abdo et al., 1998; 2000) identified a 48 kDa protein named LppQ which was found in the type strain and European, African and Australian field strains. They used the protein in immunoblotting test for the sero detection of *MmmSC* in experimentally infected cattle.

1.7.6. **Antigen detection systems:**

1.7.6.1. **Monoclonal Antibody-Based Sandwich ELISA:**

Rodriguez et al., (1996) reported a monoclonal antibody-based sandwich ELISA that could detect as little as 10 over 5 cfu/ml of both *Mmm* biotypes within two hours. Sensitivity could be improved significantly by incubating samples for 48 hours. This test does not distinguish between *M.mycoides* SC
and LC biotypes but coupled with pathological and serological information from affected animals the test could prove useful.

1.7.6.2. **Immuno Cyto-Chemistry (ICC):**

Immunocytochemistry (ICC) is increasingly used to confirm the diagnosis of CBPP particularly where the causative organism *MmmSC* is not recoverable (often following long transport distances) where animal has died of acute disease or where serology is not possible or unclear (Ferronha *et al*., 1990; Scanziani *et al*., 1997). However the sensitivity of ICC using polyclonal serum can be low and non specific results frequently occur (Bashiruddin *et al*., 1999).

1.7.7. **Molecular diagnosis:**

1.7.7.1. **Polymerase chain reaction (PCR):**

PCR is a rapid and sensitive diagnostic method. It allows detection of *MmmSC* directly in samples of lungs, bronchial lymph nodes, nasal swabs, pleural fluid and blood. Pre-incubation for 24hrs of clinical specimens in growth medium may increase test sensitivity (De Santis *et al*., 1997). If used for the identification of new isolates it reduces drastically the time required (24-48 hrs versus 2-3 weeks). The assay has been used as a diagnostic tool to detect *MmmSC* in the course of CBPP outbreaks that have affected Europe recently (Bashiruddin *et al*., 1994, 1999). However the traditional methods for isolation and identification of the causative agent of CBPP are time consuming leading to significant delay. To overcome this problem, both single and nested PCR systems have been developed for identification of *MmmSC* (Taylor *et al*., 1992; Bashiruddin *et al*., 1994, b, 1999a, b; Dedieu *et al*., 1994; Hotzel *et al*., 1996; Miserez *et al*., 1997).
Primers based on the sequence of the so-called CAP gene of *M. Capri* amplify a 0.5 kb region of *MmmSC, MmmLC* and *M. capri* but do not amplify DNA from other members of the cluster. Identification is carried out by restriction fragment analysis of PCR product with ASNI (Bashiruddin *et al.*, 1994b).

Nested (two step) PCR has improved the sensitivity of detection. Both PCR reactions were highly specific to *MmmSC*. Two nested pairs of Oligonucleotide primers which were designed by evaluation of the most heterologous segments between the P72 gene of *MmmSC* and the closely related lipoprotein P67 gene of *Mycoplasma subsp. bovine group 7* (PG50) were used (Miserez *et al.*, 1997).

Two PCR detection systems based on the 16S r-RNA genes have also been developed for identification of *MmmSC* with high sensitivity and specificity (Johansson *et al.*, 1998; Persson *et al.*, 1999). Both systems amplify the genes from all members of the *M. mycoides* cluster. In one of the systems, the final identification is performed by restriction enzyme analysis with *AluI*. The other system (PCR-Laser Induced Fluorescence, PCR-LIF) is based on a sequence length difference between the two 16S rRNA genes of *MmmSC* and the final identification is performed by fragment analysis of fluorescent labeled amplicons with a DNA sequencer. A comparatively large number of samples can also be handled by PCR-LIF (Persson *et al.*, 1999).

Colorimetric PCR has overcome the problem: specific ds-DNA produced by PCR is captured and detected enzymatically in a 96 well microtitre plate (Bashiruddin *et al.*, 1999a). The results of colorimetric detection of PCR products correlate well with those obtained by gel electrophoresis, which reduces the analysis time by 50 % and allows the use of standard equipment available for ELISA serology.
1.8. **Disease Control and Eradication:**

The most effective method of eradication of such a chronic insidious disease by testing individual animals, slaughter of all reactors encountered with compensation of the owners (stamping out), vaccination of the non reactors by reliable immunizing product and practice of stringent monitoring methods. This can only be carried out at a very large initial expense (Abdulla, 1969).

1.8.1. **Stamping out:**

Clean countries prohibit importation of living animals from countries where the disease is endemic but if the disease is introduced in the country, eradication is based on stamping out policy (Hudson, 1971). This includes: Slaughtering of all clinical cases and contact cattle, restriction of cattle movement in and around infected area and payment of compensation for cattle owners. Stamping out policy is the method of choice where the infection is detected early, where the extension of outbreak is limited and where the staff and finance permit its vigorous execution. But in cases where infection has progressed till it involved large numbers of cattle stamping out is not suitable.

1.8.2. **Testing and Elimination of Reactors:**

This method depends on quarantine of the infected herd, the slaughtering of clinical cases, bleeding for CFT and simultaneous vaccination of all contact animals. As soon as CFT results are available, reactors are also slaughtered. The cattle in the surroundings should also be vaccinated. CFT should be repeated monthly accompanied by slaughtering of reactors till the herd was clear (Hudson, 1971).
1.8.3. Vaccination:

In countries where the disease is enzootic such as most of the African countries including the Sudan in which the mode of animal husbandry is nomadic and restriction of movement is difficult; Segregation, Quarantine of the infected herd, Testing and Removal of positive animals and Annual vaccination are the routine methods for the disease control (Karib, 1958, Hudson, 1971 and Abdulla, 1975). The disease is very difficult to eradicate under African conditions due to free movement of nomadic tribes.

A joint campaign (JP28) against this disease was recommended by Directors of Veterinary Services in African countries at their meeting in Khartoum in 1973 (Abdulla, 1975). It was suggested to vaccinate cattle in East, West and Central African countries using T-1 strain of *M. mycoides* for three consecutive years or more.

1.8.4. Chemotherapy:

Although at the moment chemotherapy can not be used to eradicate CBPP, a number of drugs are available which can be used to influence the course of the disease in the individual animal. The first drug considered to be useful in specific treatment was Neosalvarsan; this was introduced early in the thirties by French workers in West Africa where it was reported to reduce losses in acute outbreaks of the disease. Turner (1961) investigated the invitro activity of some 40 preparations including antibiotics against *M. mycoides* and summarized earlier work on the subject. He found the organism relatively susceptible to a number of substances including Chloramphenicol, Streptomycin, Oxytetracycline, some Redox agents, some mercurials and Arsenicals, Nitrofurazone and Mepacrine. He confirmed its relative insusceptibility to Penicillin and found a number of other
compounds including some Sulfonamides and Sulfones that might be added to the selective media to suppress the growth of contaminating bacteria.

Hudson and Etheridge, (1965); Reported on the activity of the antibiotic Tylosin.

The expert panel (FAO, 1967); unanimously opposed the chemotherapeutic treatment of actual cases of CBPP and strongly recommended “that mass drug or antibiotic treatment of CBPP should be discouraged because it increases the number of carriers. Chemotherapy to control vaccination reactions is not objectionable.”

In spite of official condemnation, antibiotics are used so advice is necessary on which are the most effective. Ayling et al. (2000a) carried out an in vitro trial of the effects of five commonly used antibiotics on a number of strains of *MmmSC*, and concluded that tilmicosin and danaflaxacin were effective both in terms of mycoplasmastatic and mycoplasmacidal activity; florfenicol and a tetracycline provide intermediate effectiveness while spectinomycin was ineffective against some strains. The use of fluoroquinalones, such as danaflaxacin, is causing concern amongst regulatory authorities that feel these drugs should be restricted to human use because of rapid increases in microbial resistance.
1.9. **The disease in the Sudan:**

CBPP has been enzootic in the Sudan since the beginning of this century (Anon, 1912). It is considered one of the most serious diseases of cattle in the Sudan, leading to economical losses in forms of debilitation and death of sick animals and adversely affected foreign trade (Abdulla, 1975; Shallali, Elmahi, Yazeed and Abdalla, 1998).

In the Sudan the disease was first observed in 1875 in Darfur Province and later spread to Khartoum Province where it caused great losses among cattle (Anon, 1925). The disease disappeared during the Mahdi wars in 1889. In 1912 the disease reappeared again in Kordofan Province. The source of infection was from infected trade animals brought from West Africa (Anon, 1912). From there the disease spread quickly southwards and eastwards of the province. In 1913 the disease was reported in Nuba Mountains, the White Nile, Blue Nile, Upper Nile and Bahr El Gazal provinces. In 1914 the disease reached Khartoum Province and then spread to Kassala Province in 1917 and Barber Province in 1923 (Anon, 1925).

Presently, the disease is enzootic in the Western, Southern and Central Provinces of the country. Rare or almost no outbreaks were reported from Eastern and Northern Provinces and this may be due to the sedentary animals movement and limited animal numbers in these provinces (Anon, 1969).
CHAPTER TWO
GENERAL MATERIALS AND METHODS

2.1. Study Area:

This study was carried out in the Southern Sudan, in Unity State. Southern Sudan has an area of approximately 650,000 square km, bordered by Central African Republic, Chad, Democratic Republic of Congo, Uganda, Kenya, and Ethiopia. It is divided into several ecological zones; rainforest, savannah forest, flood plains, swamp and semi desert.

Unity State or El Wihda was renamed during the early 1990s in a new administrative division of Sudan. Unity state was divided into four localities; Rubkona, Meom, Pariang and Ler localities. Unity state is surrounded by Southern Kordofan, Western Kordofan, Jonglei, Warab, Northern Bahr Algazal, Lakes and Upper Nile States.

The State former name was Western Upper Nile State. Western Upper Nile State is located in the center of Southern Sudan. Most of the Unity State lands were described as low swampy lands covered with remaining water from the rainy season during most of the year. It was known as a Sudd land as well. The region is about 320 km (about 200 mi) long and about 360 km (about 220 mi) wide. It is fed by major tributaries of the north flowing White Nile, including the Bahr al ‘Arab’. Just north of Bor, the White Nile overflows onto a broad clay plain. Because the gradient of the land is so low, the rivers waters spread to form many swamps and lagoons. Papyrus and floating water plants, known in Arabic As-Sudd, grow densely in the region. The river is no longer navigable in As Sudd, as half of its water evaporates in the slow-moving waterways. The Dinka and Nuer peoples live in the grassy plains between the watercourses. Since about the 10th century, these
pastoral people have raised herds of cattle, sheep, and goats for their livelihoods. The ethnic groups of the State are mainly Nuer, Baggara and Dinka. The Nuer are divided into twelve sub-tribes or clans; Jikany, Leek, Bul, Jagei, Dok, Nyuong, Dor, Dinka (Pariang), Ghol, Lak, Thiang and Gawier. Each sub-tribe is originated and based in part of the Greater Upper Nile Area. In Unity State the Western Nuer sub tribes are the dominant. It is more often to find the Jikany, Leek, Bul, Jagei, Dinka (Pariang) and Dok sub tribes in the study area. There is also a part of the Dinka tribe in Pariang locality but considered as a part of the Nuer tribe. Some of the Western Sudan Arab or Misserya nomadic tribes (Baggara) are seasonally occupying the Northern parts of the state for the water and grazing during the dry season up to the beginning of the subsequent rainy season then they drive back to Western and Southern Kordofan states. (Figure. No.1)
Figure. No.1. Major Ethnic Groups and Cattle Movement Paths in Unity State.
2.2. **Clinical Data:**

Clinical data obtained from the reports of WN-POC\CD veterinary clinics. The clinical data was entered and analyzed by using the Statistical Package for Social Studies (SPSS) software. Descriptive data, Cross tabulation and Graphs were acquired through the SPSS software as well.

2.3. **Samples for Bacteriological and Serological Examinations:**

Tissue and serum samples were collected from different herds and locations in Unity State.

Tissue samples: Eight lung tissue samples were collected from Rubkona locality (Rubkona and Bentiue).

Serum samples: Total of 600 serum samples were collected from the four localities of Unity State (Rubkona, Meom, Pariang and Ler localities).

All serum samples were collected from cattle throughout different seasons of the years 2003 and 2004.

The lung tissues samples were cultured to isolate the *Mmm* organism, while the serum samples were examined serologically for the presence of the antibody of *Mmm*SC by both CFT (443) and C-ELISA (320) tests in the Central Veterinary Research Laboratory-Soba (CVRL).

2.3.1. **Tissue samples:**

The eight lung tissue samples were collected from suspected lungs with sequestration during post mortem examination, small portion (piece) of lung was taken by sterile forceps, placed in sterile plastic bag, labeled and then stored frozen at -20° C before shipping via air in ice containers to the Central Veterinary Research Laboratory (CVRL-Soba).
2.3.2. Serum samples:

The six hundred bovine sera were collected during this study according to the following procedure:-

Ten ml of blood were collected aseptically from the jugular vein of life animals by vein-puncture using sterile needles then drown into sterile plain vacutainers. The blood was left to stand at ambient temperature until the clot formed then the clot was removed with sterile rods, the sera were left at room temperature overnight were transferred to sterile Eppindroff tubes, the samples were labeled with animal number, date, location, sex, and age and vaccination status. Sterile syringes were also used to collect part of the samples. The serum containers were placed in a refrigerator at - 20 C° then shipped later in ice boxes to (CVRL).

2.3.2.1. Serum sample data:

2.3.2.1.1. Location of Samples:

The tested sera were collected from different locations in Unity State according to the accessibility and security of the cattle herds as follow:- Rubkona: 36.6%, Thoan: 24.6%, Kilo-15, Kilo-30 and Mala: 25.1%, Gamos and Mirmir: 5.4%, Munga and Abyamnam: 8.3%. (Table No. 1.).

2.3.2.1.2. Sex of Sampled Animals:

91% (403) of the samples were collected from the females, while 9% (40) were collected from the males.
Table No. 1. The Frequencies and Percentages of the Collected Serum Samples from different Locations in Unity State.

<table>
<thead>
<tr>
<th>Location</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubkona</td>
<td>162</td>
<td>36.6%</td>
</tr>
<tr>
<td>Thoan</td>
<td>109</td>
<td>24.6%</td>
</tr>
<tr>
<td>Mala</td>
<td>111</td>
<td>25.1%</td>
</tr>
<tr>
<td>Mirmir and Gamos</td>
<td>24</td>
<td>5.4%</td>
</tr>
<tr>
<td>Munga and Abymnam</td>
<td>37</td>
<td>8.3%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>443</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>
2.3.2.1.3. Breeds of Sampled Cattle:

91.9% (407) of the samples were collected from the Nilotic cattle breed while 8.1% (36) from the Baggara cattle breed.

2.3.2.1.4. Season of Sample collection:

30.5% (135) of the samples collected during the dry season (December, January, February, March, April) while 69.5% (308) collected during the rainy season (May, June, July, August, September, October, November).

2.3.2.1.5. Vaccination Status of Samples:

87.8% (389) of the collected samples never been vaccinated before while 12.2% (54) practiced vaccination at least once before.
2.4. Culture media:

Both liquid and solid Medium used for culturing the samples.

2.4.1. Liquid media:

2.4.1.1. Gourlay’s medium (Gourlay, 1964):

The medium consists of the following ingredients:-

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto-tryptose (DIFCO)</td>
<td>2.00 gm</td>
</tr>
<tr>
<td>Dextrose</td>
<td>0.50 gm</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>0.50 gm</td>
</tr>
<tr>
<td>Di-sodium hydrogen phosphate (Anhydrous)</td>
<td>0.25 gm</td>
</tr>
<tr>
<td>Glycerol</td>
<td>5.00 gm</td>
</tr>
<tr>
<td>Yeast Extract (DIFCO)</td>
<td>0.10 gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

All these ingredients are dissolved by steaming. After cooling, 10 ml of inactivated horse serum, 100 I.U of penicillin and 1.0 ml thallium acetate (10% w/v) were added to each 100 ml of the broth medium. The pH was adjusted to 7.6 and the medium was sterilized by Seitz filter (ESK, 14D) under negative pressure (Gourlay, 1964).

2.4.1.2. Brain Heart Infusion Broth (Difco):

The medium consists of the following ingredients:

Heart infusion broth (25g), yeast extract (5g), D.N.A (5g) and 1000 distilled water. The pH was adjusted to 7.6 after dissolving the mentioned ingredients. After that fresh yeast extract (100ml), horse serum (200ml), penicillin (200.000 IU) and 10% Thallium acetate solution (1ml) were added. The medium was prepared according to the manufacturer instruction.
2.4.1.3. **Brucella Broth (Difco):**

This media consisted of Brucella broth (Difco) (28g), yeast extract (1g) and 1ml glycerol. The pH was adjusted to 7.6, and then 200 ml horse serum, penicillin (100.000 I.U) and 0.3 ml thallium acetate were added. The medium was prepared according to the manufacturer instruction.

2.4.2. **Solid media:**

2.4.2.1. **Gourlay’s Agar Medium:**

Agar (1.5% w/v) was added to Gourlay’s broth medium mentioned in (2.4.1.1.).

2.4.2.2. **Brain Heart Infusion Agar:**

Agar was added to the brain heart infusion broth mentioned in (2.4.1.2.).

2.4.2.3. **Brucella Agar Medium (Oxoid):**

It consisted of Brucella Agar (Oxoid) (43g), yeast extract (1g), glycerol (10 ml) and 1000 ml distilled water. Then the pH was adjusted to 7.6 and sterilized by autoclaving (15 lbs/square inch for 15 minutes) and then 100 ml horse serum, 20 ml fresh yeast extract, 0.33 ml penicillin and 0.25 ml thallium acetate10% were added.

2.4.3. **Basic Cultivation Technique for Mycoplasma:**

Cultivation was done by inoculation of the specimen either directly into liquid media and subsequent transfer to agar media, or by culturing of the specimen directly into agar media.
2.5. **Buffers:**

There were different types of buffers used in both CFT and C-ELISA tests.

**2.5.1. Veronal Buffer:**

This buffer was used to dilute the reagents used in the complement fixation test. It consists of the following ingredients:

- Sodium chloride: 83.8 gm
- Sodium bicarbonate: 2.52 gm
- Sodium 5-5 diethylebarbiturate: 3.0 gm
- 5-5 Di-Ethyl Barbituric acid: 4.6 gm
- Magnesium chloride: 1.0 gm
- Calcium chloride: 0.2 gm
- Distilled water: 1000 ml
- PH: 7.4

**2.5.2. CFT-diluent tablets (Oxoid):**

One tablet dissolved in 100 ml worm distilled water and the pH was adjusted to 7.4. It is used for dilution of different reagents in CFT.

**2.5.3. Coating Buffers:**

This was used for the dilution of *Mycoplasma* antigen and it consists of the following ingredients:

- Sodium hydrogen carbonate: 2.93 gm
- Sodium carbonate: 1.59 gm
- Sodium aside: 0.20 gm
- Distilled water: 1000 ml

The pH was adjusted to (9.6)
2.5.4. Phosphate Buffer Saline (PBS):

It consists of the following ingredients:

- Di-sodium hydrogen orthophosphate: 1.4 gm
- Sodium Chloride: 7.0 gm
- Potassium chloride: 0.2 gm
- Potassium hydrogen phosphate: 0.2 gm
- Distilled water: 1000 gm

The pH was adjusted to 7.4

2.5.5. Diluting Buffer:

This buffer was used to dilute the sera samples and the conjugate. It consists of the following ingredients:

- Phosphate buffer saline (PBS): 1.0 Liter
- Tween 20: 0.5 ml

The pH was adjusted to 7.4 and stored at 4°C. This buffer should be used within three weeks from the date of preparation.

2.5.6. Substrate Buffer:

It consists of the following ingredients:

- Citric acid tri sodium dehydrate: 17.34 gm
- Citric acid: 7.84 gm
- Distilled water: 1.0 Liter

The pH was adjusted to 4.5-5.5 and it was used to dilute the ODP.

2.5.7. Washing Buffer:

This was used to wash the ELISA plates. It was kept at room temperature and used within one week. It consists of the following ingredients:
Sodium chloride: 45.0 gm  
Tween 20: 2.5 gm  
Distilled water: 1000 Liter

2.6.  Solutions:

2.6.1. Alsever’s Solution:

This solution was used for the preservation of sheep red blood cells. It consisted of:

Sodium citrate: 12.0 gm  
Sodium chloride: 4.2 gm  
D-glucose anhydrous: 20.5 gm  
Distilled water: 1.0 Liter

2.7.  Reagents:

2.7.1. Antigens:

One type of antigens was prepared and been used for the CF test.

2.7.1.1. Whole Mycoplasma Cell Antigen:

This was prepared by inoculating two liters of serum broth medium with 100 ml of a 48 hours culture of *M. mycoides* and incubated for 14 days aerobically at 37°C. After checking for purity then culture was centrifuged at 12000 rpm. The sediment was then washed three times by suspending in phosphate buffer saline (PBS) and centrifuged at 3000 rpm. The antigen produced was finally suspended in PBS at 100 concentrations (Al-Aubaidi and Fabricant, 1971) and distributed in small aliquots of approximately 1 ml and stored at 4°C.
2.7.2. **Test sera:**

Blood samples (10 ml each) were drowning aseptically from the jugular vein. Serum was separated by stand overnight in Refrigerator (4°C) and kept at -20°C till used.

2.7.3. **Preparation of Blood Cell:**

Fifty ml (50 ml) of sheep blood were collected aseptically in bottle containing 50 ml of Alsever’s solution and kept in the refrigerator overnight. 10 ml of defibrinated blood suspension were centrifuged at 3000 rpm. The RBCs given were washed three times in the buffer before being suspended in Veronal buffer in a concentration of 2% RBCs.

2.8. **Biochemical Tests:**

2.8.1. **Digitonin Sensitivity Test:**

The sensitivity to digitonin indicates the requirement for sterol; members of the genus *Mycoplasma* are digitonin sensitive, and this test is performed after the initial isolation of suspicious *mycoplasmas*.

2.8.2. **Growth Inhibition Test:**

This test carried out on agar media with specific antiserum. The test is relatively specific; it can identify the two subspecies of *M. mycoides* but cannot distinguish between SC and LC.

2.9. **Serological tests:**

Complement Fixation Test (CFT) and Competitive Enzyme Linked Immuno Sorbant Assay (c-ELISA) were prepared and performed on serum samples.
2.9.1. Lindley Plate Complement Fixation Test (CFT):

Lindley Plate Complement Fixation Test (Lindley, 1960)

The preparation of the hemolytic system, titration of the antigen and complement and the dilution of the tested sera were carried out according to Lindley (1960). The hemolytic system was prepared by the addition of 2% sheep red blood cells suspension with equal volume of the titrated hemolysin dilution and placed at 37° C for half an hour. 1/10 dilution of the tested sera were done and placed in 56° C water-bath for ½ an hour for inactivation of the complement. Complement, antigen dilutions and the sequence of the test were carried out according to Lindley (1960). The plates were either read immediately or left in the refrigerator overnight.

The essential difference between this test and Campbell and Turner test was that; instead of using series dilutions of serum against a fixed amount of complement, serial dilutions of complement were used with constant One-In-Ten (1/10) dilution of inactivated serum. Results were expressed not as serum titer but as the amount of complement fixed.

The end point was the dilution of complement giving 50% hemolysis and to facilitate reading hemolytic standards were prepared to indicate 0, 25, 50, 75 and 100% hemolysis respectively. The test was conveniently made by using Micro Plates (instead of Perspex blocks 7 x 5 ¾ x 5/8 in deep, drilled to have 80% cups, 8 per row and 10 per column).

This method has proved satisfactory when the number of sera to be tested does not exceed 100. When larger numbers were involved the Haddart technique is more suitable. The reagents can be prepared by the techniques already given or it may be more convenient to purchase them. All dilutions were made with the buffer diluent used in the Campbell and Turner test were prepared either from the formula given or from tablets. Among the various
commercial sources of complement (C’), freeze-dried negative serum, haemolytic amboceptor and preserved sheep red cells were obtained from Burroughs Wellcome and Co. The buffer tablets diluents obtained from Oxoid Ltd.

2.9.1.1. Materials:-

2.9.1.1.1. Titration of the Complement:

A preliminary titration of the C’ is necessary only when a new batch was being introduced. The minimum haemolytic dose (M.H.D.) was defined as the smallest amount that will cause 50% percent suspension of sheep red cells sensitized with the optimal amount of haemolysin. C’ with a titer of less than 1/100 was not used for the test.

The range and intervals of the C’ serial dilution were important. It has been found that a series with 0.1761 logarithmic intervals (i.e. one made by carrying over 2 milliliters to 1 milliliter of the diluent) was suitable. The final (eighth) tube contained only buffer. There were therefore only seven tubes in the series 1/10, 1/15, 1/22, 1/33, 1/50, 1/75 and 1/116. In doubling dilution (with 0.3010 logarithmic intervals) the steps were too great; such a series did not differentiate sufficiently between sera.

2.9.1.1.2. Sheep Red Blood Cell Suspension:

Cells were collected in modified Alsever’s solution as described under Campbell and Turner test. They were washed as described under the Campbell and turner test; but a final suspension of 2% percent instead of 6 percent was prepared.
2.9.1.1.3. Haemolytic Amboceptor:

This is usually obtained commercially. With each new batch a titration (1/50 to 1/3200) against the C’ dilutions used in the test have been made to determine the minimum haemolytic dose which caused 50% percent haemolysis in the presence of 4 M.H.D. of C’. In the test three times this dose was used.

2.9.1.1.4. Sensitization of Red Blood Cells:

One volume of washed red cells (2% suspension) and one volume of haemolytic amboceptor containing 3 M.H.D. of haemolysin were mixed together rapidly and then placed in the incubator at 37 C° for 30 minutes after which they were stored in the refrigerator until required.

2.9.1.1.5. Negative Bovine Serum:

Unheated negative bovine serum was usually added to the antigen as described below since it enables the test to provide more consistent results.

2.9.1.1.6. Antigen:

The dilution of antigen used was that recommended for the Campbell and Turner test by the issuing laboratory. In making the dilution one part of negative bovine serum was added to give a final dilution of 1/160 in the diluted antigen.

2.9.1.1.7. Cleansing of Micro Plates:

The plates were not cleaned with abrasives. They were washed immediately after use in tap water and then immersed in 0.5% NaOH overnight. After rinsing in tap water they were soaked in 0.5% HCL and
when this has been rinsed of they were washed in distilled water. After drying in a dust-free atmosphere they were stored inverted in a clean cupboard or drawer.

2.9.1.1.8. Measurement of Volumes:
Chemically clean dropping-pipettes delivering 0.02 milliliters were used. They could be made of glass tubing, calibrating and standardized in the laboratory.

2.9.1.2. Method:
A known positive serum, a known negative serum, a C’ titration (a row of cups containing the C’ dilutions but no serum or antigen) and a set of haemolysis standards were included in each test. An estimate was made for the volume of reagents required to test the number of samples of serum to be examined. Each serum required 2 drops of each C’ dilution, 8 drops of antigen dilution and 32 drops of sensitized red cell suspension if a complete row of an anti-complementary control was included (as in the confirmatory test for positive sera). The 1/10 dilutions of the sera to be tested were set up and inactivated at 56 C° for 30 minutes. The 2% red cell suspension and haemolysin dilution were prepared and mixed before placing in the incubator for half an hour (30 minutes). The antigen dilution was also made and the C’ dilutions were prepared and placed in the refrigerator or on ice. All the reagents were been ready before the test started and the work was carried out quickly on every occasion. One drop of 1/10 inactivated serum was placed into each cup using one row of 8 cups for each unknown serum. In a confirmatory test for a positive
serum two rows would be necessary, the second for the anti-complementary control.
The C´ was added as follow:
Starting with 8th column, one drop of the diluent was placed in the last cup of each row, working down the column of as many plates as were necessary to complete the test. Then the test proceeded with dropping the most dilute C´ into the cups of the 7th column the next dilution into the cups of the 6th column and so on.
Next antigen was added, one drop per cup (except in the confirmatory test on positive sera when antigen omitted from the cups of the second row of each such serum; instead of antigen a drop of diluent was added to each cup in these rows).
An antigen control was included when a new batch of antigen was introduced to ensure that the antigen was free from anti-complementary activity. This consisted of a row of cups containing the antigen and the C´ dilutions but no serum dilutions were added. The serum dilution in each cup was replaced by a drop of diluent.
Finally one row for the complement controls was allowed. These cups contained the dilutions of C´ but no serum or antigen and therefore required two drops of diluentent to complete the volumes.
The plates were rotated gently to mix the contents of the cups and inserted them in a humidified container in the incubator at 37° C for 30 minutes. To each cup we added 2 drops of sensitized cells and after mixing replaced for 30 minutes in the incubator. Then Removed and read. Reading might be easier if the plates were left in the refrigerator until all the Un-haemolyzed cells have settled.
Results were recorded according to the amount of C’ fixed by the 1/10 dilutions of sera. The end point was taken as the dilution of C’ in which 50% percent haemolysis had occurred. The degree of positive was indicated by the difference between the end points in the row with antigen and the row without. Pro- and anti-complementary samples were infrequent.

2.9.2. Competitive-Enzyme-Linked Immuno-Sorbant Assay:

CBPP Serum Competition ELISA-Version P05410/01 kit which has been used was produced by CIRAD-EMVT, Montpellier, France by developing a Competition ELISA (c-ELISA) based on Monoclonal Anti-MmmSC antibody (named 117/5). This test is an alternative to the CFT for the OIE and can be used for the official CBPP controls. A competitive ELISA developed at CIRAD-EMVT, Montpellier, may have advantages in terms of ease of testing and standardization of results, but it has sensitivity levels similar to CFT and thus will enable the persistence of disease as seen with CFT (Le Goff and Thiaucourt, 1998).

2.9.2.1. Materials:

CBPP Serum Competition ELISA kit, Version P05410/01 from CIRAD-EMVT/ Institute POUQUIER, FRANCE.

2.9.2.1.1. Contents:

Monowell coated micro plates, Wash concentrate (20x), Dilution buffer (24), Controls: Strong positive control (CP++), Weak positive control (CP+) and Negative control (CN), Monoclonal antibody 117/5 (anti-MmmSC)-(Mab)-(Freeze-dried), Monoclonal anti-mouse IgG peroxidase
conjugate, Revelation solution (3)-(TMB) ready to use and Stop solution (H2S04 0.5M solution).

2.9.2.1.2. **Equipment:**

ELISA reader, Centrifuge, Centrifuge tubes and micro tubes, Vortex or similar, Micro plate washing system that distribute 300 microleter per well, Tray (Boat shape), Precision Micropipettes and Multi-dispensing micropipettes, Disposable pipette tips, Distilled water, Pre-plates for pre-dilutions, Micro plate covers (lid, aluminum foil or adhesive), Incubator at +37° C (± 3° C), Plate Agitator and Printer.

2.9.2.2. **Method:**

The wells of the polystyrene microtiter plates were coated with an *MmmSC* lysate. Serum samples to be tested were diluted and incubated with the specific monoclonal antibody (117/5) in a pre-plate. This mixture was then transferred into the *MmmSC* coated micro plate. Any antibody specific to *MmmSC* in the serum will form an *MmmSC* / bovine antibody immune complex, which effectively masks the *MmmSC* sites. In this case the monoclonal antibody cannot bind to the corresponding epitope. After washing, an anti-mouse-IgG antibody coupled to peroxidase was incubated in the wells. In the presence of specific *MmmSC* antibodies in the serum that is being analyzed, the monoclonal antibody (117/5) is not fixed in the plate and the conjugate cannot bind in the wells. On the contrary, the conjugate can bind to the monoclonal antibody. After washing, the enzyme substrate (TMB) was added to the conjugate, forming a blue compound becoming yellow after blocking. The intensity of
the color is an inverse measure of the proportion of anti-\textit{Mmm}SC antibodies in the serum sample tested. The cut-off was calculated by using the results obtained from a monoclonal control (\textit{Cm}) and a conjugate control (\textit{Cc}). The positive and negative controls were delivered with the kit. They were added to each micro plate and the results were validated.

2.10. **Participatory Epidemiology:**

Participatory Epidemiology is an emerging field based on the use of participatory techniques for the harvesting of qualitative epidemiological intelligence contained within community observations, existing veterinary knowledge and traditional oral history. It relies on the widely accepted techniques of participatory rural appraisal, ethno veterinary surveys and qualitative epidemiology (Schwabe, 1984). This information can be used to design better animal health projects and delivery systems, more successful surveillance and control strategies or as new perspectives for innovative research hypotheses in ecological epidemiology.

2.10.1. **Direct Observations:**

The system of the community life in the villages and pastoral camps were observed while walking and talking to the different community members. The observational walker is definitely worth the trouble, Narayan (1996) refers to those as ‘walking surveys’.
2.10.2. **Interviews:**

Interviews with the local livestock owners, Chiefs (Salateen and Princes) and Veterinarians were conducted so as to assess and cross check the livestock situation, problems, major cattle diseases, disease outbreaks, migration routes and other useful information. The local name of the disease and the onset occasions were determined by the respondents.

2.10.3. **Mapping:**

The participants (Respondents and the Informant) were asked to sketch a map of useful resources, grazing movement, trade movements, community boundaries and origin.

2.10.4. **Seasonal Calendar:**

The informants were asked to verify their animal health problems according to the season and the climatic changes.

2.10.5. **Ranking, Scoring and proportional Piling:**

The informants were asked to construct a list of the animal health diseases for particular species in the area then the informants were asked to determine the most important diseases and rank those diseases list wise according to the importance, season, locations and prevalence. The informants were asked to score the ranked diseases in 2.10.4 above by using simple scoring and proportional piling.
2.11. Secondary Data:

The records of Animal Resources and Fisheries Director were revised so as to collect useful information about CBPP in Unity State. The director of ARF conducted vaccination campaigns during the early 1990s in Bentiu and Northern parts of the state and did not reach cattle camps towards the south due to the insecurity struggles; the records did not mention any vaccination coverage against CBPP in particular due to the vaccine quality (Lyophilized), where there was No mean to keep the vaccine frozen until use.
CHAPTER THREE
RESULTS

3.1. Clinical Data:

The analysis of the clinical data showed that there were six highly infectious diseases among cattle in Unity State: Pneumonia (Respiratory infections), FMD, H.S, Brucellosis, CBPP and B.Q. (Figure No.2.). The prevalence rates of those diseases were demonstrated in (Figure No. 3.). The percentages of the infectious cattle diseases were as follow:
Pneumonia: 41.5%, FMD: 28%, H.S: 13.8%, Brucellosis: 11.5%, CBPP: 4.8% and B.Q: 0.5%. (Figure No. 4.).

3.2. Sample Collection Year:

Twenty four point six (24.6%) out of the total numbers of samples were collected during 2003, while 75.4% of samples were collected during 2004 due to moderate improvement of security situation. (Figure No. 9.)

3.3. Isolation of the Agent:

3.3.1. Culture of the Samples:-

Eight lung tissue samples were examined by cultivation in different Mycoplasma Broth and Agar Medium (Gourlay’s, Brain heart infusion and Brucella). Two of them showed turbidity in the liquid media, later a noticeable growth and fried egg shape colonies have been clearly recognized after 7 days in the solid media.
While the two isolates were under re culturing both of them were lost due to the poor conditions in the lab at that time and the isolates were never been re-isolated successfully again from the same culture.
Figure No. 2. Bar Chart demonstrates the Highly Infectious Cattle Diseases in Unity State as obtained from the Clinical Data.
Figure No. 3. Bar Chart demonstrating the Highly Infectious Cattle Diseases in Different Seasons as obtained from the Clinical Data.
Figure No. 4. Pie Chart demonstrating the Highly Infectious Cattle Diseases Percentages as obtained from the Clinical Data.
3.4. Identification of the Agent:

3.4.1. Biochemical Tests:

3.4.1.1. Digitonin Test:

After the first isolation, the culture showed sensitivity to Digitonin and that indicated the requirement of Sterol. Genus *Mycoplasma* members are Digitonin sensitive.

3.5. Serological Tests:

3.5.1. Growth Inhibition Test:

The G.I test showed positive reactions.

3.5.2. Complement Fixation Test:

443 serum samples were tested using CFT out of the 600 been collected during the recent study.

3.5.1.1. CFT Results:

Twenty six percent (26%) of the tested samples with the complement fixation test showed positive reactions, 6.5% showed doubtful reactions and 67.5% showed negative reactions.

3.5.1.2. Sample collection year vs. CFT Result Cross tab:

Eleven point three percent (11.3%) is the positive results showed by the CFT during 2003 while the CFT positive results increased to 14.7% during 2004. (Table No. 2.). Pearson Chi-Square test showed a significant relationship, the value was (0.000).
Figure No. 5. Complement Fixation Test on Micro plate.
Table No. 2. Sample Collection Year vs. CFT Result Cross Tabulation:

Crosstab

<table>
<thead>
<tr>
<th>Sample collection year</th>
<th>CFT-Result</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Doubtful</td>
<td>Total</td>
</tr>
<tr>
<td>2003</td>
<td>50</td>
<td>50</td>
<td>9</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>11.3%</td>
<td>11.3%</td>
<td>2.0%</td>
<td>24.6%</td>
</tr>
<tr>
<td>2004</td>
<td>65</td>
<td>249</td>
<td>20</td>
<td>334</td>
</tr>
<tr>
<td></td>
<td>14.7%</td>
<td>56.2%</td>
<td>4.5%</td>
<td>75.4%</td>
</tr>
<tr>
<td>Total</td>
<td>115</td>
<td>299</td>
<td>29</td>
<td>443</td>
</tr>
<tr>
<td></td>
<td>26.0%</td>
<td>67.5%</td>
<td>6.5%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>
3.5.1.3. Location vs. CFT Result Cross tab:

Positive results in Rubkona: 9.3%, Thoan: 11.3%, K15, K30 and Mala: 3.1%, Gamos and Mirmir: 1.3% and Munga and Abyamnam: 1%

Negative results in Rubkona: 23.7%, Thoan: 11.3%, K15, K30 and Mala: 21.3%, Gamos and Mirmir: 1.3% and Munga and Abyamnam: 7.2%

Doubtful results in Rubkona: 3.6%, Thoan: 2%, K15, K30 and Mala: 0.9%, Gamos and Mirmir: 4.1% and Munga and Abyamnam: 0%

(Table No. 3.). Pearson Chi-Square test showed a significant relationship, the value was (0.000).

3.5.1.4. Sex vs. CFT Result Cross tab:

3.2% of the male samples showed positive reactions by the CFT while 22.8% of the female samples showed positive reactions by the CFT.

(Table No. 4.). Pearson Chi-Square test showed NO significant relationship, the value was (0.266).

3.5.1.5. Breed vs. CFT Result Cross tab:

In the Nilotic cattle breed 25.1% showed positive reactions by the CFT while in the Baggara cattle breed 0.9% showed positive reaction by the CFT.

(Table No. 5.). Chi-Square showed moderate significant, (0.014).

3.5.1.6. Season vs. CFT Result Cross tab:

4.7% positive reactions exhibited during the dry season which increased to 21.2% during the rainy season by the CFT (Seasonal Pattern).

(Table No. 6). Pearson Chi-Square showed a highly significant relationship, value was (0.000).
Table No. 3. Locations of Samples vs. CFT Result Cross Tabulation:

<table>
<thead>
<tr>
<th>Location of Sample</th>
<th>Rubkona</th>
<th>Thoan</th>
<th>Kilo 15, Kilo 30 &amp; Mala</th>
<th>Gamos &amp; Mirmir</th>
<th>Munga &amp; Abyamnam</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFT-Result</td>
<td>Positive</td>
<td>Negative</td>
<td>Doubtful</td>
<td>Total</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>105</td>
<td>16</td>
<td>162</td>
<td>*</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>9.3%</td>
<td>23.7%</td>
<td>3.6%</td>
<td>36.6%</td>
<td>*</td>
<td>11.3%</td>
</tr>
<tr>
<td></td>
<td>115</td>
<td>299</td>
<td>29</td>
<td>443</td>
<td>*</td>
<td>26.0%</td>
</tr>
</tbody>
</table>

* Indicates Percentages
Table No. 4. Sex of Sampled Animal vs. CFT Result Cross Tabulation.

<table>
<thead>
<tr>
<th>Sex of Animal</th>
<th>CFT-Result</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Doubtful</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14</td>
<td>25</td>
<td>1</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>*</td>
<td>3.2%</td>
<td>5.6%</td>
<td>.2%</td>
<td>9.0%</td>
<td></td>
</tr>
<tr>
<td>Femal</td>
<td>101</td>
<td>274</td>
<td>28</td>
<td>403</td>
<td></td>
</tr>
<tr>
<td>*</td>
<td>22.8%</td>
<td>61.9%</td>
<td>6.3%</td>
<td>91.0%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>115</td>
<td>299</td>
<td>29</td>
<td>443</td>
<td></td>
</tr>
<tr>
<td>*</td>
<td>26.0%</td>
<td>67.5%</td>
<td>6.5%</td>
<td>100.0%</td>
<td></td>
</tr>
</tbody>
</table>

* Indicates Percentages
Table No. 5. Types of Cattle Breed vs. CFT Result Cross Tabulation.

<table>
<thead>
<tr>
<th>Breed</th>
<th>CFT-Result</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Nilotic</td>
<td>111</td>
<td>267</td>
</tr>
<tr>
<td></td>
<td>25.1%</td>
<td>60.3%</td>
</tr>
<tr>
<td>Baggara</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>.9%</td>
<td>7.2%</td>
</tr>
<tr>
<td>Total</td>
<td>115</td>
<td>299</td>
</tr>
<tr>
<td></td>
<td>26.0%</td>
<td>67.5%</td>
</tr>
</tbody>
</table>

*Indicates Percentages.
Table No. 6. Season vs. CFT Result Cross Tabulation:

<table>
<thead>
<tr>
<th>Season of the sample collection</th>
<th>Dry season</th>
<th>Rainy season</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFT-Result</td>
<td>Positive</td>
<td>Negative</td>
<td>Doubtful</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>112</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>4.7%</td>
<td>25.3%</td>
</tr>
<tr>
<td></td>
<td>94</td>
<td>187</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>21.2%</td>
<td>42.2%</td>
</tr>
<tr>
<td>Total</td>
<td>115</td>
<td>299</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>26.0%</td>
<td>67.5%</td>
</tr>
</tbody>
</table>

* Indicates Percentages.
3.5.1.7. Vaccination vs. CFT Result Cross Tabulation:

Two point nine percent (2.9%) of the CFT positive samples appeared in the vaccinated cattle while increased to 23% CFT positive samples in the Non-vaccinated cattle. (Table No. 7.). Pearson Chi-Square test showed NO significant relationship, the value was (0.592).

3.5.2. Competitive Enzyme Linked Immuno Sorbant Assay:

3.5.2.1. c-ELISA Results:

Sixteen point six percent (16.6%) of the samples tested by the c-ELISA test showed positive reactions, 12.5% showed doubtful reactions and 70.9% showed negative reactions.

3.5.2.2. Sample collection Year vs. c-ELISA Result Cross tab:

Three point four (3.4%) is the positive results showed by the c-ELISA during 2003 while the c-ELISA positive results increased to 13.1% during 2004. (Table No. 8.). Pearson Chi-Square test showed a highly significant relationship, the value was (0.000).

3.5.2.3. Location of samples vs. c-ELISA Result Cross tab:

Positive results in Rubkona: 0.3%, Thoan: 13.1%, K15, K30 and Mala: 2.5%, Gamos and Mirmir: 0% and Munga and Abyamnam: 0.6%.
Negative results in Rubkona: 15.3%, Thoan: 18.6%, K15, K30 and Mala: 27.5%, Gamos and Mirmir: 2.1% and Munga and Abyamnam: 7.1%.
Doubtful results in Rubkona: 4.4%, Thoan: 0.6%, K15, K30 and Mala: 3.4%, Gamos and Mirmir: 1.6% and Munga and Abyamnam: 2.5%.
(Table No. 9.). Pearson Chi-Square test showed a highly significant relationship, the value was (0.000).

3.5.2.4. **Sex vs. c-ELISA Result Cross tab:**

Two point eight percent (2.8%) of the male samples showed positive reactions by the c-ELISA and 13.7% of the female samples showed positive reactions by the c-ELISA. (Table No. 10.). Pearson Chi-Square test showed NO significant relationship, the value was (0.120).

3.5.2.5. **Breed vs. c-ELISA Result Cross tab:**

In the Nilotic cattle breed 15.9% showed positive reactions by the c-ELISA while the Baggara cattle breed showed 0.6% positive reaction by the c-ELISA. (Table No. 11.). Pearson Chi-Square test showed a highly significant relationship, the value was (0.001).

3.5.2.6. **Season vs. c-ELISA Result Cross tab:**

4.1% positive reactions exhibited during the dry season which increased to 12.4% during the rainy season by the c-ELISA. (Table No. 12.). Pearson Chi-Square test showed a highly significant relationship, the value was (0.000).

3.5.2.7. **Vaccination vs. c-ELISA Result Cross tab:**

3.1% of the c-ELISA positive samples appeared in the vaccinated cattle while increased to 13.4% c-ELISA positive samples in the Non-vaccinated cattle. (Table No. 13.). Pearson Chi-Square test showed weak significant relationship, the value was (0.620)
Table No. 7. Vaccination Status vs. CFT Result Cross Tabulation.

<table>
<thead>
<tr>
<th>Vaccination</th>
<th>CFT-Result</th>
<th>Positive</th>
<th>Negative</th>
<th>Doubtful</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated</td>
<td></td>
<td>13</td>
<td>39</td>
<td>2</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>2.9%</td>
<td>8.8%</td>
<td>.5%</td>
<td>12.2%</td>
</tr>
<tr>
<td>Non-Vaccinated</td>
<td></td>
<td>102</td>
<td>260</td>
<td>27</td>
<td>389</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>23.0%</td>
<td>58.7%</td>
<td>6.1%</td>
<td>87.8%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>115</td>
<td>299</td>
<td>29</td>
<td>443</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>26.0%</td>
<td>67.5%</td>
<td>6.5%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

*Indicates Percentages.
Table No. 8. Samples Collection Year vs. c-ELISA Results Cross Tabulation.

<table>
<thead>
<tr>
<th>Sample collection year</th>
<th>ELISA-Result</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Doubtfull</td>
<td>Total</td>
</tr>
<tr>
<td>2003</td>
<td>11</td>
<td>167</td>
<td>38</td>
<td>216</td>
</tr>
<tr>
<td></td>
<td>* 3.4%</td>
<td>* 52.2%</td>
<td>* 11.9%</td>
<td>* 67.5%</td>
</tr>
<tr>
<td>2004</td>
<td>42</td>
<td>60</td>
<td>2</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>* 13.1%</td>
<td>* 18.8%</td>
<td>* .6%</td>
<td>* 32.5%</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>227</td>
<td>40</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>* 16.5%</td>
<td>* 71.0%</td>
<td>* 12.5%</td>
<td>* 100.0%</td>
</tr>
</tbody>
</table>

* Indicates Percentages.
Table No. 9. Locations of Samples vs. c-ELISA Results Cross Tabulation.

Location of Samples vs. C-ELISA Crosstabulation

<table>
<thead>
<tr>
<th>Location of Samples</th>
<th>ELISA-Result</th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Doubtfull</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Rubkona</td>
<td>1</td>
<td>49</td>
<td>14</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>.3%</td>
<td>15.3%</td>
<td>4.4%</td>
<td>20.0%</td>
</tr>
<tr>
<td>Thoan</td>
<td>42</td>
<td>60</td>
<td>2</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>13.1%</td>
<td>18.7%</td>
<td>.6%</td>
<td>32.6%</td>
</tr>
<tr>
<td>Kilo 15, Kilo 30 &amp; Mala</td>
<td>8</td>
<td>88</td>
<td>11</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>2.5%</td>
<td>27.5%</td>
<td>3.4%</td>
<td>33.4%</td>
</tr>
<tr>
<td>Gamos &amp; Mirmir</td>
<td>.00</td>
<td>7</td>
<td>5</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>0%</td>
<td>2.2%</td>
<td>1.6%</td>
<td>3.7%</td>
</tr>
<tr>
<td>Munga &amp; Abyamnam</td>
<td>2</td>
<td>23</td>
<td>8</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>.6%</td>
<td>7.2%</td>
<td>2.5%</td>
<td>10.3%</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>227</td>
<td>40</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>16.6%</td>
<td>70.9%</td>
<td>12.5%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

* Indicates Percentages.
Table No. 10. Sex vs. c-ELISA Result Cross Tabulation.

<table>
<thead>
<tr>
<th>Sex of Animal</th>
<th>Male</th>
<th>Femal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ELISA-Result</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Doubtfull</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>* 2.8%</td>
<td>6.6%</td>
<td>.6%</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>206</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>* 13.7%</td>
<td>64.4%</td>
<td>11.9%</td>
</tr>
<tr>
<td></td>
<td>53</td>
<td>227</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>* 16.5%</td>
<td>71.0%</td>
<td>12.5%</td>
</tr>
</tbody>
</table>

* Indicates Percentages.
Table No. 11. Breed vs. c-ELISA Result Cross Tabulation.

<table>
<thead>
<tr>
<th>Breed</th>
<th>ELISA-Result</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Doubtful</td>
<td>Total</td>
</tr>
<tr>
<td>Nilotic</td>
<td>51</td>
<td>203</td>
<td>32</td>
<td>286</td>
</tr>
<tr>
<td></td>
<td>15.9%</td>
<td>63.5%</td>
<td>10.0%</td>
<td>89.4%</td>
</tr>
<tr>
<td>Baggara</td>
<td>2</td>
<td>24</td>
<td>8</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>.6%</td>
<td>7.5%</td>
<td>2.5%</td>
<td>10.6%</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>227</td>
<td>40</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>16.5%</td>
<td>71.0%</td>
<td>12.5%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

* Indicates Percentages.
Table No. 12. Season of Samples collection vs. c-ELISA Result Cross Tabulation.

<table>
<thead>
<tr>
<th>Season of the sample collection</th>
<th>ELISA-Result</th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Doubtfull</td>
<td></td>
</tr>
<tr>
<td>Dry season</td>
<td></td>
<td>13</td>
<td>103</td>
<td>15</td>
<td>131</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>4.1%</td>
<td>32.2%</td>
<td>4.7%</td>
<td>41.0%</td>
</tr>
<tr>
<td>Rainy season</td>
<td></td>
<td>40</td>
<td>124</td>
<td>25</td>
<td>189</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>12.4%</td>
<td>38.8%</td>
<td>7.8%</td>
<td>59.0%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>53</td>
<td>227</td>
<td>40</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>16.5%</td>
<td>71.0%</td>
<td>12.5%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

* Indicates Percentages.
Table No. 13. Vaccination Status vs. c-ELISA Result Cross Tabulation.

Crosstab

<table>
<thead>
<tr>
<th>Vaccination</th>
<th>ELISA-Result</th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Doubtfull</td>
<td></td>
</tr>
<tr>
<td>Vaccinated</td>
<td>10</td>
<td>32</td>
<td>9</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>* 3.1%</td>
<td>10.0%</td>
<td>2.8%</td>
<td>15.9%</td>
</tr>
<tr>
<td>Non-Vaccinated</td>
<td>43</td>
<td>195</td>
<td>31</td>
<td>269</td>
</tr>
<tr>
<td></td>
<td>* 13.4%</td>
<td>61.0%</td>
<td>9.7%</td>
<td>84.1%</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>227</td>
<td>40</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>* 16.5%</td>
<td>71.0%</td>
<td>12.5%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

* Indicates Percentages.
3.5.3. CFT vs. C-ELISA Results Comparison:

3.5.3.1. Results:
The Positive reaction by the CFT was 26% while 16.6% by c-ELISA. The Negative reaction by the CFT was 67.5% while 70.9% by c-ELISA. The Doubtful reaction by the CFT was 6.5% while 12.5% by c-ELISA. (Table No. 14).

3.5.3.2. Year:
In 2003 the Positive Result by CFT was 3.4% and 13.1% by c-ELISA. while in 2004 the Positive Result by CFT was 11.3% and 14.7% by c-ELISA. (Table. No.14).

3.5.3.3. Location:
The location vs. CFT and c-ELISA Results Cross Tabulation showed a significant relationship by the CFT with value (80.710) and highly significant relationship by the c-ELISA with value (287.034) both compared with Pearson Chi-Square test.

3.5.3.4. Sex:
Sex of sampled animals vs. CFT and c-ELISA result cross tabulation checked by Pearson Chi-Square test showed a weak significant relationship.

3.5.3.5. Breed:
Breed of sampled animals vs. CFT and c-ELISA results cross tabulation revealed Moderate significant relationship with CFT and Highly significant relationship with the c-ELISA by Pearson Chi-Square test.
3.5.3.6. **Season:**

Season of sample collection vs. CFT and C-ELISA result cross tabulation checked by Pearson Chi-Square test showed highly significant relationship in both tests.

3.5.3.7. **Vaccination:**

Vaccination vs. CFT and C-ELISA results cross tabulation revealed No significant relationship with the CFT and a highly significant relationship with the c-ELISA by Pearson Chi-Square test.

3.6. **Participatory Epidemiology:**

3.6.1. **Direct Observations:**

The Nuer community used to collect the cattle dung and then burned it at night times creating smokes to prevent their cattle from the biting flies and ticks this might be indoors or outdoors. Ashes were used topically to prevent cattle from insects and ticks. In the villages the cattle were kept inside big huts called (Al Wak) at nights. Traditional cattle exchange is a common practice in the Nuer community (Marriages, Compensations etc.).

3.6.2. **Interviews:**

Doap is the local name for CBPP as referred by the different Nuer groups, which means pain in the chest. The Nuer informants mentioned a seasonal mode for CBPP especially during the rainy seasons. Nuer informants reported that some sick cattle may recover from Doap (CBPP) if an antibiotic was used. Nuer informants did not practice vaccination against epidemic diseases before but part of them reported that they had practiced
vaccination before during Pan African Rinderpest Campaign (PARC) mission in the 1990s. Vaccinated cattle had notched ears.

The Messeria informants reported that they practice vaccination annually in their origin states (Western and Southern Kordofan) against Anthrax, H.S and B.Q but not against CBPP in particular.

3.6.3. Mapping:

The Nuer groups and the Messeria groups illustrated their grazing, watering areas and the surrounding areas and their cattle movement paths.

3.6.4. Seasonal Calendars:

The majority of the Nuer informants stated that the disease Doap (CBPP) was observed after the rainfalls, part of the informants stated that disease may appear during the dry season when the cattle gathered together at the watering points.

3.6.5. Ranking, Scoring and Proportional Piling:

Nuer informants agreed on the ranking of the most common diseases in the areas as follow: FMD, H.S, Trypansomiasis, CBPP and brucellosis. Although the informants mentioned a relationship between the biting flies and the transmission of Trypansomiasis but they considered it as one of the common diseases in the area.

The Nuer groups applied proportional piling for the above ranked diseases and they mentioned the prevalence and the importance of each disease. Messeria informants did not mention CBPP as one of their major cattle diseases in their areas but they mentioned that their cattle may catch CBPP infection if get in contact with Nuer cattle. (Figure No.6.).
Table No.14. CFT Results vs. c-ELISA Results and Percentages by Locations.

<table>
<thead>
<tr>
<th>Location</th>
<th>CFT Results</th>
<th></th>
<th></th>
<th>C-ELISA Results</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Doubtful</td>
<td>Positive</td>
<td>Negative</td>
<td>Doubtful</td>
</tr>
<tr>
<td>Rubkona</td>
<td>9.3%</td>
<td>23.7%</td>
<td>3.6%</td>
<td>0.3%</td>
<td>15.3%</td>
<td>4.4%</td>
</tr>
<tr>
<td>Thoan</td>
<td>11.3%</td>
<td>11.3%</td>
<td>2.0%</td>
<td>13.1%</td>
<td>18.7%</td>
<td>0.6%</td>
</tr>
<tr>
<td>K-15, k-30 and Mala</td>
<td>3.1%</td>
<td>21.2%</td>
<td>0.9%</td>
<td>2.5%</td>
<td>27.5%</td>
<td>3.4%</td>
</tr>
<tr>
<td>Gamos and Mirmr</td>
<td>1.3%</td>
<td>4.1%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>2.2%</td>
<td>1.6%</td>
</tr>
<tr>
<td>Munga and Abyamnam</td>
<td>1.0%</td>
<td>7.2%</td>
<td>0.0%</td>
<td>0.6%</td>
<td>7.2%</td>
<td>2.5%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>26.0%</strong></td>
<td><strong>67.5%</strong></td>
<td><strong>6.5%</strong></td>
<td><strong>16.6%</strong></td>
<td><strong>70.9%</strong></td>
<td><strong>12.5%</strong></td>
</tr>
</tbody>
</table>
Figure No. 6. Proportional Piling for Ranked Bovine Diseases by Nuer.

- **Trypanosomiasis + Internal Parasites**
  - Liei

- **Foot and Mouth Disease (FMD)**
  - Dat

- **Cattle Breeder’s Pneumonia (CBPP)**
  - Doap

- **Haemorrhagic Septicaemia**
  - Yeithpin

- **Brucellosis**
  - Mullaeh
CHAPTER FOUR
DISCUSSION

The analysis of the clinical data which was obtained from WN-POC/CD vet-clinics showed that major infectious diseases in the area were: Respiratory infections, FMD, H.S, Brucellosis, CBPP and B.Q. The study covered most parts of Unity State, Southern Sudan and tested cattle from both Nuer and Messeria ethnic groups.

The Nuer tribe is the dominant tribe in the state throughout the year this result was confirmed by the study; 91.9% of the tested cattle were Nilotic cattle breed which belonged to the Nuer tribe while 8.1% Baggara cattle breed that belonged to the Messeria tribe who spent the dry season grazing and watering their cattle inside the state territories coming from Western and Southern Kordofan states. The approaching of the Messeria tribe to the state exposed their cattle to the endemic diseases in the area and spreading their diseases as well.

The majority of the samples 69.5% were collected from Nuer cattle camps during the rainy season when they assembled their cattle in the highlands between the swamps. In the study 87.2% of the sampled animals were never been vaccinated before, vaccination coverage was very low 12.2% among the tested animals; 4.1% for Nilotic cattle breed and 8.1% for Baggara cattle breed owned by the Messeria tribe.

Vaccination in general and particularly against CBPP was not practiced in Unity State due to insecurity, lack of logistic means and lack of technical support as the Animal Resources and Fisheries (ARF) director stated in the interviews.
Part of Nuer informants stated that they never had vaccinated their cattle since the beginning of 1990 during the PARC mission and that was mainly against Rinderpest; they also stated that the vaccinated cattle have notched ears. The other part of informants indicated that they had never practiced cattle vaccination before.

The Messeria tribe informants stated that they practiced vaccination in their origin states; West and Southern Kordofan against H.S, Anthrax and B.Q but not against CBPP which is not common in their origin states.

During the study two cultures out of eight (25%) showed turbidity in the liquid media, later on a noticeable growth and fried egg shape colonies have been clearly recognized after 7 days on the solid media.

After that confirmatory tests by both Digitonin and Growth Inhibition tests were applied, both showed positive reactions. That confirmed the presence of the *Mmm* SC microorganism in the area.

In this study Complement Fixation Test (CFT) results revealed that; (115) 26% out of the tested sera were positive, (29) 6.5% were doubtful and (299) 67.5% were negative via CF. Those results were similar to Mefit-Babtie (1983) who reported the results of a small CFT serosurvey including 123 Dinka, 24 Nuer and 52 Shilluk cattle and found 8%, 4% and 2% positive, respectively. Further, of 59 Dinka cattle reported to have a history of respiratory disease, 16 (27%) were positive by CFT. Out of 15 Dinka animals that had no history of respiratory, 0 (0%) were positive. Out of 59 animals reported to have had respiratory disease (*abut pio*), 21 cases occurred in the year of testing, 32 one-year previously and 6 two-years previous. Out of those 8 (38%), 7(22%) and 1(16%) were positive, respectively.
The CFT positive results illustrated in this study were increased rapidly from 3.4% during the year 2003 to 13.1% in the year 2004, those results may considered as an Early Warning System (EWS) indication of CBPP feasible outbreak due to the relative progresses in the peace situation which increased the number of cattle grazing in the study area.

In this study the CFT suggested that 25.1% out of the positive results appeared in the Nilotic cattle breed while only 0.9% appeared in Baggara cattle breed, that may be due to the none existence of direct contact between the two breeds because of Bahr Algazal River. That was also an indication that CBPP is widespread among the Nuer cattle rather than Baggara cattle.

In this study CFT exhibited 4.7% positive result during the dry season but increased to 21.2% during the rainy season suggesting a seasonal pattern for CBPP, which may be due to the stress factor caused by the assembling of large numbers of different herds in a limited flat terrain (highland) during the rainy season. The Nuer informants also mentioned that Doap (CBPP) appearance was associated with the rainy season.

Those results resembled those of Zessin et al. (1985) who noted “Because the Dinka husbandry system makes animals rotate and circulate extensively, such an endemic state with ‘disease stability’ is readily reached”. Our informants indicated that CBPP was endemic with peaks of mortality during periods of stress such as the onset of the rains and the height of the dry season. The traditional exchange of livestock would indeed appear to facilitate the establishment and maintenance of endemic stability.

2.9% of the CFT positive samples appeared in the vaccinated cattle, on the other hand 23% of the CFT positive samples occurred in Non-vaccinated cattle. The presence of the CFT Sero-Positive reactors in vaccinated samples was due to natural infection and not residual antibodies
from previous vaccination as the study suggested pear in mind that No vaccination against CBPP was particularly applied in this area.
That result can be confirmed by (Campbell and Turner, 1953) who reported neither natural nor vaccinal CFT antibodies persist except in cases of severe reactions. Antibodies induced by vaccination are not detected by CFT for more than a few weeks (Campbell and Turner, 1953).

In this study the Competitive Enzyme Linked Immuno Sorbent Assay results revealed that: (53) 16.5% of the tested sera were positive, (40) 12.5% were doubtful and (227) 71% were negative to c-ELISA.
C-ELISA positive results revealed that 2.8% were males and 13.7% were females.
The c-ELISA positive results illustrated in this study was slightly increased from 13.1% during the year 2003 to 14.7% in the year 2004 which was an indication of CBPP possible outbreak.
In this study the c-ELISA suggested that 15.9% out of the positive results appeared in the Nilotic cattle breed while only 0.6% appeared in the Bggara cattle breed, indicating that CBPP was more common among Nuer cattle.

In this study the c-ELISA exhibited 4.1% positive results during the dry season which increased to 12.4% positive during the rainy season suggesting a seasonal pattern for CBPP, which was well-matched with the seasonality of Doap (CBPP) as indicated by the Nuer informants group.
This result is consistent with Hudson’s, (1968). Who reported that the organism is very sensitive to ultraviolet light and during daylight in hot sunny weather droplets may soon be rendered harmless. Close contact at night and during dull weather may be important in the spread of infection.
In this study 3.1% of c-ELISA positive samples appeared in the vaccinated cattle while increased to 13.4% c-ELISA positive samples in the Non-vaccinated cattle.

In this study a comparison between the results which gained from the two serological tests (CFT and C-ELISA) recognized that there were obvious variations in the obtained results (Positive, Doubtful and Negative) from both tests.

Sero-Positive Reactors obtained by CFT (26%) were obviously lesser than the positive ones by C-ELISA (16.5%).

During the present study agreement between the positive results revealed by both CFT and C-ELISA was 63.5%, between the doubtful results revealed by both tests was 52% and between the negative results revealed by both tests was 95.1%.

According to the doubtful results of both tests; c-ELISA doubtful results (12.5%) were noticed to be much higher than CFT doubtful results (6.5%).

That led to miss detection of some CFT positive reactors by c-ELISA and that meant less positive results were detected by c-ELISA (16.5%) compared to CFT positive results (26%).

Those results were justified by Amanfu et al., (2000). Who reported that the c-ELISA test has been developed more recently and data is much less comprehensive but it is believed to be comparable to the CFT in terms of sensitivity for chronic carriers but less sensitive than CFT for the detection of acute cases (Amanfu et al., 2000).

Although the CFT false positive were possible as Hudson, (1971) who reported that the advantage of the CFT is the low number of false positive and negative reactions (Hudson, 1971).
In this study both CFT and c-ELISA agreed on that most of the Sero-positive results appeared in the Nilotic breeds (25.1%), (15.9%) while only (0.9%), (0.6%) appeared in the Baggara breed that may be due to the none existence of the direct contact between the two breeds but that will be an expected hazard in the future if both breeds graze near by each other.

In this study the agreement between the c-ELISA and CF Sero-positive was 63.5%. Although (Mia et al., 1993) reported 96% agreement between the CFT and c-ELISA tests over 300 positive and negative sera. Nevertheless a small number of sera from apparently healthy herds was positive in CFT but gave negative results in the c-ELISA and in both pathological and cultural examinations.

At the present work most of the sero-positive samples CFT (22.8%) and c-ELISA (13.7%) were collected from females but that has No statistical significance as proved by Chi square test.

With regards to participatory epidemiology approaches; Nuer informants mentioned the existence of Doap (CBPP) as well as the clinical signs in their cattle herds especially after the start of the rainy season, this information could be of a great value for CBPP surveillance.

Those results are compatible with those of Zessin et al. (1985) who suggested that a targeted approach to CBPP control would be warranted based on a disease intelligence system that involved the livestock owners.

In this study advantages and disadvantages of both CFT and c-ELISA were compared and evaluated from a practical point of view. CFT advantages; Complement fixing reactions were very obvious through the naked eye; results could be read directly or could be postponed overnight.
Regards to CFT disadvantages; the test preparations and procedures were time consuming, the test needed very skilled personnel to be performed properly, the test was very complicated to be carried out at the field level and the reading of reactions could be affected by personal bias. Those results agreed partially with (Hudson, 1971) who reported that an advantage of CFT; low numbers of false positive and negative reactions. The main disadvantages of CFT are that it fails to detect animals in the incubation period of the disease. Regalla, (1995); Reported that CFT is the approved OIE test, although specific, lacks sensitivity. In addition, it requires highly trained staff to perform it accurately and consistently (Regalla, 1995).

The c-ELISA advantages: Simple test, appropriate at field level, time saving, understandable results and nonexistence of personal bias. Regards to c-ELISA disadvantages: ELISA reader machine is a must, results might be lost unless been read instantly and existence of high range of doubtful results. The present advantages and disadvantages of C-ELISA results agreed to some extent with (Le Goff and Thiaucourt, 1998). Who reported that c-ELISA may have advantages in terms of ease of testing and standardization of results, but it has sensitivity levels similar to CFT and thus will enable the persistence of disease as seen with CFT (Le Goff and Thiaucourt, 1998).
CONCLUSIONS

1. Contagious Bovine Pleuropneumonia is one of the major infectious cattle diseases in Unity State together with FMD, H.S, Brucellosis and B.Q.
2. The presence of CBPP has been confirmed in Unity State through Bacteriological, Serologically and Participatory Epidemiology techniques.
3. CBPP was found to be more common among the Nilotic cattle breed rather than Bagagara cattle breed with an obvious seasonal onset.
4. The Complement fixation Test (CFT) was found to be more successful in investigating CBPP and results were obvious but the test was very complicated and rigid to be executed at the field level.
5. The Competitive Enzyme Linked Immuono Sorbant Assay was found to be useful, straightforward but has a high range of doubtful results and that may affect the validity of the test.
6. Participatory Epidemiology Approaches were found to be practical and useful in terms of anticipating CBPP outbreaks and enriching the Ethno Veterinary Knowledge (EVK).
REFERENCES


Johansson, K.E., Persson, A., Persson, M. (1998). Diagnosis of contagious caprine and contagious bovine pleuropneumonia by PCR and


pleuropneumonia in Portugal.] Patagonia e anatomic apologia de per
pneumonia contagiosa dos bovines (PPCB) em Portugal. Repositories
des Tablehops do Lab oratorio National de Investing»o Veterinaria
(Lisbon), Numerous special, 1-10.

OIE (1997). Recommended standards for epidemiological surveillance


*Mycoplasma mycoides* subsp. *mycoides* in cattle by enzyme linked

Approaches to the development of selective media for Mycoplasma
agalactiae and Mycoplasma bovis. In: Stipkovits L, Rosengarten R,
Frey J, eds. Mycoplasmas of ruminants: pathogenicity, diagnostics,
epidemiology and molecular genetics. Volume III. Luxembourg,
Germany: Office for official publications of the European
Communities, 105-108.

Parker, A.M. (1960). Contagious bovine pleuropneumonia. Production of
complement fixing antigen and some observations on its use. *Bull.


contagious bovine pleuropneumonia by PCR-LIF and PCR-REA
based on the 16S rRNA genes of *Mycoplasma mycoides* subsp.


Figure No. 2. Pie Chart demonstrates the sex distribution among samples.
Figure No. 3. Pie Chart demonstrates Types of Sampled Cattle Breed.
Figure No. 4. Pie Chart illustrates Seasons of Sample Collection.
Figure No. 5. Pie Chart illustrates Vaccination Coverage Percentages among the Sampled Animals.
Figure No. 9. Pie Chart demonstrating the Percentages of the Collected Serum Samples in 2003 and 2004.
CFT-Result

Doubtful
6.5%

Positive
26.0%

Negative
67.5%

Figure No. 11. Pie Chart demonstrating Percentages of CFT Results.
Figure No. 12. Pie Chart Demonstrating c-ELISA Results Percentages.
Table No. 15 Prevalence and Importance of Major Cattle diseases
Ranked by Nuer Ethnic Groups in Different Locations of Unity State.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Location</th>
<th>Disease (Trypanosomiasis + Internal Parasites)</th>
<th>Dat (FMD)</th>
<th>Doap (CBPP)</th>
<th>Yeithpin (Haemorrhagic Septicaemia)</th>
<th>Mullach (Brucellosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubkona</td>
<td></td>
<td>33</td>
<td>20</td>
<td>22</td>
<td>44</td>
<td>21</td>
</tr>
<tr>
<td>Thoan</td>
<td></td>
<td>24</td>
<td>20</td>
<td>20</td>
<td>27</td>
<td>14</td>
</tr>
<tr>
<td>K15, K30 and Mala</td>
<td>13</td>
<td>8</td>
<td>32</td>
<td>20</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mirmir</td>
<td></td>
<td>27</td>
<td>23</td>
<td>29</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>Khor Gamos</td>
<td></td>
<td>16</td>
<td>20</td>
<td>26</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>Munga and Abyamnam</td>
<td>10</td>
<td>16</td>
<td>29</td>
<td>41</td>
<td>15</td>
<td>07</td>
</tr>
<tr>
<td><strong>Means</strong></td>
<td></td>
<td>20.5</td>
<td>17.8</td>
<td>26.3</td>
<td>32</td>
<td>18.2</td>
</tr>
<tr>
<td><strong>Medians</strong></td>
<td></td>
<td>20</td>
<td>20</td>
<td>27.5</td>
<td>30</td>
<td>15.5</td>
</tr>
</tbody>
</table>