Thesis Ref. No. _____

ISOLATION AND IDENTIFICATION OF VIRUSES AND AEROBIC BACTERIA FROM RESPIRATORY TRACTS OF BORANA CAMELS AND OUTBREAK INVESTIGATION, SOUTHERN ETHIOPIA

MSc Thesis

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September, 2014

Bishoftu, Ethiopia

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A Thesis Submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Veterinary Microbiology

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by: **Negassa Feyissa** entitled: **ISOLATION AND IDENTIFICATION OF VIRUSES AND AEROBIC BACTERIA FROM RESPIRATORY TRACTS OF BORANA CAMELS AND OUTBREAK INVESTIGATION**, SOUTHERN ETHIOPIA and recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Science in Veterinary Microbiology.

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DEDICATION

I lovingly dedicate this thesis to my my son Ibsa whom I missed and my wife Tilaye Marara, and my brother Temesgen Feyissa, who supported me at each step of my life.

STATEMENT OF AUTHOR

First, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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Date of Submission: 25 September, 2014

AKNOWLEDGEMENTS

The realization of this work was only possible due to several people'scollaboration, to which I desire to express my gratefulness.

First and foremost, I would like to sincerely thank my advisors Dr. Gezahegn Mamo and Dr. Ashenafi Feyisa, for their guidance and tireless support throughout this study, and specially for their confidence on me. Their comment and questions were very beneficial in incompleting this manuscript. I learned a lot from their insight.

To Dr. Badaso Mamo head of MIVP department, I was grateful for his all-rounded support and advices.

I would also like to thank the staffs of Yabelo Regional Veterinary Laboratory, especially to Dr. Wubishet Zawude and Dr. Ararsa for their support in sample collection and storage in the Laboratory.

The isolation and identification of the bacteria was only possible because of the cooperation of the workers and laboratory technologists of Microbiology of CVMA and hence my thankfulness should be to all staffs specially to Ato Muluken Tekile and W/r Tesfanesh Moges for their support in advising and processing of the sample until the completion of the work.

I also thank staffs of National Veterinary Institute with special appreciation to Dr. Shiferaw Jambare, Dr. Teferi Degefa, Dr. Esayas Gelaye W/r Hawa Mohamed, Ato Bogale Gosaye and Ato Alebachew Belay for their support in sample processing and viral identification.

To all my friends, thank you for your understanding and encouragement in my many, many moments of crisis. Your friendship makes me life a wonderful experience. I cannot list all the names here, but you are always in my mind.

Lastly, but not least, my gratitude goes to my lovely wife Tilaye Merera for her all round support.

Thank you, Lord

TABLE OF CONTENTS

LIST of TABLES

PAGES

LIST OF FIGURES

PAGES

LIST OF ANNEXES

PAGES

LIST of ABBREVIATIONS and ACRONYMS

AAU	Addis Ababa University
BHI	Brain Heart Infusion
BHV	*Bovine Herpes Virus*
BRD	Bovine Respiratory Diseases
BVDV	*Bovine Viral Diarrhea*
BZPDO	Borana zone plan and development Office
CBPP	Bovine Contagious Pleuropneumonia
CCPP	Caprine Contagious Pleuropneumonia
cDNA	Complementary DNA
CoV	*Corona Virus*
CPE	Cytopathogenic Effect
CSA	Central Statistical Authority
CSD	Camel Sudden Death
CVMA	College of Veterinary Medicine and Agricalture
DEPC	Diethylpyrocarbonate
DNA	Deoxyribo Nucleic Acid
dNTPs	Deoxynucleotide Triphosphate
EIA	Enzyme Immunoassay
FA	Fluorescent Antibody
FMD	Foot and Mouth Disease
GBS	Group B *Streptococcus*
GMEM	Glasgow Minimum Essential Medium
HS	Hemorrhagic Septicemia
Ig	Immunoglobuline
IM	Intramascular
mas	meter above sea level
MERS-CoV	Middle East Respiratory Syndrome *Corona Virus*
mRNA	messanger RNA
NVI	National Veterinary Institute
OF	Oxidation Fermentation test

OIE	Office International des Epizooties
PA	Pastoral Association
PBS	Phosphate Buffered Solution
PCR	Poly Chain Reaction
PI	*Parainfluenza virus*
PPR	Peste des Petits Ruminants
RNA	Ribonucleic acid
RSV	Respiratory Syncytial Virus
RT-PCR	Reverse Transcription–PCR
SEM	Scanning Electron Microscopy
SNNPR	South Nations Nationalities and Peoples Region
SPSS	Statistical Package Software for Social science
TEM	Transmission Electron Microscopy
UV	Ultraviolet ray

ABSTRACT

A cross-sectional study was conducted from December 2013 to March 2014 to isolate and identify bacteria and virus from respiratory tracts of apparently healthy and ill camels, evaluate antimicrobial susceptibility pattern of the bacterial isolates and assess risk factors for respiratory infections (*Camelus dromedarius*) in Borana zone of Oromia Regional State, Ethiopia. A total of 371 camels (175 from camels at field and 196 from camels at Akaki abattoir were sampled. All of the field samples were nasal swabs; 35 of them were from camels with respiratory problems for isolation of virus. The abattoir samples include 196 nasal swabs, 196 tracheal swabs and 196 lung swabs for bacteriological isolation and identification. One hundred camel owners were also interviewed to identify risk factors of camel respiratory disease. 80% of field samples and 79.1% of abattoir samples yielded at least one type of bacteria. Of different respiratory organs sampled at abattoir, 90.3% of nasal, 78.1% of tracheal and 68.9% of lung swabs gave bacteria on culture. As the result, total of 274 isolates from 140 field samples and 817 isolates from 155 nasal swabs at abattoir were obtained. The Most frequently isolated pathogens from field samples were *Pasteurella maltocida* (22.3%), *Staphylococcus aureus* (21.7%), *E.coli* (20.6%), *Streptococcus pyogenes* (14.3%) and *Mannhemia hemolytica* (12.6%). Whereas, the abattoir samples result indicated that *Streptococcus pyogens* (24.0%), *Staphylococcus aureus* (21.4%) and *Bacillus spp,* (20.4%) from nasal swabs, *Streptococcus pyogens* (12.2%), and *Pasteurellaa maltocida* (11.2%) from tracheal swabs, and *Pasteurellaa maltocida* (15.3%), *Mannhemia hemolytica*(11.2%) and *Streptococcus pyogens* (15.3%) from lung swabs were the most frequently isolated pathogens. Isolation frequency of the bacteria was significantly associated with lung lesion ($p<0.05$) that is indicative for their taking part in causing lung lesion in camel. The antibiotic susceptibility result of *Staphylococccus spp, Srteptococcus spp, Pasteurella maltocida and* and *Mannhemia hemolytica* indicated that the Gram positive cocci were resistant to most commonly used antibiotics such as penicillin, streptomycin, cloxacillin, ampcillin but were relatively susceptible to nalidixic acid and vancomycin. *Pasteurella maltocida* was highly resistant to streptomycin and cloxacillin at rate of 100%, but susceptible to chloramphenicol (71.4%) and trimatoprim-salphomethaxazole (87.5%). Similarly, *Mannhemia hemo-*

lytica was found resistant to streptomycin (83.3%) and cloxacillin (75%) but relatively susceptible to trimatoprim-salphomethaxazole (58.3%). Out of 35 nasal swabs from clinically sick camels 13 (42.9%) samples exhibited morphologic alterations (CPE) on VERO cell monolayer. Five of these CPE positive samples were checked for the presence of Parainfluenza 3 virus and were all positive by PCR. The isolation of the bacteria from both health and clinically sick camels could be the indicative for the residence of the microflora in upper respiratory tract and involve in causing diseases under some difficult conditions such as viral infection. The isolation of PIV3 from camels with respiratory sign is suggestive for the distribution of the virus in the studied area and the involvement of it in causing respiratory disease outbreak in Borana. The pastoralist interview result showed that respiratory diseases in camels were common, there have been interaction of camels with wild and domestic animals, which might expose them to share similar pathogens, and antibiotic administration has been practiced by the pastoralists themselves that is important in evolving of antibiotic resistant strain of different bacteria.

Keywords: Bacteria and Virus, Borena zone, Dromedary camels, Respiratory tract

1. INTRODUCTION

Camels and llamas are the two genera classified under suborder Tylopoda of order *Artiodactyla*. Camels are assumed to be originated, before 56 to 33.9 million years ago, from Protylopus, an animal that occupied the North American continent. The dromedary or one-humped camel *(Camelus dromedarius)* is one of two species within the genus *Camelus*, the other being the Bactrian or two-humped camel *(Camelus bactrianus* (Palmer, 1999).

As of 2010, out of 25.9 million camel population of the world, 90% of them are dromedaries (Karen, 2010). More than 85% of the dromedary camels are found in Africa, and the rest in the Indian subcontinent and the countries of the Middle East (FAOStat, 2011). The greater portion of camel population in Africa is mainly concentrated to the horn of Africa (Somalia, Sudan, Ethiopia, Kenya, Djibouti, and Eritrea). Those countries alone account for 58% of the total camel population of the world. One-humped camel (*Camelus dromedarius)* is also widely distributed in North African countries, the Arab peninsula, and some countries of Asia (Faye *et al.*, 2012).

One-humped camel, due to its ability to survive under the extremely harsh climate conditions of desert, it has been providing life in a place uninhabited by most animals (Al-Tarazi, 2001). This potential is because of its physiology is adapted to desert environment where it survives with little water and poor feed. It can walk 5-7 days with little or no food and water and can lose a quarter of its body weight without impairing its normal functions. Due to this peculiar adaptation nature, it is the least affected among livestock during long drought conditions (Kebede and Gelaye, 2010, Ouajd and Kamel, 2009).

In Ethiopia, current camel (*Camelus dromedarius)* population is estimated to be about 2.5 millions that with this amount, it ranks 4th in the world (CSA, 2013). They are mainly distributed in arid and semi arid areas of northeast, east, south, and southeast of the country and are one of the livelihoods of most pastoralists of Afar, Somali, and Oromia regions. In these regions, Somali, Afar, and Oromo of Kereyu and Borana areas are the main eth-

nic groups involved in camel husbandry in the country. In this area, camel raising is nearly always coupled with sheep and goats, and sometimes cattle rarely with asses and horses (Dawo, 2010). Borana rangeland of southern Ethiopia is the third important camel production area in the country, the first and second being Somali and Afar respectively.

The one- humped camel (*Camelus dromedarius*) plays an important role as a primary source of subsistence in the lowlands of Ethiopia. It provides food (milk and meat), and used as a reliable means of transport. Furthermore, they are a means of investment, long-term savings, a source of prestige for their owners and their families (Dawo, 2010). Other importance of the species over the rest of livestock is due to its efficacy in converting poor quality fodder into energy for work and consumable products. For these reasons, it sustains the life of pastoralists in harsh environment where, in some areas, impossible to raise other livestock animals and/or produce crop productions (Dirie and Abdurahman, 2003, Raziq and Younas, 2006).

Camel seems to be spared from the devastating epidemic infections such as rinderpest, contagious pleuropneumonia and foot and mouth disease which threaten other livestock species in the same region. It is, however, affected by many other diseases, which are economically important and even unknown to date (Dirie and Abdurahman, 2003). The health constraints in camel farming are well known and have been listed for a long time under the main classical diseases such as trypanosomosis, camelpox , gastro-intestinal parasitism, contagious skin necrosis, pneumonia, tuberculosis and mange mite infections (Demeke, 1998; Odeh *et al.,* 1999; Bekele, 1999). However, at least in the past decade, new cases marked by severe symptoms, high mortality and impossibility to form a precise diagnosis have been emerged in several countries (Wernery and Kaaden, 2002; Nardone *et al.,* 2010; Wall *et al.,* 2011). Among the numerous emerging diseases, respiratory diseases are the major threats to the camel population. Recently, these diseases have been becoming an important and serious problem of camel production in most African countries. They have important and serious impact on camel production by causing economic losses resulted from death, decreased performance of diseased animals, lowered weight gain, reduced carcass value and increase treatment costs (Fulton *et al.,* 2002).

In Ethiopia, camel respiratory disease outbreaks have occurred in different parts of the country over times. However, it has received little consideration, even though it is an emerging disease causing considerable loss of production and deaths. For instance, in 1995, an outbreak of respiratory disease, which was contagious in nature with high rate of morbidity and a variable rate of mortality, has occurred in Somali region. Later in 2005, 2006 and 2007 outbreaks suspicious as respiratory system involvement, characterized by sudden death, have killed hundreds of camels in Afar, Somali, and Borana and Karayu of Oromia region. Nevertheless, investigations of the causes of these outbreaks, made by a number of veterinary institutions and laboratories in the country, have failed to isolate the exact etiological agents of the disease (Awol *et al.,* 2011; Roger *et al.,* 2000, 2001; Bekele, 2008).

A definitive etiology of most respiratory diseases of camels has not yet been determined as a variety of viruses, fungi, bacteria and parasites are the possible causes of respiratory outbreaks (Kebede and Gelaye, 2010). Previous works in Ethiopia have identified the etiological agents of camel respiratory diseases only from animals slaughtered at abattoir and sero-epidemiological survey.

However, complimentary information and data are needed from isolation of causative agents from samples collected at level of residential areas. In addition, in spite of the continuous occurrence of outbreaks of sudden death in camels and existence respiratory related clinical signs, so far in depth microbiological examination based on isolation of bacteria and virus combined with assessment the local knowledge and perception of the outbreak through the participation of the pastoralist at village level has not been carried out particularly in Borona pastoral area.

Therefore the objective of this study was:

- To isolate and identify aerobic bacteria from respiratory tracts of apparently health, clinically ill and lung of slaughtered camels;
- To investigate the drug susceptibility profile of aerobic bacterial pathogens of camel respiratory tracts in the study area;
- To isolate and characterize viruses from clinically ill camels with respiratory diseases; and
- To assess the risk factors for viruses and opportunistic bacterial infections in camel respiratory tract.

2. LITERATURE REVIEW

2.1. Distribution of camels (*Camelus dromedaries*)

The scientific assumptions about the historic origin and distribution of camel are that it was instigated from *Protylopus*, an animal that occupied the North American continent during the Eocene period. The assumptions, speculated that the one-humped species probably evolved in one of the hottest and most arid areas of western Asia. Camelid was probably among the last of major domestic species to be put to regular use by man. The most likely time of domestication is about 4000 years before or slightly earlier. The presumed area of domestication is the southern Arabian Peninsula, probably the area of Yemen and Oman. It was from this presumed center of domestication that dromedary has subsequently been distributed to almost the rest of the world (Palmer, 1999).

Then after, environmental, social, and cultural factors have greatly influenced the distribution and production of the species. The greatest cultural influences in recent distribution of camels was the advent of Islam, when Arabs spread their gospel, consolidating its ranges north and east wards in Asia, and along the Mediterranean littoral. There have been many attempts to introduce camels outside the "normal" ranges, in Brazil, Colombia, USA, Cuba, Spain, Italy, and France. Generally, there has been steady increase in camel population since about 1980s. (Bekele, 2004)

Arid and semi-arid zones of tropical and subtropical countries of Africa and Asia are convenient ecology for one humped camel production. As the result, the Horn of African region has the largest concentration of the species in the world (Karen, 2010) that about 80% of Africa and 60% of the world camel population is found in it (Abebe, 2000). The recent expansion of camel within Africa has been thought as related with climatic change results in recurrent droughts. As the consquence, the productivity of the African savanna grasslands has reduced by changing the balance between trees, shrubs and grasses, and accelerating the advancement of woody and invasive plant species. The decline in pastoral resources is fueling violent conflicts among competing pastoralists and agro-

pastoralists throughout the African continent. However, the affected pastoralists have developed various indigenous coping strategies to minimize the risks. Diversifying herd composition by acquisition of drought-tolerant livestock genotypes and species such as camel and goat is one among the strategies (Raziq 2009). Recently Borana Oromos in Southern Ethiopia and many semiarid and arid regions of other countries of the Horn of Africa increasingly practice camel production. Cattle numbers are decreasing in such regions while camel numbers are increasing and are likely to play more significant role for human nutrition in the future (Raziq, 2009).

Subsistence camel production is practiced in dry areas of Ethiopia, which covers 61% to 65% of the total land area (Abebe, 2000). There are four camel breeds namely milk, meat, dual purpose and baggage camels in the country. Except in western lowlands, the distribution of camel in Ethiopia have been follwed with that of the dry lands, and of *T.evansi* and overlaps with the area occupied by Muslim societies from northeastern through eastern to southern arid and semiarid low lands. Ownership varied from several to hundred, 50-100, or 5-10 or even less camels. Mostly in the large herds, females are dominant (more than 75%). Males are sold as pack animals and for meat. While in small herds, males are dominant in number and they are used for transport of goods (Melaku and Getachew, 2012).

Borana pastoralists probably started camel production in early 1560 in the Gedda period of Abbay Orro (Hukka, 1998). It has also been speculated that the Muslim Gebra communities were said to be the means to introduce camels to the Borena plateau (Coppock, 1994). Thereafter, changes in social interactions, extensive seasonal migration and change in rangeland ecosystem (decreasing pasture for grazers and increasing browse vegetation species) are major factors for the expansion of dromedaries into the area (Biffa and Chaka, 2002).

2.2. Potential Importance of Camel

Camels are more important over other livestock species living in the same environment for many reasons. First, it is the least affected animal by climatic changes and drought (Kebede and Gelaye, 2010; Ouajd and Kamel, 2009).This arises from economical use of water in almost all metabolic functions. Second, it utilizes wide range of feed resource which enables exploits different utilization mechanism of the savanna perennial herbaceous and woody species. They have thick, very strong, and rubber-like lips that make them easier to eat prickly and thorn desert plants. Furthermore, their strong digestive system digests everything it comes across like plastic plates or copper wires. As the result, camels can change poor quality pasture into milk and meat. Camel might be an exceptional animal, which can be used worldwide as a tool of natural modifier and can work ideally under nourishment instability scenario (Getahun and Kassa, 2002). In addition, its long neck enables the animal to reach and feed on the leaves, which are three meters high above the ground. Hence, in mixed species, the camel feeds on plants or part of plants that are not eaten by other conventional livestock due to its size to browse the highest strata, thus reducing competitions and enhancing complementarities (Wilson *el al.*, 1990).

The dromedary plays economic, social, and ecological roles. From a global point of view, the economic significance of camel production is negligible in comparison with that of other domestic animals. Nevertheless, in Africa (especially in East Africa) and Sahel countries, the camel makes a significant contribution to national economies. However, it is difficult to evaluate this economic contribution as most of the camel products are traded by informal sectors (Getahun and Kassa, 2002). However, the market integration of camel products represents the most outstanding change in the last twenty years (Faye *et al.,* 2012). Unfortunately, data on these animal transfers, volumes and values are lacking, contrary to cattle market (Turner and Williams, 2002)**.**

Camel is primarily among the domestic animals of pastoral communities that ensure food security. As it is well-adapted livestock species, which survives and produces in extreme climatic conditions, it is acknowledged for its implication in the pastoral economy of dif-

ferent countries. It lives in arid and semi-arid areas, which are not suitable for crop production and where other livestock species hardly thrive. Now days, the increasing human population and declining per capita production of food in Africa, are indicative for an urgent need to develop marginal resources, such as arid land, and optimize their utilization through appropriate livestock production systems of which undoubtedly, camel production is the most suitable (Dawo, 2010; Faye *et al.*, 2012).

Even though, the primary reasons for keeping dromedary camels vary from country to country and from one place to the other, many pastoral groups and communities in diverse ecozones throughout the world are depending on camels for their livelihood. Their dependence consists of utilization of camel meat, milk, leather and wool, cultural status and financial reserve, exportation of live camels, uses as an important sport and tourism resources, and as animals for packing, transport and riding. Camels are kept mainly for riding (sport) and tourism resources in countries of the Arabian Peninsula (Wilson and Bourzat, 1988), for transportation in Eritrea (Gebrehiwet, 1999) and for milk and meat production and transportation in Ethiopia and Somalia (Schwartz, 1992; Bekele, 1999).

In Ethiopia, camels play diverse roles in livelihood of the poor pastoralists that they are the subset of huge livestock resource when considered from national economic point of view. The roles include providing meat and milk, the building of assets, insurance against unexpected events, and have spiritual and social values. In additon, camels are means of traction and movement of goods and human, income generation in pastoralists, and very recently, it plays pronounced role in the export revenue of the country in both live animal and carcass export (Simenew *et al.,* 2013). In Borana, camel rearing is part of livestock diversification, which have economic and ecological advantages that it represents a minimal competition with other ruminants. In the zone, camels supply the households with milk, and reduce vulnerability to food insecurity during the dry periods, while other animals could not do. It is also used as income generation from live animal sale (Bekele, 2010).

Milk and meat are the important products that camels produce elsewhere. They are very reliable milk producers during dry seasons and drought years when milk from cattle sheep and goats is scarce (Tefera and Gebreab, 2001; Getahun and Kassa, 2002,). Long lactation and ability to maintain milk production over long dry season are important facets of camel productivity (Schwartz and Dioli, 1992). In recent years, the picture of moving nomads has changed to some extent. With growing urbanization, the demand for milk among the urban population has been increasing. On the other hand, the demand for a number of goods such as grain, oil, sugar and clothes increased among the pastoralists and the milk sales become the most important part of cash income for many camel owning pastoralists. Therefore, apart from home consumption, majority of the households sell the produced milk to generate cash income in Ethiopian pastoralists (Getahun and Kassa, 2002).

Camel meat is another important product mainly as a source of income. Sale of live camels, usually males and unproductive females for slaughter, is very common in East Africa and there are now increasing numbers of camel meaat consumer in the region. There is also a growing export trade of slaughter camels to the Arabian Peninsula (Getahun and Kassa, 2002).

Until the arrival of motorized transport in the arid and semi-arid zones, camels have been the sole means of transport in the areas where they are adapted. They are also used for domestic use for drawing water from wells, rivers and dams, and source of power for oil mill. Camel racing and other leisure activities such as camel safaris and trekking have recently become a tourist attraction and luxurious in some parts of the world (Bekele, 2010).

In medical world, camel has been becoming important animal for different purposes. Animal studies that have been published provided evidence on camel milk therapeutic activity. Among the protective proteins in camel milk are lysozyme, lactoferrin lactoperoxidase, and peptidoglycan recognition protein. These proteins have anti diarrheal /anti bacterial action as well as high titers of antibodies against rotavirus, and they impact on the

immune system. For hundreds of years camel milk has been used to treat diarrhea even though the identity of the active substance in the milk was not known. Significant improvement has also attained in reduction of insulin doses to obtain glycaemic control along with significant improvement in glycohemoglobin (HbA$_{1c}$) level in type-I diabetic patients taking raw camel milk for more than one year (Agrawal *et al.*, 2005). Moreover, camel immunoglobulins are a tenth size of the human and are highly potent. These properties have been put to use by the Homeland Security Department of the United States government to create biosensors for determining which is being used in biological welfare attack (Yagil, 2013). Alshazly *et al.* (2010) has also determined that camel urine contains nano-particles, which might be effective against certain types of cancerous cells. The active ingredient in camel urine was identified as PM701, which is the codename of the researcher, has already been tested in human.

2.3. Constraints of Camel Production

Majority of camels in the world are raised by pastoral societies of the third world who dwell in dry marginal areas. Due to the facts that migratory system of the pastoralists summed up with living in remote areas with harsh living conditions and poor infrastructure, the animals are probably inaccessible for research. This affects the depth of our knowledge on the general aspects of camels. Generally, there was negligence towards the promotion of camel health and production until recent after which the camel become the subject of more intensive and systematic interest (Baumann and Zessin, 1992; Schwartz and Dioli, 1992).

In addition, pastoralism is always associated with risks, because the life is based on unreliable climatic conditions, border and marginal land conflicts, recurrent drought, and epidemic livestock diseases. The impact of climate change on animal health could also have either direct effects as a result of heat stress and shortage of water and feed which lead to over-mortality, or indirect effects as a result of more favorable conditions for microbes and parasites or vectors to spread (Rass, 2006; Seifu, 2009).

Camel production in Ethiopia is constrained by a number of factors of which feed and water shortage and prevalence of disease, veterinary services, lack of governmental or private drug stores, and lack of professionals support towards improvements of production and productivity of their camels, and absence of a pastoral-friendly market system and structures are the most important ones (Seifu 2009; Simenew *et al*, 2013) . Participatory epidemiological discussions made with pastoralists in Borana has spotted that most important constraints to camel productions were identified to be widespread diseases, poor veterinary service and lack of attention from government. Among these, camel diseases were the most important one (Bekele, 2010).

2.4. Camel Diseases

Despite camels are uniquely adapted to hot and arid environment and seem to be spared from devastating epidemic infection that threaten other animal species sharing the same habitat, there are a number of economically important diseases by which they are affected (Dia, 2006; Intisar *et al.,* 2010). The dromedary has been commonly suffering from different diseases like trypanosomosis, camel pox, mangemites, respiratory disease complex, hemorrhagic septicemia, cephalopsis, pustular dermatitis, dermatomycosis, gastrointestinal parasites, tick infestation, dermatomycosis, ocular problems, rabies, facial paralysis, wry neck syndrome, stiff neck saddle sores, wounds, abscesses and acute plant poisoning for the past so many years (Tefera, 2004).

In Ethiopia, even though there is huge number of camels, it has been given little research and development attention (Seifu 2009; Dawo, 2010). Camel pox, trypanosomiasis, calf scours, respiratory diseases, and camel reproductive problems such as abortion, still birth, uterine prolapse, retained foetal membrane, dystocia and endometritis were the most commonly reported diseases. Among these, respiratory diseases, camel pox, anthrax, and trypanosomiasis were the most prevalent diseases in the country (Semenew *et al.*, 2013).

Even though, camel diseases that are shared with other species of livestock are comparatively well-known, other camel-specific diseases, although well-known to pastoralists for

generations, still remain a mystery to the scientific community. This indicates that camel diseases have not been very extensively researched in comparison with those of other domesticated species. This could be, owing partly to the non-sedentary nature of the herds that constantly moving in search of grazing and water. It is only in a few places, where the animals are found in favorable environments, often alongside other species, that attempts have been made to study camel diseases. Therefore, these diseases need to be studied and characterised for appropriate cures to be developed (Dirie and Abdurahman, 2003).

Published data have revealed that at least in the past decade, new camel diseases marked by severe symptoms, high mortality and then impossibility to form a precise diagnosis have emerged in several countries. For instance, an epizooty prevailed in the Horn of Africa in 1995-1996, characterized by febrile attacks, a highly contagious respiratory syndrome with a high morbidity (up to 90%) and a mortality varying from 5 to 70% depending on the herd and treatment administered has occurred in different countries (Roger *et al.,* 2000). In the early 2000s, in Sudan and Kenya deadly camel disease, without clear causal agent was occurred, although PPR viruses were suspected at the time. The pastoralists of these countries considered those diseases as new ones (Khalafalla *et al.,* 2005, Khalafalla *et al.,* 2010). In 2003-2004, a large number of dead camels were also reported in Mali, Niger and Chad. The symptoms were not specific and death occurred quickly. Some witnesses called it a "stunning death". No quantitative data was available because no exhaustive survey was carried out, but the death rates seemed very high. Anthrax was suspected for a moment as well as an acute form of trypanosomosis, but the results of laboratory analyses did not confirm those (Olwoch *et al.,* 2007). In 2005, unusual high numbers of camel deaths were reported from Afar and Oromia Region in Ethiopia. Similarly, Sudden deaths of camels were observed in early 2006 in the same regions of the country (Dawo, 2007).

In 2006 and in 2007, in Somalia, Ethiopia and North Kenya, sudden deaths affecting hundreds of camels were reported. The then assumed causes include plant intoxication and mineral deficiencies. But later main viral diseases such as PPR, blue tongue, foot-

and-mouth disease and Rift Valley fever were suspected but they have not yet been confirmed (Faye *et al.*, 2012; Gluecks *et al.*, 2010). The 2007 camel mortality in the Borana zone and neighboring Guji zone of Oromia regional state in Ethiopia, affected mainly lactating and pregnant camels, a few breeding males of more than three years of age, and some heifers. At the time, many more camels were found dead also in other pastoral areas of Oromia and Somali regions of the country (Dawo, 2007).

The disease syndrome named 'Camel Sudden Death' (CSD) mainly affected adult camels, especially lactating and pregnant females, breeding bulls and pack camels has been reported. To pace the causative agents, investigations were carried out in Ethiopia in 2006 and jointly in Somalia and Kenya in 2007. Field investigations using participatory epidemiology and data analysis supports the hypothesis that CSD is a new disease entity in camels, as it does not have any traditional disease names in many local pastoralist languages of the region. Laboratory findings indicated that *Bacillus anthrasis* and other bacteria and parasites commonly found in camels were not involved in CSD. Inflammatory cell responses seen in histology and results from attempted virus isolation carried out in Kenya indicated involvement of a viral agent (Gluecks *et al.*, 2010).

Despite all efforts have been done to identify the real possible causes of the disease, so far, there was no substantial result obtained from the investigation of the disease. These outbreaks affected large numbers of camels and therefore, required examination of the causative agents and assessments of the economic impact as well as a risk factor study remained in order to control future outbreaks by tailoring control measures to the problem (Dawo, 2010; Kebede and Gelaye, 2010).

Thus the emergence of new diseases in this species, although famous for its resistance to be diagnosed, occurred in many cases in the present decade. The causes of these diseases are not necessarily identical because the symptoms are not always the same. Multiple factors are also probably involved, and immuno-depressive elements, including certain viruses, can aggravate parasite infections. Unfortunately, investigations have been hindered by the difficult access to sick animals, as often located in remote areas (Faye *et al.*, 2012).

The presence of oedema in the lung and massive froth/foam in lung and trachea are indicative for CSD disease involves pulmonary problem (Gluecks *et al.,* 2010).

2.4.1. Camel Respiratory Diseases

Pulmonary diseases are among most frequent camel diseases that result considerable loss in production (Zubair *et al.,* 2004; Kane *et al.,* 2005). As causative agents, varieties of viruses, fungi, bacteria, and parasites have been found associated with the problems among camels (Schwartz and Dioli, 1992). Although the diseases are among the many diseases of camels that are rampant in Ethiopia, and the single most important disease posing a huge threat to the camel population, few studies were conducted on the extents of the problems on camels as compared to other species of livestock animals (Bekele, 2008).

2.4.2. Importance of Camel Respiratory Diseases

2.4.2.1 .Economic importance of camel respiratory diseases

Respiratory tract diseases are among the most important and commonly encountered disease of camel. They are among the emerging health hazards to camels worldwide that are incurring considerable loss of life and production (Khan, 2012). The economic impacts of camel respiratory diseases are because of that they result in death, decreased performance of animals, lowered weight gain, reduced carcass value and increase treatment costs (Fulton *et al.,* 2002). Death of camel due to camel respiratory disease has been reported as high as 70% in horn of Africa (Bekele, 1999; Mochabo *et al*., 2006). In addition on the basis of information gleaned through participatory appraisal, one of the respiratory diseases, hemorrhagic septicemia (HS), have been become apparent to be among the five important camel diseases and valorem economic losses incurred by it has been reported from Pakistan. These losses are due to high fatalities, loss of productivity and heavy cost on compulsory medication and vaccination (Khan, 2012). Moreover, despite low mortality and morbidity rates, if any, the recovery period is quite long having negative impact on

overall productivity (Bekele, 2010). The influence of the disease, hence, can impair not only the pastoralists but also the national economy of a country in which the outbreaks have been occurred.

2.4.2.2. Public health importance of camel respiratory diseases

Variety of viruses alone or concurrent with other microorganisms have been isolated from camels suffered from respiratory disease. These agents may represent risk to not only camels, but also to other livestock and even to human population (Bardonnet *et al.,* 2002). For instance, recently, camel is suspected as the reservoir for a new *betacoronavirus* known as Middle East Respiratory Syndrome *Corona Virus* (MERS-CoV) which has public health importance (Reusken, *et al.,* 2013). There is growing evidence that the dromedary camel is a host species for the MERS-CoV and that they play an important role in the transmission to humans. The first evidence of dromedary camels being part of the transmission chain was the detection of high rates of antibodies against MERS-CoV in dromedary camels on the Arabian Peninsula. Evidence of infection in camels precedes the first evidence of human infection. Now days, RNA of the virus has been detected in different specimens from camels. The virus has also been isolated from nasal and faecal samples obtained from camel. Detection of MERS-CoV in dromedary camels imported from Sudan and Ethiopia for slaughter to Egypt have been reported. In strengthening of the evidences, serologic findings of previous MERS-CoV infection in dromedaries in Nigeria, Ethiopia and Tunisia, suggest that the virus could be geographically widespread in the dromedary camel populations in African continent. This might draw the scientists to estimate that until now undetected transmission to humans may occur outside of the Arabian Peninsula (ECDC, 2014). In addition, *Respiratory syncytial virus* which is one of the well-known causes of respiratory infection in human and various animal species (Murphy *et al.,* 1999), has been isolated from infected lungs of camels slaughtered at Akaki abattoir in Ethiopia at a rate of 83.3% (Ayelet *et al.*,2013).

Camel tuberculosis, of which the causative agents could have zoonotic importance, have been reported in Egypt, United Arab Emirates, Pakistan and Australia (Melaku and Geta-

chew, 2012). *Mycobacterium tuberculosis*, *M. bovis* and atypical mycobacteria such as *M. kansasi*, *M. aquae*, *M. furtuitum*, and *M. semegmatis* have been isolated in camel as causative agent of camel TB. In Ethiopia, few studies have been conducted to understand the distribution of camel tuberculosis. Mamo *et al.*, (2009) conducted the first study using cross sectional sampling and reported 5.017% prevalence in postmortem examination from Dre Dawa abattoir. Recently, Mamo *et al*., (2011) conducted on camel TB to investigate the pathology of camel tuberculosis and characterize its causative agents using postmortem, mycobacteriological culturing and multiplex poly chain reaction (PCR). In the research, 4 of 14 samples were found positive. for the growth of mycobacteria on pyruvateenrich Loewenstein-Jensen medium indicating the presence of *Mycobacterium bovis* have also observed Mycobacterium, in pneumonic camel lungs by acid fast (Ziel Neelson) staining technique at rates of 5.3% (Awole *et al.*, 2011). Public health important bacteria such as *Staphylococcus aureus*, *Bacillus spp*, and *E.coli* have also been isolated from camel lungs by various researchers (Abubakar *et al.*, 2010; Awol *et al.*, 2011). Moreover, an important pathogen affecting humans and livestock species, *Streptococcus agalactiae*, a Group B *Streptococcus* (GBS) isolated from African camel have been found 34% resistant to Oxytetracycline (Fischer *et al*., 2013).

Hydatid disease or cystic echinococcosis caused by infection with the larval (metacestode) stage of *Echinococcus granulosus*, (*E. granulosus*) is considered to be one of the most important helminth zoonoses (El-Mahdi *et al*. 2004). It is known that the camel is one of common intermediate hosts of *Ecchinococcus granulosus* and hydatid cysts have been frequently reported from camel of almost all countries (Hosseini and Eslami, 1998). According to different literature reviews, hydatid cysts are found in the lungs, liver and spleen of infected camels, and losses of what would otherwise have been parts for human consumption were considerable. In a survey conducted in Borana zone of Ethiopia indicated that 25.7% of the investigated camels were found infected with hydatidosis (Bekele, 2010).

2.4.3. Status of Camel Respiratory Diseases in Ethiopia and other countries

Respiratory disease is common among camels, as showed by the reports from bronchopneumonia and pneumonic lesions at abattoirs, although little is known of the complex etiology of these diseases. This is because camel respiratory problem has received little consideration, even though it has been an emerging disease causing considerable loss of production and deaths (Kebede and Gelaye, 2010).

It has been frequently reported that in the Horn of Africa since 1995 highly contagious respiratory syndrome with a high morbidity and varying mortality rate have occurred. The rate could be even higher than the usual during the rainy season, even though the causative agents were not precisely identified (Roger *et al.*, 2000). Agab (2006) has reported respiratory complaints in a camel dairy farm in Saudi Arabia at rate of 1.8%. Camel respiratory disease outbreak, of which *parainfluenza virus 3* and *Mannhemia hemolytica* were determined as causative agents, has been observed in Afar region of Ethiopia (Kebede and Gelaye 2010). In Borana zone of southern Ethiopia, respiratory infections have been found common in camels and occur in two forms: acute and chronic forms. The chronic form with long time coughing is locally called "*Dhuguda, or kufa"* while the acute form with nasal discharge is named as "*Furi."* The most important and severe form is the acute respiratory infection, which has occurred in the form of outbreak during the major wet season. The respiratory disease involves all age group in the area. It results in disastrous death losses causing up to 20% morbidity and over 50% mortality in affected herds (Bekele, 2010).

Bekele (1999) described the outbreak of pneumonia in camels that involved camel herds from as far Northern East as Afar regions through Ogaden to Borana, southern part of Ethiopia causing up to 30% morbidity and 6.4% mortality. Roger *et al.*, (2001) observed higher seroprevalences to *Morbilli virus* in convalescent camels due to respiratory diseases in Ethiopia and this increases the weight of the virus to involve in camel respiratory diseases. Moreover, Simenew *et al*. (2013) have reported that respiratory diseases were one of the most prevalent diseases of Somali camels in Eastern Ethiopia with prevalence

rate of 4%. Camel sudden death occurred in different African countries has been evidenced that it involves pulmonary problem. This is because the postmortem conducted on the dead camels had edema and froth/foam in their lung and trachea (Gluecks *et al.,* 2010).

2.4.4. Etiology of camel respiratory diseases

A definitive etiology of most respiratory diseases of camels has not yet been determined. This is because, most of the infectious agents, which cause respiratory disease, are ubiquitous in the environment and are present as normal residents in the nasal cavities of normal animals. This often creates difficulty in the interpretation of the microbiological findings in outbreaks of respiratory diseases. Several infectious agents could commonly be isolated from the respiratory tract of clinically ill and health camel. A variety of viral, bacterial, fungal and parasitic microorganisms have been associated with respiratory disease problems in the species (Abbas and Omer, 2005).

2.4.4.1. bacteria

Pasteurella multocida is the most commonly isolated pathogen from pneumonic camels (Wernery and Kaaden, 2002). *Pasteurell*a (*Mannhemia) haemolytica* has also isolated in association with severe pneumonia. Bekele (1999) isolated *Paseurella haemolytica* from the lungs, thoracic fluid and whole blood of camels with an epidemic of respiratory disease caused 29.6% morbidity and 6.4% mortality in camels of Ethiopia. Moreover, *Streptococcus spps* are suspected in causing camel respiratory diseases were even suspected as causing camel sudden death even though not confirmed in previous studies (Bekele 2010). Al-Tarazi, (2001) also found that *Staphylococcus aureus, Actinomyces pyogenes* and *hemolytic streptococci* were the most frequent isolates from lung abscess cases.

Numerous other bacteria including, *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Rhodococcus equi,* and *Neisseria spp* have been incriminated in the aetiology of pneumonia in camels, (Alhendi,1999; Abubakar *et al.,* 2010)). Lung abscesses due

to *Arcanobacterium pyogenes* and *Corynebacterium pseudotuberculosis* were reported by Abu-Bakr *et al*. (1999) as affecting camels. However, there is very little in the literature with regards to the role played by mycoplasmas and other mollicutes in the etiology of pneumonia in camels (Wernery and Kaaden, 2002). However, Abbas *et al*. (2002) isolated *M. arginini* along with several other bacteria from camel lungs with lesions suggestive of causing chronic interstitial pneumonia in the species.

In Ethiopia, the micro flora of upper respiratory tracts particularly from nasal cavity of camels have not so far isolated and identified even though bacteria have been isolated from pneumonic lungs and lungs of apparently health slaughtered camels at different abattoir in the country. The findings of Awol *et al*. (2011) from pneumonic lungs of camels slaughtered at Akaki abattoir showed that a total of 54 bacterial species were isolated and identified from 50 samples. Mohammed (2011) also identified a total of 37 bacterial species from 32 pnuemonic lung samples collected from Oromia zone of Amhara regional state. In addition, Ahimed (2010) conducted a study at Jigjiga to isolate and identify bacterial species from lung of apparently healthy camels slaughtered at abattoir. Table 1 summarizes the bacterial isolates recovered from respiratory tracts of camels slaughtered in different abattoir in Ethiopia.

Table 1: Bacterial Isolates from Slaughtered Camels at Different Abattoir in Ethiopia

Isolates	Prevalence (%) Akaki	Prevalence (%) Oromia zone	Prevalence (%) Jigjiga
Coagulase negative *staphylococcus spp,*	21.1	-	48.7
Streptococcus species,	19.3	5.4	20.5
E. coli,	17.5	21.62	12.8
Francisella tularensis,			
Flavobacteria species,	5.3	-	-
Rhodococcus equi,	5.3	2.7	-
Bordetella bronchoseptica,	5.3	2.7	5.1
Aeromonas hydrophila,	3.5	-	-
Neisseria species,	3.5	-	-
Streptococcus agalactia,	108	5.4	-
Staphylococcus aureus,	1.8	24.32	-
Pasteurella trehalosi,	1.8	-	-
Pasteurella antipestifer,	1.8	-	-
Psuedomonas aeroginosa,	1.8	-	-
Micrococcus species,	1.8	2.5	-
Mycobacterium species,	5.3	-	-
Klebsiella pneumoniae,	-	8.1	-
Mannhemia hemolytica,	-	5.4	7.7
Actinomysis pyogens,	-	5.4	-
Salmonella species,	-	2.7	-
Proteus spicies,	-	2.7	-
Corynebacterium species	-	-	5.2

2.4.4.2. virus

Though the causative etiologies of camel respiratory diseases are remained mysterious, studies have found out the involvement of different viruses as one of the causative agents. Several researchers have reported high antibody titers against numerous respiratory tract viruses such as *Adenovirus, Parainfluenza virus 3, Respiratory syncytial virus*, and *Morbili virus* in camel sera, but the pathological and epidemiological significance of these findings is not clear (Roge *et al.,* 2001). Thus, studies insisted that a continuous and detailed molecular epidemiologic investigation is needed to determine the viral species prevailing in dromedary camels in a particular geographic area (Kebede and Gelaye, 2010).

Other viruses most frequently associated with respiratory diseases of camel include a type 1 *bovine herpesvirus (BHV1), influenza viruses A and B, bovine viral diarrhea virus (BVDV)* and *peste des petits ruminants (PPR) Virus*. *Adenovirus,* and *coronavirus (CoV)* are viruses which may be involved in the disease but could be underestimated (Wernery and Kaaden, 2002; Wunschman *et al.,* 2002). Among known camel respiratory disease causing viruses, *Bovine virus diarrhea (BVD), Bovine herpesvirus type 1 (BHV-1),* and *Parainfluenza type 3 (PI3)* viruses have all been incriminated in the etiology of acute respiratory diseases in camel (Durham and Hassard 1990). They are known to cause serious losses due to the control programs in different countries (Elbayoumy *et al,* 2013).

Among these viruses, *Adenovirus* and *Respiratory syncytial virus* have been isolated and identified from lung lesion of Ethiopian camel (Ayelet *et al.,* 2013). Moreover, the evidence of a study conducted by Kebede and Gelaye (2010) was suggestive for *Parainfluenza virus-3* being the primary pathogenic agent in the camel respiratory disease outbreak in Ethiopia.

2.4.4.3. fungi

Mycotic pneumonia in camels caused by fungi as *Aspergillus fumigates, Aspergillus flavus* and *Candida albicans* were recorded. The isolated fungi were mostly mixed with bacteria (Abo-Elnaga and Osman, 2012). According to Soliman and Darwish (2001) Pathological studies of camel pneumonic lungs investigation revealed that granulomatus pneumonia are occurred due to Aspergillus fumigatus infection, but haemorrhagic pneumonia occurred due to other fungal infection such as *Penicillium spp., Fusarium spp., Mucor spp.* and yeasts and mixed bacterial and fungal infection. In other study conducted in India, mycotic pneumonia was found in 5.09% of total cases observed in an investigation carried out on 157 lung samples of camel (Bhardwaj *et al.*, 2005).

El-Khouly *et al.* (1992) observed fungal hyphae and, occasionally, the characteristic conidial morphology of *Aspergillus fumigatus* by direct smears from lesions in the respiratory and alimentary tracts of racing camels (*Camelus dromedarius*). Clinical signs observed in the cases were pyrexia, coughing, lachrymation, oedema of the throat and submandibular region and enlargement of submandibular lymph nodes. In all the cases, *Aspergillus spp.* was the most prevalent type of the recorded fungi. However, there have not been published documents regarding the mycotic pneumonia in Ethiopian camel lungs.

2.4.4.4. parasites

Several studies have been conducted to determine the prevalence and types of gastrointestinal tract parasites in camel. The seasonal occurrence of the diseases due to the parasites has also understood. However only few studies have been conducted on parasites those cause polmunary diseases in this animal. Chhabra and Gupta (2006) assumed that the lungworms, *Dictyocaulus* or *Protostrongylus* spp. are rare in camels due to its typical browsing habit. However, Radfar *et al*. (2006) isolated *Dictyocaulus filaria* at rate of 10% from lung and *Cephalopina titillator* larvae at rate of 63.3% from nasal cavity of slaughtered camels in Egypt. Swai *et al.* (2011) also isolated *Dictyocaulus spp* from fecal sample of camels in Tanzania. In addition a study conducted on entire lung of slaughtered

dromedaries in Iran revealed that out of the 50 camels examined, 24 % were positive for lungworm infection that 91.6% and 8.3% were showed infection to hydatidcyst and *Dipetalonema evansi*, respectively (Ebrahimi *et al*., 2012). In Ethiopia, majorities of the studies on the pulmonary parasites have focused on lung hydatid cyst. A study conducted on pneumonic lungs in Dredwa, Ethiopia, showed that parasitic bronchopneumonia and hydatidcyst have been revealed at the rates of 0.96% and 30.80% respectively (Bekele, 2008). Giro *et al*. (2013) have also found that the prevalence of hydatidcyst in slaughtered camel at Akaki abattoir in Ethiopia was 40.39%.

2.4.5. Pathogenesis of Respiratory Diseases

According to the developing concept of polymicrobial diseases, some diseases in both animals and humans result from infections by multiple pathogens. Polymicrobial diseases are defined as those diseases that can occur with organisms from different kingdoms, from different genera in a kingdom, from different species in a genus, from different strains in a species, and /or from different sub strains in a strain. Polymicrobial diseases can be categorized into diseases that originate from polyviral infections, polybacterial infections, viral and bacterial infections, and polymicrobial mycotic infections, and those that result from microbe-induced immunosuppression (Hodgins *et al.*, 2002).

In many experiments using animal models, bacterial infections have been superimposed at various times after inoculation of viruses. Enhancement of bacterial infection was demonstrated by comparisons of the bacterial contents of appropriate tissues with those of animals receiving bacteria alone. More-severe lesions showed exacerbation of disease or higher death rates than for animals receiving either the bacteria or viruses alone (Oleszak and Leibowitz, 1990).

Clinical and epidemiological observations indicate that viruses and bacteria can cooperate to produce more severe respiratory disease in animals than occurs with either infection alone. Experimental studies of mixed infection in animals have confirmed this. The mechanisms involved have been probed, and it has been demonstrated that viruses can aid

bacteria in all aspects of their pathogenesis, namely, infection and penetration of mucous surfaces, growth in the host environment, interference with host defense, and the causation of damage to the host. Conversely, bacterial proteases may help viruses such as influenza virus to infect cells of the respiratory tract by cleaving the viral HA protien. To cause disease, microorganisms must infect mucous surfaces, penetrate into the tissues, grow in the tissue environment, inhibit host defense mechanisms, and cause damage to the host. Viruses can increase the ability of bacterial pathogens to achieve one or more of these steps (Smith, 1995).

It has long been recognized that secondary bacterial infections are a primary complication of viral respiratory tract infection. In all areas of the world, upper respiratory tract infections and their complicating sequelae are the leading causes of acute infectious morbidity in animal and human. These situations of mixed microbial superinfection often result in increased morbidity or mortality over that caused by the primary viral or bacterial infection and are found on synergistic processes in which specific diseases exist only if several microbes are present. Prior to having an understanding of the exact mechanisms that contribute to such microbial synergy, evidence of the etiology of viral-bacterial superinfections could be discerned from close examination of epidemiological data. These data often depict regular, and sometimes seasonal, patterns of peak periods of viral isolation (or detection of viral infection diagnostically) preceding those of bacterial disease (McIntosh *et al.,* 1993).

Moreover, commensal bacteria such as *Pasteurellaceae* which inhabit the upper respiratory tract of mammals are originated from which they are normally inhaled in small numbers and rapidly cleared by the mucociliary escalator of the trachea and large bronchi. For instance, one direct result of the mixing of animals from different farms of origin is the transmission of respiratory viruses from apparently healthy carriers to their naive penmates. Many of those viruses, which are known to predispose animals for development of pneumonic pasteurellosis, have direct effects on bacterial clearance mechanisms in the lung, affecting especially the ability of virus-infected alveolar macrophages to take up and kill inhaled bacteria (Laegreid *et al.,* 1989).

However, this clearance may be impaired when the animals are stressed by changes in weather, change in feed or housing, or transport. Endogenous release of stress hormones has been showed to increase mucus production and decrease ciliary activity, impair recruitment of neutrophils into pulmonary tissue, and reduce the phagocytic activity of resident alveolar macrophages (Frank, 1989).

The upper respiratory tract contains many normal flora that include *Streptococcus species, Haemophilus species, Neisseria species, Corynebacterium species, Staphylococcus species* and many anaerobes such as *Bacteroides*. The normal bacteria of a healthy animal can even be altered by several factors such as the nutritional and immunological status of the animal or the environment. The suppression of the normal bacteria frequently allows the development of potential pathogens, leading to the presentation of a variety of pathologies (Herthelius *et al*., 1989). Therefore, although the normal bacterial flora is generally harmless and beneficial to the host, they can cause diseases when the host defenses are impaired. Bacteria from the upper respiratory tract are washed downwards towards the lower respiratory tract, but the action of the ciliated epithelium and sticky mucus that covers the lining of the bronchial tubes keeps the lower respiratory tract free of these microorganisms.

Viruses may however, interfere with the ciliary function, allowing themselves or other microorganism invaders such as bacteria, to gain access to the lower respiratory tract (Doe, 2009). It has been proved that, the numbers of bacteria isolated from the nasopharynx and tonsillar regions increase during transport or similarly stressful changes in environment (Frank, 1989).Although, the mechanism for this enhanced bacterial colonization is not known, the result is an increase in the numbers of inhaled bacteria that is coincident with reduced nonspecific defenses (Laegreid *et al.,* 1989). Pneumonia can be induced experimentally with bacteria alone but only by direct pulmonary inoculation (intrabronchial instillation or transthoracic injection) using larger numbers of bacteria than are needed for aerosol or intranasal challenge following viral prechallenge (Martin *et al.,* 1998). Once the normal pulmonary function is impaired or pulmonary tissues are damaged, potential

bacterial pathogens that normally reside in the upper respiratory tract could establish a foothold to initiate respiratory disease. This is particularly true for *pasteurella* infection (Moiser, 1994).

Apart from the direct involvement of the microorganisms, the antigen specific immune response to the virus results in killing of virus-infected epithelial cells and macrophages, further compromising clearance and rendering the host extremely vulnerable. The importance of the antiviral immune response in facilitating bacterial pneumonia is clearly demonstrated in experimental models of bovine pneumonia in which the interval between viral challenge and bacterial challenge is critical for production of disease and corresponds directly to the point when antiviral antibodies are first detected in serum (Hodgins *et al.*, 2002).

Respiratory diseases are common in camel. Pathogenesis is multifactorial, and the diseases appear due to the interaction of infectious microorganisms (bacteria, mycoplasma, viruses and fungi), host defence, environmental factors (Lacasta *et al*., 2008) and stress (Roy, 1990 and Wikse and Baker 1996).Mode of infection and spread depends solely on the infectious agent. Despite low morbidity and mortality rates, the recovery period of affected animals is quite long. The negative impact on overall productivity could not be underestimated due to the long recovery period (Schwartz and Dioli, 1992).

2.4.6. Risk Factors

2.4.6.1. host factors

The relationship between a host and a pathogen is dynamic, since each modifies the activities and functions of the other. The outcome of such a relationship depends on the virulence of the pathogen and the relative degree of resistance or susceptibility of the host, due mainly to the effectiveness of the host defense mechanisms. Certain pathogens infect only certain species of animal. Although other explanations are possible, these observations might be explained by the existence of specific interactions between microorganisms and eukaryotic tissue surfaces which allow microorganisms to become established on the surface (Todar, 2009).

Even though there have not been evidences for host factors specific for camel respiratory diseases, studies have showed that sex and age are a significant factor in determining the susceptibility to and severity of pulmonary diseases in both humans and animals. A study conducted on murine respiratory mycoplasmosis for the development of acute or chronic inflammatory lung diseases has found that male mice were found more susceptible and develop serious pneumonic complex than the female did (Yancey, 2001). Other study conducted on bovine respiratory disease (BRD) by van der Fels-Klerx *et al*., (2000) in UK have concluded that there has been significant differences in susceptibility to BRD among different age groups of the animal. In this research, the study animals were grouped into three strata as <3, 3-6 and 6-24 months age range. The then study has reported the youngsters developed sever pneumonia, the middle age group developed mild problem outbreaks while no BRD outbreaks in the highest age groups. Furthermore, general truth of the hardness of camels only refers to adult camel. But very young camels are susceptible to several problems leading to high morbidity and mortality (Khan *et al*., 2003).

2.4.6.2. environmental and climatic factors

Respiratory diseases in food animals are due to complex of factors that often interact to produce disease. The management and environmental stress placed on these animals are often the decisive factors between the development of clinical diseases or the diseases remaining sub clinical. In camels, environmental and management stresses like overwork, undue exposure to cold winds, and rain, sudden change of climate, insufficient food have been listed to influence the susceptibility of the animal to respiratory diseases. Camels under other forms of stress such as overcrowding, unsanitary conditions and those suffering from other health problems and young stock are the classes most at risk to have respiratory diseases (Schwartz and Dioli, 1992. Chronic diseases particularly trypanosomosis play an important role in disarming the respiratory defense mechanisms through dehydration and increased level of circulating glucocorticoids as well as increasing in number and virulence of the agents (Radostitis *et al*., 2007).

The studies conducted particularly on camels of Borana origin have been carried out after taking them by track to high land. However, a study on camel (Camelus dromedary) indicated that transporting animal by vehicle induces stress on the animal (Baghshani *et al.*, 2010). Stress is of course one of the factors for camels to be affected by respiratory diseases (Bekele, 2010). Moreover, dust storms emerging during the first weeks of the rainy season in the African Sahel can also contribute to respiratory disease in camels. Hansen *et al*. (1989) described an outbreak of pneumoconiosis in Somali camels and reported the presence of large numbers of dust-laden macrophages in the lungs camels that had pneumonia. The crowding of pastoralist camels around limited watering points during the summer also contributes to the spread of respiratory pathogens as camels from different geographical regions often use the scanty open water sources (Bekele, 2010).

In Borana camels, several risk factors including sudden climatic changes as well as the stress of migration from the south to the north during early rainfall were reported to be associated with pneumonia. Higher incidence of respiratory infection during the wet season was associated with change of climatic condition from warm dry period to wet, pre-

disposing factors being draft, cold, rain, poor nutrition, as well as migration that may distress the animals. It has been suggested that the long treks undertaken by camels during the rainy season (Abebe, 1991; Schwartz and Dioli, 1992; Agab and Abbas, 1999) as well as the housing of camels in unsheltered pens (Chauhan *et al.*, 1987; Abbas *et al.*, 2002) are major predisposing factors in Borana.

2.4.7. Immune Responses in Camel Respiratory Diseases

The upper respiratory tract is continuously exposed to air that contains dusts, bacteria, fungi, viruses and various noxious agents and downward spread the bacteria which commonly colonize the nose and throat. Under normal conditions, the airways and the lung parenchyma prevent the entry of microorganisms and neutralize or remove infectious agents so that the lung contains very few, if any, organisms beyond the terminal lung units. Hence the effect of the infection here are more sever and more ominous than in the upper respiratory tract. The respiratory system has developed a series of defensive measures to counter against these agents. These complex protective mechanism of the upper and lower respiratory tracts are broadly categorized as physical defenses, cellular and secretory defenses (Melaku and Getachew, 2012).

2.4.7.1. physical defense mechanism

Histology of trachea of camel (*Camelus dromedarius*) studied using light, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) have revealed that, it consists of incomplete cartilaginous rings of hyaline. The lamina epithelium is composed of pseudostratified-ciliated columnar epithelium with many goblet cells of which functions are to protect the underlying tissues and to secrete mucus that line and trap pathogenic agents not to be accessible to the mucosal layer. Submucosal layers were loose connective tissue with many elastic fibres. Submucosal glands were tubuloalveolar with mucous (acidic and neutral) secretions that can kill microbes. Trachealis muscle was attached to the inside sheet of tracheal cartilage. Ultrastructural studies showed that surface epithelium is pseudostratified with mucus-producing goblet cells, ciliated and basal cells,

similar to other mammals. The ciliated cells contained many mitochondria, oval nucleus and many big granules. The cilia expel the agents not to enter the lower respiratory tracts. In scanning electron microscopy (SEM) studies, viscoelastic layers were observed on the epithelial surface of trachea, and there were highly condensed cilia under this layer (Raji and Naserpour, 2007).

2.4.7.2. humoral immunity

The immune system is a network of cells, tissues, and organs that work together to defend the body against attacks by "foreign" invaders. These invaders are primarily bacteria, viruses, parasites, and fungi. The immune system consists of two components called humoral and the cell mediated systems. The humoral system consists of antibodies (immunoglobulins) which are formed by the B lymphocytes. There are five classes of immunoglobulin (Ig). These are IgG, IgM, IgA, IgE and IgD. In the course of an infection, Immunoglobulin G is the predominate antibody in secondary immune responses and constitutes the defense against bacteria and viruses. Immunoglobulin M is the dominant antibody produced in primary immune responses and is very effective in defense against bacteria and viruses, while IgA is the main immunoglobulin in secretions of the respiratory tracts. It protects mucous membrane from attack by bacteria and viruses (Sproul *et al*., 2000). IgE production occurs locally in the nasal mucosa, without the involvement of lymphoid tissue (Takhar, 2005). IgD's function is to signal when the young B cells in the spleen are ready to be activated.

The humoral immune response of *Camelus dromedarius*, and llamas includes functional antibodies formed by two heavy chains and no light chains. The amino acid sequence of the variable domain of the naturally occurring heavy-chain antibodies reveals the necessary adaptations to compensate for the absence of the light chain. In contrast to the conventional antibodies, a large proportion of the heavy-chain antibodies acts as competitive enzyme inhibitors (Muyldermans and, Lauwereys 1999).

The survival of the animal depends on the successful body defense against microbial invasions. Despite a general reputation of camel for hardiness and resistance, which largely

is true for adult camels, very young camels are susceptible to several problems leading to high morbidity and mortality (Khan *et al*., 2003). The camel calf comes to life almost deprived of serum immunoglobulin and depends on colostrum for virtually all its humoral passive immunity. Failure to achieve adequate transfer of passive immunoglobulin has been associated with increased risk of diarrhea, respiratory affections and motility of neonates (AL-Sultan, 2008).

Passive immunity to many diseases is not transmitted to young camels via the placenta of the dam and therefore has to be acquired after birth. Colostrum does, however, carry antibodies to diseases to which the dam has been exposed and passively transfers resistance to the same diseases to the newborn camel. Many camel owners, especially in certain East African countries, do not allow the young to suckle the colostrum, considering it bad for them. This practice certainly contributes to the high morbidity and mortality rate, which may be as much as 40% before weaning (Khan *et al*., 2003).

2.4.7.3. cellular immunity

The other component of the immune system is cell-mediated immune system, which involves the T cells. The cell-mediated immune responses require that the T cells be presented with foreign antigen bound to host proteins. The cell-mediated immunity is central in host defense against intracellular pathogens such as viruses (Adair *et al*., 2000).

Respiratory virus infection may result in considerable lung injury, and host immune responses may be an important contributor to this. Important factors that determine the magnitude of immunopathologic tissue damage include the degree of distal distribution of infection into alveolar cells, the overall viral load, the magnitude of the T-cell responses, the effector mechanisms employed by the T cells, and regulatory mechanisms that may come into play. CD8+ T cells are important contributors to viral clearance, utilizing contact-dependent effector functions (Bruder *et al.*, 2006).

2.4.8. Diagnostic Methods

2.4.8.1. clinical methods

A further disadvantage of clinically diagnose camel respiratory diseases arises from imperfect knowledge of normal physiological values and their variations. Furthermore, the diseases of camelidae, are often difficult to deal with, having very similar and non-specific signs. Sero-muco-purulent nasal discharge, lacrimation, productive coughing, and abdominal breathing, with elevated body temperature, mostly characterize camel respiratory diseases due to infection. However, fluctuations are commonly observed in the body temperature of the camel, which is able to adjust its own body temperature. (41-42^{o}c). The species differences between the camel and other domestic animals necessitate some specific examination techniques. Young camels are examined in the standing position, while adults require restraint. Restraining procedures, both standing and in sternal recumbency, are described. New equipment and a crush were designed. The body temperature of the camels examined fluctuated from 35.7 to 38.9 degrees C, being lowest in the morning and highest in the afternoon; high temperature in the morning is indicative of fever, while high afternoon temperatures could be hyperthermia. It was difficult to take the pulse rate for routine procedures (Vogel, 2005).

The heart rate ranged from 35 to 50 per min; there was no difference between the heart rate in the morning and in the afternoon. The mean respiratory rate was 11 per min and respiration was of thoracol-lumbar type. The mucous membranes of the eye were an important site for appreciating signs of discoloration, while those of the mouth, rectum, and vagina were unsuitable. The left flank was the best site for determining the rate of rumen contractions, which was 3+/- .2 every 5 min, as determined by auscultation, counting the contractions by the application of the fist was difficult. The palpable external lymph nodes were the parotid, maxillary, prescapular, inferior cervical, thoracic, cubital, ilial and popiteal; they are large and can be seen on inspection in healthy animals, so that was not indicative of disease (Tefera, 2004).

Typical clinical signs of acute onset of lower respiratory diseases are a change in respiratory rate and depth, wheezing, coughing, uni-or bilateral nasal discharge (serous, purulent or haemorrhagic), increased temperature, anorexia, reluctance to move or work, hyperlacrimation, abnormal posture such as abduction of the elbows, extended neck, head to neck angle is wider than usual, swelling above the sinus frontalis. Abortion can occur in pregnant animals, particularly during mid pregnancy. Chronic cases of respiratory disease are characterized by weight loss and intermittent fever despite grazing. General immunodepression makes the affected animals more prone to other infections. Typical signs of respiratory diseases of viral origin are often masked by secondary bacterial invasion. Bacteriological and histological examination should be performed if clear-cut etiological diagnosis is needed (Schwartz and Dioli, 1992).

In animal with respiratory problem, there are many different types of nasal discharges on the bases of their consistency, contents, and involvement of the nostrils. Discharge can be serous (clear, watery), mucoid (yellow and mucous-like), purulent (green-yellow, thick, looks like pus), sanguineous (bloody), or contain feed material and saliva. Nasal discharge can be unilateral or bilateral, which helps to identify the source of the discharge. Discharge that is unilateral typically comes from the nasal passage, the sinus, or occasionally the guttural pouch. Discharge that is bilateral can arise from the guttural pouches, the pharynx, or the lower respiratory tract (trachea and lungs). Discharge can be acute in onset (within hours to a couple days) or chronic (lasting more than 2-3 days). Nasal discharge may be the only clinical sign (symptom) or there may be other clinical signs such as ocular discharge, enlarged lymph nodes (which is nonspecific and present with many types of nasal discharge), fever, cough, facial deformity, abnormal noise when breathing or exercising, exercise intolerance or poor performance, poor appetite, difficulty eating, lethargy, or weight loss. Sometimes nasal discharge can have a foul odor, which can be specific to certain types of bacterial infections, tissue damage, or sinus infections secondary to tooth root infections (Grenager, 2009).

2.4.8.2. laboratory methods

For prevention and control of diseases, early detection of cases and correct disease diagnosis is very important. In order to arrive at correct diagnosis, some clinical samples/specimens collected from live animals are analyzed in local hospital laboratory or sometimes, required to be sent to disease diagnostic laboratories for examination. Usually a swab in a protective plastic sleeve is used to take a throat for bacterial cultures and transported in transporting media. Nasal swabs are the easiest specimens to collect for respiratory bacteria and are also the best specimens (i.e., they contain the most virus) for the majority of the respiratory virus isolation. Swab samples need to be collected after cleaning and disinfecting the external part of the nose using 70% alcohol and put into sterile, screw capped, leak proof, containers containing about 3ml bacterial/viral transport media. Swab specimens intended for viral and bacterial isolation must always be kept cold and moist, which requires preparation ahead of time (Omer *et al.,* 2012).

Microorganisms such as bacteria must have a constant nutrient supply if they are to survive. When trying to grow microbes in the lab adequate nutrition must be provided using artificial media. Media may be liquid (broth) or solid (agar). Any desired nutrients may be incorporated into the broth or agar to grow bacteria. The optimum temperature at which most pathogenic bacteria prefer to grow is 37°C with appropriate atmospheric composition. Organisms grown in broth cultures cause turbidity, or cloudiness, in the broth. On agar, masses of cells, known as colonies, appear after a period of incubation. Certain techniques will allow bacterial cells to be widely separated on agar so that as the cell divides and produces a visible mass (colony), the colony will be isolated from other colonies. Since the colony came from a single bacterial cell, all cells in the colony should be the same species. Isolated colonies are assumed pure cultures (McKerrell, 2004).

One of the most fundamental tasks that someone working with microorganisms must perform is to identify microorganisms. The presence of pathogenic microorganisms can be confirmed by examination of smears, cultural and biochemical characteristics and detection by immunological and molecular methods (Hogg, 2005). There are a number of

ways that this can be done. Firstly, the microorganism can be stained and viewed under the microscope. This gives information regarding the shape (morphology) of the micro-organism. Another way of identifying microorganisms is to find out the reactions that the microorganism carries out. This is known as biochemical testing. Once the morphology and biochemistry of the micro-organism is known, identification keys and tables can be consulted to work out the identity of the micro-organism being investigated (Cappuccino and Sherman, 2002)

The cornerstone of bacterial genetics has been, until recently, the isolation of specific mutants, i.e. strains in which the gene concerned is altered (usually in a deleterious fashion). This alteration shows up as a change in the corresponding characteristics of the organism. It is this change in the observable properties of the organism (the phenotype) that is used to follow the transmission of the gene. The genetic nature of the organism (the genotype) is inferred from the observable characteristics (Dale and Park, 2004).

The search for suitable culture systems for viruses led to the discovery, that viruses could be grown on the chorioallantoic membrane of embryonated chicken eggs. The use of embryonated chicken eggs then became routine for propagation efforts, not only for avian viruses, but also for viruses infecting mammalian species. Viruses in most of the animal virus families can be grown in embryonated eggs, probably because of the wide variety of cell and tissue types present in the developing embryo and its environment. In some cases, embryonated eggs entirely replaced research animals for the growth of virus stocks, and if the viral infection resulted in the death of the embryo, this system also provided a quantitative measure of the amounts of virus in individual stocks (Fenner, 2011)..

Later, cell culture has largely replaced the egg system, which is labor-intensive and expensive, but the embryonated egg is still widely used for the isolation and growth of influenza viruses, in addition to many other viruses. Viral growth in cell cultures is most often detected based on the development of microscopically visible cytopathic effect (CPE). Some viruses, however, can grow to high titer without producing visible CPE and must be detected by other means. When viruses grow in cell culture, they often produce

changes in the infected cells, or cytopathic effect (CPE). A number of viruses reveal distinctive morphologic changes resulting from CPE that can be visualized microscopically and that allow their recognition in the culture with considerable confidence. Important characteristics of CPE include which cell culture types are affected, the timing and rate of progression, the distribution (focal, diffuse, limited to the margins of the cell culture), and the nature of the morphologic changes. The CPE may be sufficiently distinctive to allow unequivocal identification of the virus, but in other cases, techniques such as FA staining performed on cells scraped from the cell culture are used to confirm the presence of the suspected virus (Leland and Ginocchio, 2007).

The detection of viral antigens directly in clinical specimens has become an essential component of the methodologic repertoire of diagnostic virology. Antigen detection methods can provide diagnostic information within a few hours of the receipt of the specimen in the laboratory. The lack of requirement for virus viability is another important advantage over viral culture, especially when specimen transport time is prolonged or otherwise suboptimal. Antigen detection methods can be applied when the conditions. First, when viral antigen is expressed and present in an accessible specimen. Second, when an appropriate antibody (usually, but not necessarily, monoclonal) is available. Third, when antigenic variability does not preclude recognition by immunologic reagents of different strains of the target virus., Lastly, when the antigen being detected is sufficiently stable that it does not degrade during transport and processing of the specimen are met (Storch, 2001):

Methods used for viral antigen detection include FA staining, immunoperoxidase staining, and enzyme immunoassay (EIA). The presence of antibodies to respiratory diseases against organisms which are of major concern in other domestic animals but about which similarly little is known in relation to camel includes *influenza, parainfluenza,* and *pasteurella spp, Bovine virus diarrhea*, *Adenovirus* and *respiratory syncytial virus*. These antibodies are present over wide geographical areas and in varying proportions of animals (Khan *et al*., 2003).

Diagnostic virology is being revolutionized by the application of nucleic acid detection techniques. These techniques detect specific nucleic acid sequences and can be applied to the detection of virtually any virus. Depending on the target sequence, the assays can be specific for a single virus species or for a group of related viruses. The latter characteristic is particularly advantageous because it allows nucleic acid detection techniques to be applied to groups of viruses, for which antigenic diversity has precluded successful application of antigen detection techniques. In such cases, highly conserved region of the genome have been exploited to develop reverse transcription–polymerase chain reaction (RT-PCR) assays (for RNA viruses) that can simultaneously detect almost all serotypes of a virus (Chapman *et al.,* 1990).

The earliest attempts at nucleic acid–based diagnosis involved direct hybridization of nucleic acid probes to viral nucleic acids present in clinical specimens. Direct hybridization was never widely adopted because it lacked adequate sensitivity, requiring the presence of 10^4 to 10^5 copies of the target nucleic acid. The development of PCR and other nucleic acid amplification techniques overcame the sensitivity barrier and have led to the development of nucleic acid–based diagnostic tests for many viruses. Nucleic acid amplification assays are particularly attractive for viruses that are difficult or impossible to cultivate, viruses that grow slowly in culture, and viruses for which antigen detection cannot be applied because of antigenic diversity or because the level of viral antigen in clinical specimens is too low to permit successful detection. Because nucleic acid amplification techniques can often be performed on a small volume, they are also uniquely useful for specimens that may be available only in small amounts. Still another advantage of nucleic acid detection as a diagnostic method is the stability of DNA, so that detection of viral nucleic acids can be done even when conditions lead to loss of virus viability (Kainz, 2000).

Nucleic acid amplification assays can be divided into those that amplify target nucleic acid, those that amplify the probe itself, and those that amplify the detection signal (Storch, 2001). PCR is the prototype of target amplification assays. PCR employs short oligonucleotide primers and a thermostable DNA polymerase, such as Taq polymerase, to

amplify a segment of target DNA that is typically 100 to 1000 base pairs in length. The PCR itself includes 25 to 40 cycles, each consisting of denaturation, primer annealing, and extension steps that take place at different temperatures. An instrument called a thermal cycler that controls the temperature of the reaction controls progression through the steps of the cycle. After PCR amplification, gel electrophoresis or one of several probe-hybridization techniques, such as Southern blot, detects the PCR product, or amplicon. The analytic sensitivity of PCR can be as low as 1 to 10 copies of target DNA. Because of its simplicity and broad applicability, PCR has been widely used in viral diagnosis (Abd-Elsalam, 2003).

Another important target amplification technique is called transcription-based amplification. These techniques use three enzymes: reverse transcriptase (RT), RNase H, and T7 RNA polymerase to amplify a target RNA sequence, employing a series of reactions that mimic the retrovirus replication scheme. An advantage of transcription-based amplification assays is that they are isothermal and do not require complicated instrumentation. The assays begin with synthesis of a DNA strand that is complementary to the RNA target, using a primer that contains a T7 polymerase–binding site. The resulting DNA–RNA hybrid is converted to double-stranded DNA by the action of RNase H and a second primer that also contains a T7 polymerase–binding site. The double-stranded DNA product then serves as a template for transcription brought about by T7 RNA polymerase. The newly synthesized RNA transcripts serve as templates for additional cycles of the reaction. (Fenner, 2011).

Because Taq polymerase uses only DNA as a template, the use of PCR to detect viral RNA sequences requires the inclusion of an RT step before PCR. This can be done in the same reaction tube in which PCR will take place, and the combined reaction is called RT-PCR. The RT reaction can be performed using a devoted enzyme such as Moloney murine leukemia virus RT or avian myeloblastosis virus RT. Alternatively, heat-stable multifunctional enzymes, such as recombinant Thermophilus DNA polymerase, are available that can carry out RT as well as DNA polymerase reactions. Special care must be used in specimen processing because of the susceptibility of RNA to digestion by ribonucleases

that may be present in clinical samples. RT-PCR has been applied to the detection of numerous RNA viruses, including RSV, influenza and parainfluenza viruses, rotavirus, and other RNA viruses. RT-PCR can also be applied to the detection of viral messenger RNA (mRNA). This may be particularly useful in the diagnosis of infection caused by viruses that have a latent phase in their life cycle. For these viruses, detection of viral DNA might not distinguish between latent and productive infection, whereas detection of an mRNA expressed only in productive infection would be evidence of active viral infection. Transcription-based amplification assays are ideally suited for amplification of RNA (Fenner, 2011).

The measurement of antiviral antibodies was one of the first methods used for the specific diagnosis of viral infections and remains an important tool in the diagnostic virology laboratory. The role of serology may be for the diagnosis of acute or current infection or for the determination of immune status to specific viruses. Serologic diagnosis is important for viruses that cannot be readily cultured or for which culture is slow or otherwise impractical. Serology is uniquely useful for defining immunity with respect to specific viruses. It is important to use sensitive assays, such as EIA, for this purpose because antibody levels indicative of past infection may decline to low levels years after the infection. The specific assay used must also be selected with care to ensure that it measures antibodies that correlate with immunity (Storch, 2001).

2.4.9. Control and Prevention of Camel Respiratory Diseases

2.4.9.1. treatments

The treatment of camel respiratory diseases depends on the types of causative agents. Principal treatment of affected animals includes antimicrobial therapy, improved management practices such as better housing, hygiene, and good nursing care. High doses of long-acting broad-spectrum antibiotics have been recommended to be used in case of bacterial infections. In case of nasal airway obstruction due to purulent discharge, relief can be achieved by regular cleaning and flushing of the nasal cavity with saline solution. The might need caution because can be fistula formation between the sinus and the nasal

cavity is a common sequel in most cases of sinus infection. If treated early, prognosis is usually good (Schwartz and Dioli, 1992).

In practice, the use of drugs in camels, including dosage regimens and withdrawal times, has been mostly based on data obtained from other ruminants, mainly cattle. This approach may lead to irrational drug use in the camel, resulting in toxicity, or decreased e¡ectiveness. However, the last decade has witnessed a surge in pharmacological research in the camel, almost entirely in the field of pharmacokinetics. This stemmed from the need to establish drug dosage regimens in the camel, as in most cases drug manufacturers give no specifc dosage recommendations for this species. (Alquarawi and Ali, 2000)

In Ethiopia, Oxytetracycline and Penstryptomycin are the most frequently used medicines for camel respiratory diseases caused by bacteria, Albendazole and Ivermectin for vermic pneumonia. As some respiratory diseases such as Haemorrhagic septicaemia (HS) also called pasteurellosis are acute and quite often fatal disease, early treatment is essential. Treating with antibiotics such as amoxycillin, tetracyclines or sulphonamides are recommendable. Recently being used more effective treatment is the injection, popularly known as Doctor Jin. It is injected IM at the rate of 1ml/10 kg body weight for HS (Khan *et al*., 2003).

However, the uncontrolled distribution and usage of antibiotics to treat bacterial livestock infections in the Horn of Africa is likely to contribute to the emergence and transmission of antimicrobial resistance genes and requires further investigation. For instance, an important pathogen affecting humans and livestock species, *Streptococcus agalactiae*, a Group B *Streptococcus* (GBS) isolated from African camel have been found 34% resistant to Oxytetracycline (Fischer *et al*., 2013).

2.4.9.2. prevention of camel respiratory diseases

Vaccines are a common way to provide individual and herd immunity to a variety of bacteria or viral agents. Vaccines is needed used as complement, but ought not be as replacement of good management techniques for disease prevention such as biosecurity, herd health checks, nutrition, stocking densities, etc. There is a lot of confusion about

vaccines especially for camelids since there are not any developed specifically for these species. All of the vaccines used in camelids have been developed for cattle, small ruminants, and horses. Therefore using these products is considered "off label" in camelids and the manufacturers do not guarantee effectiveness and safety. Some vaccine against camel respiratory disease causing viruses such as *Equine herpes virus-1* (Rhinopneumonitis) has been recommended only for specified geographical area.

3. MATERIAL AND METHODS

3.1. Study area

The study was conducted in four selected districts (Yabelo, Arero, Dirre and Moyale) of-Borana Zone of Oromia Regional State from November 2013 to June 2014. Borana Zone is located at altitude and longitude of $03^0$37' 23.8" to 05^0 02' 52.4" North and 37^0 56' 49.4" to 39^0 01' 101"East respectively in the Southern part of Ethiopia. Boran zone is located at about 600 km South of Addis Ababa with altitude of 970 masl in the south bordering Kenya to 1693 masl in the Northeast.The area receives a mean annual rainfall of 500 mm3 in the South to over 700 mm3 in the North and annual temperature ranging from 19 ºC to 24 ºC. The area is generally a semi arid whose topography gave a characteristic climatic condition that is conducive for the adaptation of camel (Bekele, 1999).
Generally, the Borena area (Annex 6) represents a vast lowland area of southern Ethiopia covering an area of about 95,000 km^2. The area is bordering with Kenya to the South, Somali region of Ethiopia to the East, Guji zone to the North and Southern nations and Nationalities and People region (SNNPR) to the West. The Borana plateau gently slopes from high mountain massifs in the North (1650 m. a.s.l) to the South bordering Kenya (1000 m.a.s.l) with slight variation due to central mountain ranges, and scattered volcanic cones and craters (Coppock, 1994).

The climate is generally semi-arid with annual average rainfall ranging from 300 mm in the south to over 700 mm in the north. The rain pattern is bimodal type with the main rainy season locally “ganna” extending from March to May and small rainy season “haggaya” from mid-September to mid-November. Annual mean daily temperature varies from 190C to 240C with moderate seasonal variation. The other two seasons are the cool dry season “adoleessa” extending from June to August and the warmer dry season “bonna” December to February. Seasons affect herding strategies due to its effect on forage and water source availability (Coppock, 1994). Consequently, herd splitting is practiced to cope up with shortage of resources during dry season (Demeke, 1998, Desta, 2000).

The vegetation is dominated by savannah type containing mixture of perennial and woody plants. The savannah community varies from open grassland to bush encroached areas. There is shift in composition in response to heavy grazing, browsing, burning, and drought. Grazing shifts the community to more trees where as browsing and burning favors the grass. Several plant species in the area are recognized as valuable livestock forage. Acacia are the dominating bush species in the area (Coppock, 1994; Desta, 2000).
Surface water is a serious problem in the area. Traditional deep wells "ellas", ponds, perennial springs, permanent rivers (Dawa and Genale), and seasonal sources (streams, ephemeral ponds and shallow wells) are water sources for both human and livestock. Deep wells and large ponds (machine excavated) are used in dry seasons while seasonal streams, ephemeral ponds, and shallow wells are used in wet seasons (Helland, 1982).

Animal husbandry is characterized by extensive pastoral production system and seasonalmobility. Cattle are the dominating animal species followed by goats, camels and sheep.Camel and cattle herd splitting into mobile "forra" and home-based "warra" is practised asstrategy to mitigate forage and water shortage (Desta, 2000). Camel herd movement may bemoving the whole herd to water point and to relatively high altitude where green forage isavailable or partial herd away from home base (Demeke, 1998). Table 1 shows thedistribution of camels and other livestock in Borana, and the number of camels of the zone was 199,993 (BZPDO, 2013). For the current study, Yabelo and Arero were selected from comparatively mid highland areas whereas, Dirre and Moyalerepresenting low land areas.

3.2. Study animal

According to data obtained from BZPDO (2013), there were 23,326, 35,165,19,286 and 13,305 camels being kept in Yabello, Arero, Dire, and Moyale districts respectively (Table.1). A total of 199,993 camel population existed in Borana was considered as study population.

Table 2: Number and distribution of livestock in the districts of Borena zone in 2013 (BZPDO, 2013)

	Species and number of Livestock							
Districts	cattle	Goat	Sheep	Camel	Donkey	Mule	Horse	Poultry
Dugda Dawa	303316	207359	107139	21929	28019	1959	131	60307
Yabello	238032	98867	39140	23326	2880	380	525	40141
Taltalle	230866	188371	66939	1884	9193	148	0	37541
Dire	153650	59083	27767	19286	4386	808	1092	8875
Moyale	49040	38476	11544	13305	2672	13	2	35202
Dilo	116543	66708	97422	7204	14122	450	100	76
Miyo	61093	54000	20000	7012	3600	800	215	14114
Malka Soda	160475	124288	65000	48320	12310	2096	102	92230
Arero	175000	80758	58467	35165	5774	1403	23	11800
Dhas	173661	67891	22631	22562	23951	320	150	100
Total	1661676	985801	516049	199993	106907	8377	2340	300386

Source: BZPDO (2013)

3.3. Study Design, District and Herd Selection and Sampling Strategy

This study has two components: investigation of the possible viral causes of respiratory problems and isolation of bacteria from respiratory tracts at field and abattoir. Four districts were selected purposively due to easier accessibility by vehicle, camel population, and security (there was ongoing conflict among tribes in the zone during sample collection). Similarly, peasant associations (PA's) were also purposively selected for the same reasons. PA is the lowest administrative unit within a district that was considered during the sample collection. Camel herds in 3 PA`s from Yabelo, 1 from Arero, 3 from Dire and 1 from Moyale were sampled. Households were randomly selected and on average 22 camels from a household were sampled. All clinically sick animals regardless of their sex

and age difference have been sampled after confirming that it had not been treated with antibiotics recently. All age groups have been included in the sampling group. A total of 175 nasal swabs for bacteriological study and 35 nasal swab samples for viral isolation have been collected during sampling time.

3.4. Sample Collections and Laboratory Analysis

3.4.1. Questionaire Survey

One hundred twenty four randomly selected camel owners from the four districts were interviewed by using structured questions along with sample collection. The questionnaire consisted of 25 questions and were pre-tested on some camel owners (Anex 1).
The information gathered among other include, herd composition, the major livestock health problem including camel and the presence of camel suddon death.

3.4.2. Field Swab Sample Collection

Randomly selected camels from a herd were examined for presence of respiratory signs and their history was asked and sampled for bacterial isolation. From camels with respiratory signs, a pair of samples, one for bacterial (if not commenced antibiotics) and the other for viral isolations were collected.Briefly, the external part of the nose of the animal to be sampled was cleaned using cotton wool moistened with 70% alcohol. A 15-inch long sterile swab was gently inserted into the nasal cavity. It was then rubbed andgently rotated against the wall of the nostrilto obtain sufficient amount of microbes. The swab was put into the tubes to which 3ml Stourt's transport medium or Viral transporting medium had been added based on the type of agent suspected to be isolated. The test tube containing the swab was labeled with date, Sample ID, and respiratory health status of the sample, kept in icebox containing ice pack, and transported to Yabelo Regional Veterinary Laboratory within four to six hours. The swab samples for bacterial isolation were stored at -20^{O}C while those for viral isolation were stored at -70^{O}C in refrigerator until they were processed according to the protocol stated in OIE (2012).

The samples for bacteriological examinations were transported to the Microbiology Laboratory of College of Veterinary and Agriculture (CVMA)Addis Ababa University(AAU). Whereas, Samples for viral isolation were taken to the virology laboratory of National Veterinary Institute (NVI) and stored accordingto OIE (2012) until processing. The samples were stored as same condition as stated in OIE (2012).

3.4.3. Swab Sample Collection From Abattoir

Nasal samples were collected from Borana camels slaughtered in Akaki slaughter house, Addis Ababa abattoir enterprise from February to April 2014. Before slaughter, general physical examination was conducted on each camel in the lairage for the presence of respiratory signs. After slaughtering, *postmortem* examination was made by visual examination and thorough palpation of the trachea, lungs, and the associated bronchial lymph nodes for the presence of any lesion. Sampling was done following the procedures given by OIE (2012). The external part of the nose of the animal was cleaned using cotton wool moistened with 70% alcohol. A 15 inch long sterile swab was inserted into the nasal cavity. It was then rubbed and rotated against the wall of the nostril. With regard to trachea and lung swabs, the surface of the tissue to be sampled was burned by hot scalpel blade. The area was incised with sterile scalpel blade. The inner part of the area was then swabbed using 15 inch sterile swab. The swab samples were put in to test tube in which about 3 ml brain hearth infusions (Annex 2) had been added. The test tube containing the swab was labeled with date of collection, sample ID and organ from which the sample was collected. Samples were then transported to the laboratory under cold chain in icebox to the Microbiology Laboratory of College of CVMA, AAU, Bishoftu.

3.4.4 .Bacteriological Isolation and Identification

The swab samples from field were inoculated into Brain Heart Infusion (BHI) broth for the primary enrichment and then incubated aerobically for 24 hours at 37^0C. While samples from the abattoir were directly incubated because they were collected in test tubes containing BHI at similar temperature with the field samples for 18-24 hours. The growth in BHI was streaked onto sheep blood agar enriched with 5-7 % (V/V) sheep blood in such a way that cultured broth samples were thoroughly agitated and mixed. A loop full of the cultured broth was streaked onto agar plate (Annex 2).

All cultured media were incubated aerobically at 37°C for 24-48 hrs. The inoculated plates were checked for growth with 18-24 hours interval for maximum 48 hours. Mixed colonies were subcultured on blood agar to obtain similar colony. Primary bacterial identification of the isolates was conducted based on colony characters such as colony morphology, colour, size, elevation, types of haemolysis on blood agar. Gram stain characteristics of the pure colony was examined in order to determine the Gram reaction, cellular morphology and arrangement (Annex 4). The gram negative rod bacteria were subcultured on MacConkey agar (Annex 2). Garam positive cocci bacteria were in turn differentiated by catalase and Oxidation-Fermentation (OF) tests and were identified to genera level. Pure colonies were transferred to further secondary biochemical tests. Different tests on agar media, broth sugar and salts have been used for biochemical tests to reach on definitive species of certain genera of the isolates. The bacterial isolation and identification were conducted following standard procedures described by Quinn *et al.* (1999) and Jousimies-Some *et al.* (2002) for identification of cultured bacteria to genera level and species level (Annex 3 and 5).

3.4.5. Drug Sensitivity of Bacterial Isolates

The methods used to determine drug sensitivity profile of the isolates were according to the guidance described by Quinn *et al*. (1999) and Cockerill *et al.* (2012). Disc diffusion method on Mueller- Hinton agar was used to study the antimicrobial susceptibility profile

of *Streptococcus spp, Staphylococcus spp, Pasteurellaspp* and *Manhemiahemolytica* isolates. As the cultures used were old, the log method was used to prepare inoculums suspension. Briefly, the surface of 3 to 4 pure similar appearance colonies of the identified bacteria were touched with a sterile loop and inoculated into BHI broth to make a suspension. The suspension was incubated at 37^{O}C aerobically for 2 to8 hours. The production of moderate turbidity was examined by comparing the turbidity with 0.5 McFarland standards, which was prepared by adding 0.5ml of 1.17% $BaCl2H_2O$ solution to 99.5ml of 0.36N sulfuric acid in the laboratory.

Inoculation of the plate, using the inoculum prepared by the above methods, was carried out by saturation of a sterile cotton swab with the bacterial suspension. The bacterial suspensions were streaked evenly over the surface of the Muller Hinton agar medium with the swab (Annex 2). Muller Hinton agar plates were enriched with 5% sheep blood for *Streptocuccusspp*isolates. The agar medium was streaked in three directions and the swab was run around the circumference of the plate to ensure that the entire surface is covered. The inoculated plates were allowed to dry for 15-30 min at room temperature before application of the antimicrobial discs.

Commonly used antimicrobial discs Chloramphenicol (30µg/disc), Trimethoprim- Sulphamethoxazole (1.25/23.75µg/disk), Ampicillin (10µg/disc), Nalidixic Acid (30µg/disk),Penicillin (10 units/disc), Doxacillin (30µg/disk), Cloxacillin (1 µg/disk), Vancomycin (30 µg/disk) and Streptomycin (10 µg/disk) were placed on the inoculated agar surface with sterile forceps by taking care that the discs no closer than 10 to 15mm to the edge of the plate and sufficiently separated from each other. The discs were pressed gently to ensure the contact with the surface of the agar. The plates were incubated at 37^{O}C within 15 minutes after placing the discs on the surface of the inoculated agar. The plates were incubated aerobically upside down. The zone of inhibition for each drug wasmeasured after overnight incubation (18-24 hours) and interpreted.

3.5.6. Viral isolation and Characterization

Most viruses can be grown in different type of cell cultures and can be seen their destructive effect in infected cell culture which provide a better understanding about various viral infections under laboratory conditions. In order to isolate and determine cell distraction nature, called cytopathic effects (CPE) of viruses in the samples collected at field level, the following procedure has been used.

After 2% GMEM media, calf serum and PBS were Kept warm at 37^{o}C in water bath, they were put on light, cleaned and disinfected laminar flow hood. The media from monolayer formed cells was discarded and 0.5ml or 1ml from sample suspension was inoculated, into 25 or 75cm^2 VERO cell culture flasks respectively. The inoculated flasks were incubated at 37^{0}c for one hour to adsorb the viruses on to the cell. The suspension was discarded from monolayer formation inoculation and then washed with PBS and lastly 2% GMEM media was added. The inoculated cell cultures were incubated at 37^{O}C after the stopper had been closed loosely. Inoculated samples were daily observed by using inverted microscope for any contamination or growth. After 2 days, the media was changed and repeated every two days for growth negative samples. Viral growth on the cell was checked every day with the microscope and was waited until 10 days. The cultures were read and the interpreted results were recorded on record book.

3.5.7. Molecular Identification method

Five viral cultures with CAPE, which were indicative for the presence of Parainfluenza were tested by molecular method (PCR). Parainfluenza virus was identified by molecular techniques to determine the presence and types of the virus involved in the respiratory diseases of camels in the study area. The viral nucleic acid extraction method used during this study was mainly based on viral capsid purification techniques described previously (Allander *et al.,* 2001, Nanda *et al.,* 2008).

The growth medium was removed from the cell culture and 0.6ml lysis buffer with 2-mercaptoethanol was added. The cell pellet was vortexed until the pellets were dispersed and the cell appear lysed. The lysates were homogenized at room temperature using rotor-stator homogenizer for at maximum speed 45minutes. The homogenates were centrifuged at 26000 xg for 5minutes and then the supernatant was transferred to RNase-free tube.

The RNA was purified by adding equal amount of 70% ethanol to the volume of cell homogenate. The alcohol-homogenate suspension was vortexed thoroughly to mix and disperse any visible precipitate that may form after ethanol addition. Seven hundredµl of the sample including any remaining precipitate was transferred to the spin cartridge. Then it was centrifuged at 12000 x g for 15 seconds at room temperature and the flow-through was discarded and the pin cartridge was reinserted into the same collection tube. This step was repeated until the entire sample has been processed. The spin cartridges were washed by wash buffer II three times.

The RNA was eluted by adding 100µl RNase-free water to the center of the spin cartridge, and incubated at room temperature for 1minute. The spin cartridge was centrifuged for 2 minutes at 12000xg at room temperature to elute the RNA from the membrane into the recovery tube. The purified RNA was stored at -80^{O}C until PCR amplification process will be carried on.

3.5.8. Complementary DNA synthesis

The following procedure was used to convert viral RNA into first strand cDNA. The components (sample, primer, dNTP mix and DEPC treated water) were mixed and briefly centrifuged before use. The mixture was incubated at 65^{O}C for 5minutes and then placed on ice for about 1min.

The cDNA synthesis mix was prepared by adding in the order of 10XRT buffer, 25mM $MgCl_2$, 0.1MDTT, RNaseOUT^{TM}(40U/µg), and SuperScript^{TM} IIIRT. cDNA synthesis

mix was added to RNA/primer mixture, mixed gently and collected by brief centrifugation. It was then incubated at $50^{O}C$ for 50 minutes. The reactions were terminated by placing at $85^{O}C$ for 5min and chilled on ice. The reactions by brief centrifugation. RNase H was added and to each tube and incubated for 20 min at $37^{O}C$. cDNA synthesis reaction was stored at $-20^{O}C$ until used for PCR.

3.5.9. cDNA Amplification

Parainfluenza virus 3 (PIV3) was investigated using PIV3 specific forward (PIV3PR5) and reverse (PIV3PR3) primers sequences: 5'- GATCCACTGTGTCACCGCTCAATACC -3' and 3'-ACCAGGAAACTATGCTGCAGAACGGC-5'), respectively (Osiowy, 1998). PCR reaction was carried out in a total volume of 20µl in a 0.2 ml reaction tube containing 2 µl RNase free water, iQ^{TM} supermix (2x) which contained antibody-mediated hot-start iTaq DNA polymerase, dNTPs, $MgCl_2$, enhancers and stabilizers, forward and reverse PIV3 primers 2 µl each, and Template (cDNA) 4 µl. Briefly, the iQ^{TM} supermix (2x) and other frozen reaction components were thawed to room temperature and were thoroughly mixed and centrifuged briefly to collect solution at the bottom of the tubes and were stored on ice protecting from light. Assay master mix was prepared one for all sampls by adding all required components together except the DNA template according to the volume aforementioned. The assay master mix was thoroughly mixed to ensure homogeneity and equal aliquots were dispensed into each quantitative PCR (qPCR) tube.

In all of the work good pipetting practice has been employed to ensure assay precision and accuracy. DNA templates were then added to the PCR tubes and the tubes were cealed with flat caps. The tubes were vortexed for 30 at least 30 seconds to ensure thorough mixing of the reaction components. The tubes were spinned to remove any air bubbles and the reaction mixture was collected in the vessel bottom. The thermal cycling protocol on the PCR instrument (machine) was programmed in such a way that first polymerase activation and DNA denaturation was carried out at $95^{O}C$ for 5 minutes for 1^{st} cycle. Denaturation at $95^{O}C$, anneling at $65^{O}C$ and extension at $72^{O}C$ for 30 seconds

were carried out. These steps were repeated for 15 cycles. Lastly, denaturation at $95^{O}C$ and anneling at $62^{O}C$ were carried out for 30 seconds each and extension was done at $72^{O}C$ for 5 minutes. These steps were in turn repeated for 20 cycles.

3.5.10. PCR product analysis by gel electrophoresis

The PCR products were analyzed with 1.5% agarose gel containing specified volume of gel red. Briefly, 10µl of PCR products mixed with loading buffer and loaded to wells in pre-prepared gel and run at 130 volt for 1:10 hours in parallel with DNA 100 bp molecular weight marker in electrophoresis apparatus using 1x TAE buffer. The DNA band was visualized by UV illumination and the size will be determined by the DNA molecular weight marker standard. The expected size of the amplicon is 234 bp.

3.6. Data Entry and Management

Questionnaire and biological data were stored in Excel® spreadsheets. Data analysis was done using statistical packages SPSS (statistical package software ver. 20 for Windows, SPSS Inc, Chicago, IL). The data were first validated, and then descriptive analysis was conducted. To study association between variables chi square test and logistic regression analysis were used. The confidence level was set at 95% (α=0.05).

4. RESULTS

4.1. Case History and Clinical Observation

Total of 34 camel herds (175 animals):12 herds (50 animals) from Yabelo, 4 herds (16 animals) from Arero, 16 herds (71 animals) from Dirre and 2 herds (38 animas) from Moyale were sampled. Of the examined camels, 37.7% of them were from the mid-high land (name of the ditricts) and the rest 62.3% were from the low-land areas (nameof the ditricts). In visual inspections, 33.7% of the samples were from camels with one or more respiratory signs including coughing, dyspenia, nasal discharge, increased respiratory rate (21-25 per min), and some of them were in poor body condition and weak and remained laid down (Annex 8). The symptoms were found to be associated with districts ($P<0.05$).

Observation of respiratory signs do not significantly associated with sex, age and parity level ($p>0.05$), but it does with districts ($p<0.05$) (Table 3). Dirre camels were the least affected group and Arero's were the highest one with odds ratio of 4.420 taking the least affected group as reference. However, as ages increase the proportion of the respiratory disease symptoms seemed slightly increase with rates of 26% in camels with age of less or equals to three years, 36% in age group of above three years but less or equals to eight years, and 39% in age group above eight years.

Table 3: Univariate logistic regression analysis of association of risk factors with respiratory signs

Factors		Examined (n=175)	Camels with respiratory signs (%)	Odds Ratio	95% CI		p-value
					Lower	Upper	
Districts	Dirre	71	16 (22.5)	Ref			
	Yabelo	50	20 (40.0)	2.29	1.03	5.06	0.041
	Arero	16	9 (56.2)	4.42	1.42	13.73	0.010
	Moyale	38	14 (36.8)	2.00	0.84	4.75	0.114
Age	<3	58	15 (25.9)	Ref			
(years)	3-8	69	25 (36.2)	1.62	0.75	3.50	0.212
	>8	48	19 (39.6)	1.87	0.82	4.28	0.134
Sex	Male	40	14 (35.0)	Ref			
	Female	135	45 (33.3)	1.07	0.44	1.95	0.845
Parity	No calf	42	10 (23.8)	Ref			
	1-3	58	25 (43.1)	1.87	0.77	4.550	0.170
	>4	35	16 (45.7)	1.89	0.71	5.074	0.206

4.2. Isolated and identified bacteria

4.2.1. Isolated bacteria from field samples

From175nasal swabs collected at Borena,140 (80%) showed bacterial growth on culture and identified to genera level. *Staphylococcus spp, Streptococcusspp and Pasteurella spp* were identified to species level (Table 4).*Staphylococcusaureus* and *Streptococci pyogen* were the most frequently recovered Gram positive bacteria with rates of 21.7% and 14.3% respectively while*Pasterella maltocida* (22.3%) and *E. coli* (20.6%) were the predominant Gram negative isolates.The isolation frequiences for most of the isolates significantly associate with health status of camels (Table 5). *Staphylococcus aureus, S. hyicus,Streptococcus equi subsp.equi, S. equi subsp.zooepidermicus, , S. pnuemoniae, S. pyogenes,Corynebacterium spp, Mannhemia hemolytica, Pasteurella maltocida* and *E.coli* were found to associate significantly with respiratory signs ($p>0.05$). But*Bacillus spp Actinomyces spp.,* and *Micrococcus spp* other *Pasteurella spp* other than *P. maltocida* were not found to associate with respiratory problems.

Table 4: Bacterial isolates from camel nasal swab collected from Borana

Isolates (Gram positive)	**Frequency (%)**	**Isolates (Gram Negative**	**Frequency (%)**
Staphylococcus aureus	38 (21.7)	*Mannhemia hemolytica*	22 (12.6)
S.epidermidis	14(8.0)	*Pasteurella maltocida*	39 (22.3)
S. hycus	9 (5.1)	*Pasteurella spp* other than *p. maltocida*	9 (5.1)
Micrococcus spp	10(5.7)	*E. coli*	36 (20.6)
S.equi subsp equi	10 (5.7)	*Proteus spp*	5 (2.9)
S. equi subsp zooepidemicus	16(9.1)		
S. pneumoniae	8 (4.6)		
S. pyogen	25 (14.3)		
Bacillus spp	7 (4.0)		
Actinomyces spp	9 (5.1)		
Corynebacterium spp	17 (9.7)		

Table 5: Univariate logistic regression analysis of association between bacterial isolates and respiratory problems

Bacterial agent (s)	Respiratory Symptoms	No. of Isolates (%)	Odds Ratio	95% CI		p-value
				Lower	Upper	
S. equi subsp.equi	Healthy	3 (2.6)	Ref			0.022
	Sick	7 (11.9)	5.07	1.26	20.39	
S. equi subsp. zooepidemicus	Healthy	6 (5.2)	Ref			0.015
	Sick	10 (16.9)	3.74	1.29	10.87	
S .pnuemoniae	Healthy	2 (1.7)	Ref.			0.025
	Sick	6 (10.2)	6.45	1.26	33.03	
S. pyogenes	Healthy	10 (8.6)	Ref			0.004
	Sick	15(25.4)	3.61	1.51	8.66	
Pasteurella spp	Healthy	4 (3.4)	Ref			0.168
	Sick	5 (8.5)	2.59	0.67	10.04	
Proteus spp	Healthy	4 (3.4)	Ref			0.519
	Sick	1 (1.7)	0.48	0.53	4.42	
S. aureus	Healthy	10 (8.6)	Ref			0.000
	Sick	28 (47.5)	9.57	4.19	21.86	
S. hiycus	Healthy	3 (2.6)	Ref			0.046
	Sick	6 (10.2)	4.26	1.03	17.71	
S. epidermidis	Healthy	11 (9.5)	Ref			0.318
	Sick	3 (5.1)	0.53	0.137	1.91	

Continued

Bacteria	Respiratory Symptoms	No. of Isolates (%)	Odds Ratio	95% CI		p-value
				Lower	Upper	
Corynebacterium spp	Healthy	6 (5.2)	Ref			0.007
	Sick	11 (18.6)	4.201	1.469	12.017	
Bacillus spp	Healthy	4 (3.4)	Ref			0.604
	Sick	3 (5.1)	1.500	0.325	6.934	
Actinomyces spp	Healthy	5 (4.3)	Ref			0.488
	Sick	4 (6.8)	1.615	0.417	6.252	
Mannhemia hemolytica	Healthy	10 (8.6)	Ref			0.031
	Sick	12 (20.3)	2.706	1.093	6.702	
Pasteurella maltocida	Healthy	17 (14.7)	Ref			0.001
	Sick	22 (37.3)	3.463	1.463	7.236	
E.coli	Healthy	16 (13.8)	Ref			0.002
	Sick	20 (33.9)	3.205	1.507	6.815	
Micrococcus spp	Healthy	6 (5.2)	Ref			0.666
	Sick	4 (6.8)	1.333	0.361	4.921	

Most of the bacteria were isolated regardless of sex, age and parity of the camels. However, the isolation frequency of *Streptococcus pneumonia* is higher in camels below three years than camels older than 8 years. However, most of the isolates were recovered at highest rate from camels of age between three and eight years compared to the two age extremity groups. The recovery rate of *Streptococcus equi subspp equi was* significantly associated with Districts ($p<0.05$): low rate from Yabelo (0%) and Moyale (5.3%) but high rate from Dirre (18.8%) and Arero (10%).

4.2.2. Isolated bacteria from Slaughter house samples

The bacteria isolated from abattoir samples are summarized in Table 6. Except *S. epidermidis* which was not isolated from lung, *Corynebacterium spp.,E.coli, S.aureus* and *kllebseilla spp* which were not isolated from trachea, *S. intermedius* and *S.pneumoniae* which were isolated only from lung, other bacteria were isolated from the three parts of the respiratory tracts. The distribution of bacterial isolation significantly decreased with advance from nasal tract to lung (P<0.05). Each isolates with their corresponding organs are showed in Table 7. Table 8 summaries the comparison of bacteria isolates from field nasal swabs with abattoir nasal swabs. *S. epidermidis, Enterococcus spp* and *Klebsiella spp* were isolated only from abattoir samples where as *S. pneumoniae* was not isolated from nasal swab collected from abattoir.

Table 6: Logistic regression analysis of bacterial isolates from different parts of camel respiratory tracts of abattoir samples

Organs	**Cultured sample**	**Positive Samples (%)**	**Odds Ratio**	**95% CI**		**p-value**
				Lower	**Upper**	
Nasal	196	177 (90.3)	4.209	2.40	7.380	0.000
Trachea	196	153 (78.1)	1.608	1.02	2.21	0.040
Lung	196	135 (68.9)	Ref			

Table 7: Bacterial isolates from respiratory tracts of abattoir samples

	Number (%) of isolates (n=196)				Number (%) of isolates (n=196)		
Isolates	**Nasal**	**Tracheal**	**Lung**	**Isolates**	**Nasal**	**Tracheal**	**Lung**
Staphylococcus aureus	42 (21.4)	0 (0.00)	10 (5.1)	*Enterococcus spp*	24 (12.2)	66 (33.7)	45 (23.0)
S. huyicus	14(7.1)	4 (2.0)	13 (6.6)	*Bacillus spp*	40 (20.4)	18 (9.2)	10 (5.1)
S.epidermidis	6 (3.1)	8 (4.1)	0 (0.0)	*Corynebacterium spp*	6 (3.1)	0 (0.0)	11 (5.6)
S. intermedius	26 (13.3)	0 (0.0)	0 (0.0)	*Actinomyces*	12 (6.1)	0 (0.0)	0 (0.0)
Micrococcus spp	22 (11.2)	12 (6.1)	6(3.1)	*Mannhemia hemolytica*	28 (14.3)	18(9.2)	22(11.2)
Streptococcus pyogens	47 (24.0)	24 (12.2)	30 (15.3)	*Pasteurellaa maltocida*	16 (8.2)	22 (11.2)	30(15.3)
S. equi subsp equi	14 (7.1)	16 (8.2)	14 (7.1)	*E. coli*	30 (15.3)	0 (0.0)	4 (2.0)
S. equi subsp zooepidemicus	67 (34.2)	23 (11.7)	35 (17.9)	*Proteus spp*	14 (7.1)	4 (2.0)	1 (0.5)
S. pneumoniae	0 (0.00)	0 (0.00)	13 (6.6)	*Klebsiella spp*	14 (7.1)	0 (0.0)	4 (2.0)

Table 8: Bacterial isolates from Nasal Swabs Collected at Field and Abattoir Level (n=175 and 196 respectively)

				C.I. 95%		
Bacterial Isolates	**Sample source**	**Positive Sample (%)**	**Odds Ratio**	**Lower**	**Upper**	**p-value**
Staphylococcus aureus	Abattoir	42 (21.4)	Ref			0.947
	Field	38 (21.7)	1.017	0.620	1.669	
S. hyicus	Abattoir	14 (7.1)	1.419	0.598	3.364	0.427
	Field	9(5.1)	Ref			
S.epidermidis	Abattoir	6 (3.1)	Ref			0.043
	Field	14 (8.0)	2.754	1.034	7.331	
S. intermedius	Abattoir	26 (13.3)				
	Field	0 (0.0)				
Micrococcus spp	Abattoir	22 (11.2)	2.086	0.959	4.539	0.064
	Field	10 (5.7)	Ref			
Streptococcus pyogens	Abattoir	47 (24.0)	1.893	1.108	3.233	0.020
	Field	25 (14.3)	Ref			
S. equi subsp equi	Abattoir	14 (7.1)	1.269	0.549	2.935	0.577
	Field	10 (5.7)	Ref			
S. equi subsp zooepidemi-	Abattoir	67 (34.2)	5.161	2.853	9.337	0.000
cus	Field	16 (9.1)	Ref			
S. pneumonia	Abattoir	0 (0.0)				
	Field	8 (4.6)				

Continued

Bacterial Isolates	Sample Source	Positive Sample (%)	Odds Ratio	C.I. 95%		p-value
				Lower	Upper	
Enterococcus spp	Abattoir	24 (12.2)				
	Field	0 (0.0)				
Bacillus spp	Abattoir	40(20.4)	6.154	2.678	14.142	0.00
	Field	7(4.0)	Ref			
Corynebacterium spp	Abattoir	6(3.1)	Ref			
	Field	17 (9.7)	3.407	1.312	8.848	0.002
Actinomyces	Abattoir	12 (6.1)	1.203	0.494	2.927	0.684
	Field	9 (5.1)	Ref			
Mannhemia hemolytica	Abattoir	28 (14.3)	1.159	0.636	2.112	0.630
	Field	22 (12.6)	Ref			
Pasteurellaa maltocida	Abattoir	16 (8.2)	Ref			
	Field	39 (22.3)	3.226	1.730	6.015	0.000
Pasteurella spp	Abattoir	0 (0.0)				
	Field	9 (5.1)				
E. coli	Abattoir	30 (15.3)	Ref			
	Field	36 (20.6)	1.433	0.840	2.445	0.187
Proteus spp	Abattoir	14 (7.1)	2.615	0.922	7.417	0.071
	Field	5 (2.9)	Ref			
Klebsiella spp	Abattoir	14 (7.1)				
	Field	0 (0.0)				

The results of isolates with association of lung lesion were indicated in Table 10.*Staphylococcus spp, Streptococcus spp, Micrococcus spp, Bacillus spp* and *Crynebacterium spp* were Gram positive isolates from lung lesion whereas *Mannhemia hemolytica, pasteurella maltocida, E.coli* and *Kllebseilla spp* were the Gram negative isolates. There is significant difference in isolation rates between lesioned and normal lungs for both Gram positive and Gram negative bacteria (P<0.05). Table 9 depicts the association between isolation rate and lung status.

Table 9: Chi square test of association between isolation frequency of bacteria and lung status

Isolates	**Lung**				
	Normal(%)	**Lesion (%)**	**Pearson Chi-square**	**df**	**p-value**
Enterococcus spp	1 (1.8)	44 (31.4)	19.871	1	0.000
S. equi subsp zooepidemicus	0 (0.00)	35 (25.0)	17.043	1	0.000
S.equi subsp equi	0 (0.0)	14 (10.0)	6.013	1	0.014
S. pyogen	0 (0.0)	30 (21.4)	14.169	1	0.000
S.pneumoniae	0 (0.0)	13 (9.3)	5.569	1	0.018
S. aureus	0 (0.0)	10 (7.1)	4.215	1	0.040
S.hyicus	1 (1.8)	12 (8.6)	2.974	1	0.085
Micrococcus spp	0 (0.0)	6(4.3)	2.476	1	0.116
Bacillus spp	2 (3.6)	8 (5.7)	0.379	1	0.538
Corynebacterium spp	1 (1.8)	10 (7.1)	2.167	1	0.141
Mannhemia hemolytica	0 (0.0)	22 (15.7)	9.913	1	0.002
Pasteurella maltocida	0 (0.0)	30 (21.4)	14.169	1	0.000
E. coli	0 (0.0)	4 (2.9)	1.633	1	0.201
Proteus spp	1 (1.8)	0 (0.00)	2.513	1	0.113
Kllebseilla spp	0 (0.0)	4 (2.9)	1.633	1	0.201

4.3. Drug Susceptibility Tests of the Isolates

A total of 101 isolates (i.e. 8 *Pasteurella maltocida,* 24 *Manhemia hemolytica,* 47 *Streptococcus spp* and 22 *Staphylococcus spp)* were tested for antibiotic susceptibility to determine their drug susceptibility profiles *(Table 10)*. *Pasteurella maltocida* and *Manhemia hemolytica* showed highly resistant to streptomycin and cloxacillin at rate of 100% to each, however, *Pasteurella maltocida* were susceptible to chloramphenicol and trimatoprim-salphomethaxazole than to other drugs. *Streptococcus spp* were highly resistant to Ampicillin, Cloxacillin, and Penicillin at rates of 97.6%, 90.9% and 89.9% respectively. But they were relatively susceptible to Doxacillin at rate of 42.6%. *Staphylococcus spp* were found resistant to penicillin (90.5%) and cloxacillin (86.4%), but 54.5% of them was susceptible to doxacillin.

Table 10: The results of the antibiotic sensitivity test of *Streptococcus spp, Staphylococcs spp, M.hemolytica and P. maltocida* isolates from camel respiratory tracts

Drug	**Susceptibility**	**Isolates (%)**			
		Streptococcus spp	***Staphylococcus spp***	***M. hemolytica***	***P. maltocida***
STR	Susceptible	2 (4.3)	N.A.	0 (0.0)	0 (0.00)
	Intermediate	2 (4.3)	N.A.	4 (16.7)	0 (0.00)
	Resistant	43 (91.4)	N.A.	20 (83.3)	8 (100)
DOX	Susceptible	20 (42.6)	12 (54.5)	N.A.	N.A.
	Intermediate	3(6.4)	5 (22.7)	N.A.	N.A.
	Resistant	24 (51.1)	5 (22.7)	N.A.	N.A.
NAL	Susceptible	17 (36.2)	7 (30.4)	N.A.	N.A.
	Intermediate	14 (29.8)	10 (43.5)	N.A.	N.A.
	Resistant	16 (34.0)	6 (26.1)	N.A.	N.A.
PEN	Susceptible	4 (11.1)	2 (9.5)	N.A.	N.A.
	Intermediate	0 (0.0)	0 (0)	N.A.	N.A.
	Resistant	32 (89.9)	19 (90.5)	N.A.	N.A.
CLOX	Susceptible	0 (0.0)	3(13.6)	0 (0.0)	0(0.0)
	Intermediate	3 (9.1)	0 (0.0)	6 (25)	0 (0.0)
	Resistant	30 (90.9)	19 (86.4)	18 (75)	8 (100)
VAN	Susceptible	15 (35.7)	N.A.	N.A.	N.A.
	Intermediate	27 (64.3)	N.A.	N.A.	N.A.
	Resistant	0 (0.0)	N.A.	N.A.	N.A.

Cont'd

Drug	Susceptibility	Isolates (%)			
		Streptococcus spp	***Staphylococcus spp***	***M. hemolytica***	***P. maltocida***
AMP	Susceptible	1 (2.4)	10 (45.5)	N.A.	N.A.
	Intermediate	0 (0.0)	3 (13.6)	N.A.	N.A.
	Resistant	41 (97.6)	9 (40.9)	N.A.	N.A.
CHL	Susceptible	N.A.	N.A.	10 (41.7)	5 (71.4)
	Intermediate	N.A.	N.A.	3 (12.5)	0 (0.0)
	Resistant	N.A.	N.A.	11(45.8)	2 (28.6)
SXT	Susceptible	N.A.	N.A.	14 (58.3)	7 (87.5)
	Intermediate	N.A.	N.A.	3(12.5)	0 (0.0)
	Resistant	N.A.	**N.A.**	7 (29.2)	1 (12.5)

N.A.=Not applied**,** DOX= Doxacillin, STR= Streptomycin, AMP=Ampcillin, NAL=Nalidixic acid, PEN= Penicillin, CLOX=Cloxacillin, VAN=Vancomycin, CHL=Chloramphenicol, SXT=Trimethoprim-sulfamethoxazole

4.4. Viral Isolation and Identification Results

4.4.1. Viral Isolation

Thirty five samples taken from camels with respiratory signs were cultured for viral isolation on confluent grown VERO cell monolayer. Thirteen of them (42.9%) exhibited morphologic alterations (CPE) on VERO cell monolayer (Table 11).

Table 11: Results of viral isolation on VERO cell monolayer and chi-square test of association with sex and age of camels

Factors		**Tested Samples**	**CPE Positive (%)**	**Person chi-square**	**df**	**p-value**
Sex	Male	11	4 (26.7)	0.276	1	0.599
	Female	24	11 (73.3)			
	Total	35	15 (42.9)			
Age	$\leq$ 3 years	10	4(26.7)	1.789	2	0.471
	3<x$\leq$ 8 years	15	5 (33.3)			
	> 8 years	10	6 (40)			
	Total	35	15 (42.9)			

The presence of virus in the nasal swab samples was evidenced by initial swelling and rounding of infected VERO cells. The most predominant and frequently observed type of CPE was the aggregation of infected cells with syncytia formation Fig.1

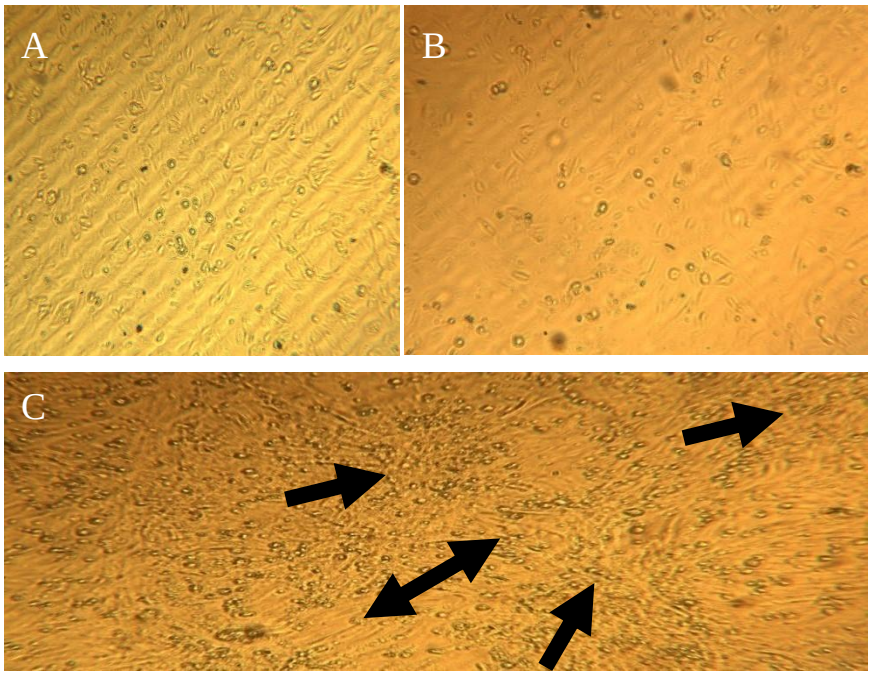

Figure 1: Digital photography of CPE formation of the viruses on VERO cell monolayer.

(A): Not inoculated (B): No CPE formation (C): CPE positive:- sample (⟶) syncytial formation, (⟷) ploughing.

4.4.2. Molecular Identification of Isolated Viruses

Total of Five CPE positive samples were tested for the presence of *Parainfluenza virus* 3 genome. All samples tested were positive for *Parainfluenza viruses* 3 (Figure 2).

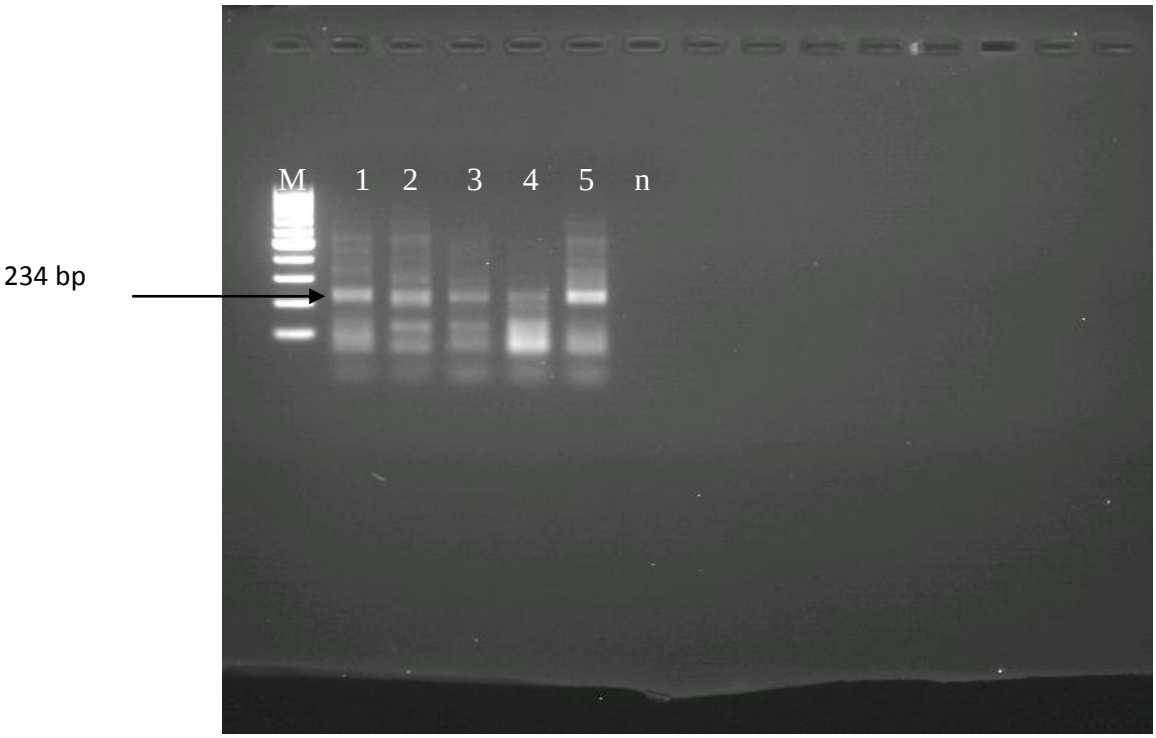

Figure 2: Digital photography of Gel red stained agarose gel electrophoreses PCR amplified PIV3 products samples analyzed by UV. M= Molecular Weight Ladder, **1-5=** Samples, n= negative control

4.5. Results of questionnaire survey

A total of twenty four structured questionnaires were delivered to one hundred Borana pastoralists in order to assess general possible sources and managements of camel respiratory diseases in the study area. Family heads or member were interviewed at their localities (Hola).The pastoralists in the study area keep 16 camels on average, of which 46.5 are adult male.

Majority of the pastoralists were illiterate and only 5% of them completed secondary school. Their camels were most of the time kept separately during day and night, even though, they had other livestock such as cattle, shoats and equines.As camels are considered as most resistant animal for feed shortage, none of them had the habit of storing feed for their camels. Risk factors thought to be associated with camel respiratory diseases and sudden death described by the respondents are as summarized in Table 13.

Table 12: Summary of the findings of questionnaire survey regarding possible risk factors for respiratory diseases in camels of Borana, Southern Ethiopia

Questions	**Categories**	**No. of Respondents (%)**
Keeping camels with others	Keeping separately	99 (99)
	With cattle	0 (0.0)
	With Shoats	1 (1)
	With Equines	0 (0.0)
	With Pets	0 (0.0)
Staying camels in kraal at night with others	No	98 (98)
	With cattle	0 (0)
	With Shoats	1(1)
	With Equines	1(1)
	With Pets	0 (0)

‘Continued’

Questions	**Categories**	**No. of Respondents (%)**
Camel wild life interaction	Yes	100 (100)
	No	0 (0.0)
New comer camel disease	Yes	97 (97)
	No	3(3)
Age	Adult	78 (78)
	Young	30 (30)
Sex	Male	28 (28)
	Female	75 (75)
Production status	Pregnant	59 (59)
	Lactating	67 (67)
Suspicion from where the new camel disease come from	Somali Region	68 (68)
	SSNP	0 (0.0)
	Guji Zone	1(1)
	Kenya	6(6)
	Within Borana	1(1)
	Unknown	28(28)

According to the respondents, major diseases include CCPP, CBPP, FMD, Black leg, circling diseases, sudden death, respiratory diseases, botulism, external parasites and diarrhea were the most repeatedly mentioned ones (Annex 7).

The respondents' complains, indicated that there were more than fifty camel diseases based on the signs observed, camel respiratory diseases were highly complained by 79% of the respondents followed by camel pox with rate of 41%. The major symptoms of camel respiratory diseases observed by the pastoralists were coughing, sneezing, dyspenia, unilateral or bilateral nasal discharge, lacremation, anorexia and weakness.Young camels remain laid down (Anex 8). Other camel diseases known in the study area were abortion, botulism, puss, tetanus and trypanosomosis.

But 13% of them called the disease with different names locally given to it. 6% of them called it Dhaqachisa, 4% Dhiba Dhiga, 2% chalisa and 1% said Gagabsa which mean that 'abruptly laid down', 'blood pressure', 'silent killer' and 'coma' respectively. Although majority of the respondent (87%) did not observe any sign that the animal die as soon as laid down, 13% of them observed some signs such as coughing, dyspenia, bloating, profusion of saliva, stiffiness of the neck and increasing of milk yield than usual for lactating camels.

Table 13: Camel respiratory disease stage, and control and prevention methods by pastoralists in Borana

Question	**Categories**	**No. of Respondents (%)**
Status of camel respiratory disease	Common	79 (79)
	Not common	21 (21)
Ethno-drugs	No	23 (23)
	Mineral salt	75 (75)
	Plant product	4 (4)
	Animal product	2 (2)
	Mixed	89 (89)
	Branding	6 (6)
Result of Ethno-drug	No response	0 (0)
	Aggravation	1(1)
	Cure	99 (99)
Vaccination given	Yes	0 (0.0)
	No	100 (100)
Clinic taking habit	Yes	41(41)
	No	59 (59)
Veterinary drugs given by local farmers	Yes	99 (99)
	No	1(1)

Out of one hundred respondents, fifty nine of them did not take sick camels to veterinary clinic even though for 56% of them veterinary clinics are situated with 8km of their area. There was no association found between clinic distance and taking sick camels to clinic ($p>0.05$) (Table 15). All of the respondents explained that they have been administering for their animals by themselves. The drugs mostly available around were Oxytetracycline (10% and 20% concentration), Penstreptomycin, Intradine (Sulphadimadine),Ivermectine and Albendazole (2500mg and 300mg). The pastoralists explained that they have been giving 10 ml of Oxytetracycline, Penstreptomycine and Sulphadimidine (Intradine) for all

adult large animals such as camel, cattle and equines and 5ml for young large animal and small ruminantsregardiless of their body weight and reproductive status of the animal.

5. DISCUSSION

5.1. Case history and clinical observation

In the present study, frequently observed camel respiratory signs were coughing, sneezing, dyspenia, unilateral or bilateral nasal discharge, and increase in respiratory rate (18-20 per minute). Bekele, (2010) explained that respiratory infections are common in Borana camels. The disease appeared as acute and chronic forms. The acute form, locally called "Furi", could show mild or severe symptoms. The mild form is characterized by sneezing and serous nasal discharge whereas the sever form is characterized by coughing with profusion of purulent nasal discharge. The chronic form locally named as "Dhukuda" revealed as long time coughing, decreasing feed intake and emaciation.

The scientific suggestions given to an outbreak of camel respiratory diseases with signs as dullness and depression, nasal blockage with thick scar formation on nasal orifice opaque in color nasal discharge, coughing, mouth breathing and gradual losing of body weight in Pakistan in 2011 was that respiratory viral infection with bacterial complication. This assumption was by taking into account the contagiousness nature of the outbreak. However, a definitive diagnoses could only be reached laboratory method (Kakar 2011).

With agreement to this, similar symptoms were observed by Bekele, (1999) in Somali region of Ethiopia on an outbreak of camel respiratory disease. Respiratory disorder concurrent with high fiver with 90% morbidity and 5-70% mortality has also been determined by Roger *et al*. (2000) in Ethiopian camels and by Abraham *et al*. (2005) in camel, cattle and sheep in the same country. The signs observed in our study are similar with the outbreak occurred in Borana in 2007 (Bekele, 2010). Moreover, camels in Saudi Arabia showed signs of cough, bronchitis, mucopurulent discharge, inappetance, fever, pulmonary consolidation and rales on auscultation due to fungal infection (Na'ma *et al*., 2011).

The present study does not demonstrate association between host risk factors such as age, sex and parity with respiratory signs. However, there is slightly increase with the ages of the camels; 25.9% in camels up to three years, 36.2% in camels between three and eight years and 39.6% in camels above eight years. Our observation agrees with findings of Bekele (2010). The reason for the decrement of the respiratory sign rate withage could be due to the fact that the young camels might not develop an infectious component to its etiology or as the result of prevalence of reactive airway obstruction associated with allergy to molds and spores, which increases with age (Dixon *et al.,* 1995).

In this study, significant association was observed between the respiratory diseases and districts in which Dirre camels were less affected compared with other districts. Moyales', however, were roughly as two times affected as Dirres'. The crowding of pastoralist camels around limited watering points during dry season has been suspected to contribute to the spread of respiratory pathogens and hence prevalence difference can occur among camels living in similar ecology as camels from some areas often use the scanty open water sources. Other risk factors including sudden climatic changes as well as the stress of migration from the south to the north during early rainfall were reported to be associated with respiratory problems of camel in Borana (Bekele, 2010).

5.2. Bacterial Isolates

Detailed investigation was carried out to determine bacterial composition inhabiting the upper respiratory tract of apparently healthy and sick camels. Out of 175 nasal swabs collected from field and 196 respiratory tract swabs (nasal, trachea and lungs) from abattoir, 140 (80%) and 177 (90.3%) respectively yielded at least one type of bacteria. As a result, a total of 274 (representative of 274 different colony morphologies) isolates from 140 field samples and 422 (representative of 422 different colony morphologies) isolates from 177 abattoir samples were obtained. This reveals that more than one bacteria were recovered per study animal. On average 1.96 and 2.4 bacteria were seen per sample (animal) respectively for the two sample category (abattoir and residential area) i.e. a corresponding bacterial burden per animal was about 2 in this study.

Variety of bacterial species was found to colonize the nasal passageways of apparently healthy camels. Out of 116 apparently healthy camels, 82 (70.7%) of nasal swab samples collected from field were positive for bacterial growth. Fifty eight (98.3%) of samples from clinically sick camels yielded bacterial colony of which majority of them were similar types of isolates with those isolated from apparently health camels. The invariable isolation of these organisms from the nasal cavity of apparently healthy and clinically sick camel in this study reflects their possible role in respiratory syndrome. The normal bacteria of healthy individual animal can be altered by several factors such as the nutritional and immunological status of the animal or the environment.. The suppression of the host immunity frequently allows the advancement of opportunistic bacteria to potential pathogens, leading to the presentation of a variety of pathologies (Azizollah *et al.*, 2009).

The bacterial yield of the current finding is higher than the previous bacterial isolation rate from camel respiratory tracts by Moustafa (2004) who isolated bacteria from camel with respiratory problem and the normal ones at rates of 88% and 28.88% respectively. Even though the recovery rates of Mustafa (2004) was less than the current isolation rates, higher proportion of bacteria were recorded in clinically sick than the healthy in both studies. The result was also in agreement with previous findings of Yimer and Asseged (2007) who recovered different bacteria from sheep nasal swab and got similar result with the current research.

Both Gram negative and Gram positive bacterial genera were found to be associated with the respiratory symptoms in samples from the field .Out of 157 bacterial isolates from camels with respiratory signs, 97 (61.8%) were Gram positive and 60 (38.2%) were Gram negative. However, of 117 bacteria isolated from apparently healthy camels, 66(56.4%) were Gram positive and 51(43.6%) were Gram negative. In general, the isolation rate of Gram positive bacteria (60%) was significantly higher than Gram negative bacteria (48.6%) ($P < 0.05$). Bacteria in apparently healthy animal can become opportunistic pathogens during stress to animals and thus resulting in endogenous infection. Predomin-

ance of Gram-positive bacteria from diseased camels might show their significant role in being opportunistic camel respiratory pathogens as similar to equines stated by Mir *et al.*, (2013). The current finding is in agreement with previous reports by Azizollah *et al.*, (2009) from camel respiratory passage way, Mir *et al.*,(2013) and Omer *et al., (2012)* from equines and sheep nasal swabs respectively.

The results of this study showed the presence of *P. multocida* and/or *M. haemolytica*, *Staphylococcus spp*, *Streptococcus spp.* and other bacteria in healthy and clinically sick camels. This may support the previous bacteriological evidences by Awol *et al.*, (2011), Mohammed (2011) and Ahimed 2010) for the involvement of multiple agents in causing camel pneumonia in Ethiopia. It has been repeatedly determined that bacterial infections are among the main causes of pneumonia in camels (Alhendi, 2000 and Seddek, 2002).

Moreover, in conjunction with viruses such as parainfluenza viruses, influenza viruses and morbili viruses, most of these bacteria have previously been incriminated to cause camel sudden death (CSD) (Yigezu *et al.*, 1997, Bekele, 1999 and Roger *et al.*, 2001). In support of previous studies, the current study isolated *Pasteurella maltocida, Streptococci equi subspp equi* and *E.coli* from an active case sudden death in Kawa pastoralist association of Arero district. El-Moez *et al.* (2013) stated that respiratory disease important bacteria that have been incriminated to cause sudden death include Gram positive bacteria such as *Staphylococcus aureus, Streptococcus equi* and acid fast bacilli, and Gram negative bacteria such as *P. multocida, P.pneumonia* and *E.coli*. Sudden death in farm animals may occur due to several causes, among which, bacterial causes are the most predominant. Age, immune status, pregnancy, nutritional, management factors and altitude may affect the response of animal when subjected to sudden death causing bacteria. It is not uncommon to detect pulmonary mixed infection since the animal respiratory air passages act as reservoirs for potential pathogenic microorganisms, which develop pneumonia on the onset under stress factors, poor sanitation and climatic conditions (Moustafa, 2004).

The predominant species among the isolates from nasal swab samples collected from field were *Streptococcus pyogen* (14.3%), *Staphylococcus aureus* (21.7%), *Mannhemia hemolytica* (12.6%), *Pasteurella maltocida* (22.3%) and *E. coli*(20.6%). Whereas *Enterococcus spp*. (23.0%), *Streptococcus equi subsp zooepidemicus* (21.3%), *Streptococcus pyogen (17.2%), Bacillus spp.(11.6%), Pasteurella maltocida* (11.6%), *Mannhemia hemolytica (11.2%),* and *S. aureus* (8.8%) were the most frequently recovered bacteria in abattoir swabs from different parts of repsiratort tract. In addition to these bacteria,S. *epidrmidis, S. intermedius, S. hyicus, Micrococcusspp., Streptococcus equi subsp equi, S. pneumonia, Corynebaxterium spp., Actynomyces spp. Proteus* and *Klebseilla spp* were also isolated. Studies have also isolated similar bacteria from nasal passage way of camels (Alhendi 1999, Azizollah *et al.,* 2009 and Ismail *et al.,* 2014). This finding was also in agreement with previous findings who isolated those bacteria from nasopharyngeal as commensals residing in upper respiratory tracts and are all capable of causing infection when the body defense mechanisms are impaired (Zamri-saad *et al*., 2006).

Although the types of the pneumonia were not identified, in the current study, out of 196 lungs of slaughtered camels examined, 135 (68.9%) had at least one lesion of which 129 (95.6%) yielded bacteria on cultured media. Various bacterial agents were found associated with the lung lesions. Both Gram negative and Gram positive bacteria were isolated from the lesions. Except *S. epidermidis* which was not isolated from lung, *Corynebacterium spp., E.coli, S.aureus* and *kllebseilla spp* which were not isolated from trachea, and *S. intermedius* and *S.pneumoniae* which were only isolated from lung, the majority of the isolates colonized all the anatomical sites investigated. *Enterococcus spp, Streptococcus equi subsp equi, S. equi subsp zooepidemicus, S. pyogen S. pneumoniae* and *Staphylococcus aureus* were the Gram positive isolates found to assocaite significantly with lung lesion ($p<0.05$). But among Gram negative isolates only *Mannhemia hemolytica* and *Pasteurella maltocida* had significant association with the lesion ($P<0.05$). Other bacteria isolated from lung lesion but not significantly associated include, *S.hyicus, Micrococcus spp, Bacillus spp, Corynebacterium, E.coli,* and *Kllebsiella spp*. *Proteus spp* was isolated from normal lung but not from the lesion (Table 10).

Our finding was in agreement with previous reports that *Staphylococcus aureus*, *Streptococcus spp.*, *Pasteurella spp.* and *Staphylococcus spp*. were frequently isolated from pneumonic lungs of camel (Abubakar*et al.*, 2010, Awol*et al.*, 2011, Mohamed and Abdelsalam, 2008). Moreover, Al-Tarazi (2001) and Azizollah *et al.*, (2009) isolated most of these bacteria from normal camel lung tissues. However, the isolation of the latter group from lesioned lung tissue, without significant isolation rate between normal lung tissue and lung lesion, suggests that they represent the normal flora. However, under conditions of stress, poor sanitation, and immunosuppression, they may involve in pneumonia in camels.

In comparison with this findings, different researches have isolated similar bacteria from tonsil (Azizollah *et al.*, 2009) and trachea of camel (Azizollah *et al.*, 2009). Other studies also isolated these bacteria from upper and lower respiratory tracts of domestic animals such as donkeys (Gutema *et al*., 2009), horses (Mir *et al.*, 2013), sheep (Yimer and Asseged 2007, Omer *et al.*, 2012), and goats (Megra *et al.*, 2006). Azizi *et al., (*2013), in their investigation of bacteria causing lower respiratory tracts, isolated *Pasteurella multocida* and *S. aureus* from sheep lungs affected with interstitial pneumonia and stated them as being possible secondary invaders and/or environmental contaminants because viruses had been suspicious to be normally the etiologic agents of this type of pneumonia. However, according to Al-Tarazi (2001), bacteria such as *M. haemolytica*, and *Pseudomonas aeruginosa* might be the main cause of chronic bronchopneumonia in camels while *E. coli* and *Klebsiella* spp are considered as secondary invaders.

Staphylococcus aureus was among the frequently isolated bacterial species recovered from nasal swabs collected at field level at rate of 21.7% and 21.4% at abattoir level. The odds ratio of the sick camels to have *S. aureus* is 4 times more than that of the healthy one. *S. aureus* was isolated at rate of 21.4% from nasal swab and 5.1% from lung samples but it was not recovered from trachea. It was recovered from lung lesion at a rate of 7.1% which is higher than that of Al-Tarazi *et al., (2001)* finding who isolated the bacteria at a rate of 4% but the current finding is in comparison with Abubakar *et al,*. (2010) while less than that of Moustafa (2004), Awol *et al., (2011)* and Abo-Elnaga and Osman,

(2012) who isolated *S. aureus* at rates of 7%, 18.64%, 21.1% and 28.6% from lung lesion respectively. With agreement with the report of Abubakar *et al.,* 2010, *S. aureus* was associated with lung lesion in this study ($p<0.05$).

S. aureus has also been frequently isolated from lung lesion and nasal swab of different animals including camels with respiratory problems (Al-Tarazi, 2001, Awol *et al.*, 2011, Muhammad *et al.*,2012, Kebede and Gelaye, 2010). Moreover, Wood *et al.,* (2005) have reported it as a secondary bacterial pathogen in horses suffering from primary viral infection of respiratory tracts and stated it as the most common pneumonic bacteria isolated from lung tissue. It has also been isolated from respiratory tracts of health sheep (Yimer and Asseged 2007) and from upper respiratory tracts of donkeys apparently healthy and clinically with respiratory problems (Gutema *et al.,* 2009).

The predominance of *Staphylococcus aureus* in this study was in agreement with other findings who have isolated it from different respiratory organs of camels. In their research on pneumonic camel lung Abo-Elnaga and Osman, (2012) and Abubakar *et al.,* (2010) have isolated *Staphylococcus aureus* predominantly. The high isolation rate of the bacteria summed up with the possibility of it to be recovered from the healthy one is indicative for the opportunistic involvement in camel respiratory diseases.

The results of bacterial isolates from nasal swabs collected at field level indicated that there was mixed infection in camel respiratory diseases. *S.aureus* was isolated concurrent with *S.equi subsp equi* (10.7%), *S.equi subsp zooepidemicus* (21.4%), *S.pneumoniae* (14.3), *S.pyogen* (35.7%), *Corynebacterium spp* (28.6%), *Actinomyces spp* (7.1%), *Mannhemia hemolytica* (10.7%), *P. maltocida* (35.7%) and *E.coli* (32.1%). Wood *et al*. (2005) explained that there was significant correlation between the presences of bacterial species, consistent with clinical observations that mixed infections in the presence of disease were common in equine respiratory disease symptoms.

Moreover, the current study found that 80% of *S.aureus* isolates from lung lesion were found mixed infection with *Streptococcus spp.* of which 40% were with *S.pyogens* and *S.*

equi subsp zooepidemicus. It was also isolated mixed with *Corynebacterium spp* (20%), *Actinomyces spp* (100%) and *Pasteurella maltocida* (20%). This finding was in accordance with Abo-Elnaga and Osman, (2012), Taha *et al., (* 2007*)*and Sayed and Zaitoun (2009) who reported pneumonic mixed pathogens have been mainly associated with *S. aureus* and other organisms, which demonstrate the complexity of the disease where *S. aureus* may predispose the animals to infection by other pathogens. Those researches isolated *S.aureus* in mixed infection of lung lesion with *S. pyogens, Corynebacterium pyogenes, Escherichia coli, Pasteurella multocida, Klebsiella species, Bacillus spp*. and *Actinomyces pyogenes* but at lower rate than the current results. Pneumonia in other animals due to mixed infection caused by *staphylococcus spp* and many researchers have reported it with other bacteria. Momin *et al* (2011) isolated *Staphylococcus spp* mixed with *pasteurella spp* in goats with respiratory problems at rate of 24%. Berge *et al.* (2006) isolated *P. multocida* and *P. hemolytica* from the respiratory tract of sheep and goats. Yimer and Asseged (2007*)* also reported *Staphylococcus* spp*, E. coli, Corynebacter* spp*, Klebsiella* spp and *Bacillus* spp in the respiratory tract of sheep.

Apart from the respiratory infection, *S.aureus* has also been incriminated in causing different diseases in different animal and human being. it is more common for infections to result in many manifestation of disease in various animal and human being such as endocarditis, pneumonia, skin infections, bone infections, sepsis, food poisoning have been mentioned (Klein *et al.*, 2007). It has been recovered from camel mastitis in Ethiopia (Husein *et al.*, 2013), in Nigeria (Muhammad *et al*., 2012) and in Keniya (Younan 2001). Furthermore, Abdul-Gader *et al.* (2005) reported *Staphylococcus aureus* to be the main pathogenic bacteria found in camel milk.

Except *S. aureus*, most of *staphylococcus spp* isolated from camel respiratory tracts in previous study have been reported as a general *Staphylococcus spp.* and so that difficult to refer each species. In this study, other *Staphylococcus spp.* isolated include *Staphylococcus hyicus, S. intermedius* and *S.epidermidis*. The coagulase positive *S. intermedius,* and the coagulase-variable *S. hyicus* are important pathogens of domestic animals (Quinn *et al.,* 2004).

In current study, *S. hyicus* was isolated at rates of 5.1% from nasal swab collected at residential area and 7.1 from nasal swabs of abattoir samples. It was also isolated from trachea and lung samples of slaughtered camels in this study at rates of 2.0% and 6.6% respectively. It was recovered both from 8.6% of lung lesion and 1.8% of normal lung swabs from abattoir. *S. hyicus* has been known with association with mastitis of different animals. Abdul-Gader *et al.,* 2005 has isolated it from clinical mastitic cases of Ethiopian camels. It was isolated from camel urine from Sudan by Ahmed *et al.* (2008). Its significance is with exudative epidermitis in pig. It has also isolated from bovine mastitic milk but its pathogenicity is doubtful (Quinn *et al.,* 2004).

S. intermedius was isolated from nasal swabs collected abattoir at rate 13.3% but was not from swabs collected at residential area. It was only recovered from nasal swabs. This finding was higher than Mir *et al.*(2013) report who isolated *S.intermedius* at rate of 9.65% from upper respiratory tracts of equines. This bacteria was not isolated from lung lesion in this study. They isolated the bacteria at higher rate from apparently health equines than from diseased ones. Boguta *et al.* (2004) did not find any variation between the two cases in foal upper respiratory tracts. It has also been reported as one of among staphylococci, most common species isolated from oral cavity in dogs and incriminated in causing pyoderma in cat (Abrahamian and Goldstein, 2011).

S.epidermidis was isolated significantly at higher rate (8%) from nasal swabs at residential area than at abattoir (3.1%). This result was less than the report of Omer *et al*. (2012) who recovered the bacterium from nasopharyngeal passageway of sheep at rate of 15.6%. The isolation rates from camels at residential area indicated that the bacteria were isolated at higher rate from apparently health camel (9.5%) than was from sick (5.1%). With the current finding agreement, Omer *et al*. (2012) isolated *S.epidermidis* at higher rate from clinically sick sheep than the health sheep. Regarding to the samples collected from abattoir, it was isolated from nasal and tracheal swabs at rates of 3.1% and 4.1% respectively but was not isolated from lung swab samples.

Micrococcus spp. was isolated from 11.2 % of nasal swabs at abattoir but only from 5.1% of camels at residential area. With agreement of the abattoir result, Kurćubić *et al.*(2013), Mir *et al.*(2013) and Omer *et al.* (2012) isolated the bacteria from nasal mucosa of beef cattle (10%), equines (9.96%) and sheep (9%) respectively. Al-Ani *et al.* (1998) also isolated the bacteria from nasal cavity of both clinically ill and apparently health camels. In the present study, the recovery rates of the bacterium from apparently health and clinically sick camels were 5.2% and 6.8% respectively. This proportion might not be in comparison with Mir *et al.* (2013) result who isolated Micrococcus spp., at low rates from clinically sick equines (4.72%) than from apparently health camels (13.4%). The current study also isolated it from all anatomical sites of camel respiratory tracts at rates of 11.2%, 6.1% and 3.1% from nasal, tracheal and lung swabs respectively. In agreement with this *Micrococcus spp* was isolated from all anatomical sites of sheep respiratory tracts but trachea and lung were more frequently involved (Yimer and Asseged, 2007). But Megra *et al.* (2006) isolated these bacteria from nasal cavity, and tonsil of goat at rates of 4% and 2% respectively, but not from trachea and lung. The bacterium was not recovered from normal lung, but was isolated from lung lesion at rate of 4.3%. This finding is in contrast with Abubakar *et al.* (2010) who isolated it at rates of 2.2% from normal lung and 1.9% from lung lesion and Awol *et al.* (2011) who recovered it from lung lesion at a rate of 1.8%.

Although *Micrococcus* species were isolated from nasal cavities of camels (Shemsedin 2002) and normal rabbits (Ajuwape and Aregbesola 2002), nasal cavity and tonsils of goats (Megra *et al.,* 2006) and nasal cavity, trachea and lung of camel in the present study, they are assumed to be non pathogenic (Quinn *et al.*, 2002). However, from the fact that this bacterium has been frequently isolated from lesioned lungs of camels in previous studies (Abubakar *et al.*, 2010 and Awole *et al.*, 2011) and in the current study, it could have a role in the development of respiratory infections.

Streptococcus spp, in this study, was isolated at a rate of 25.7% in nasal swab collected from camels at field level. It was isolated at rates of 17.2% from health camels and 42.4% from clinically sick camels. The recovery rate of *Streptococcus spp* from sick camels was

about 3.5% times more than that of from healthy one, which is indicative for their association with respiratory problem. Comparable to this, Azizollah *et al.* (2009) isolated it from nasal cavity apparently health camels but at higher rate than the current report (35%). Al-Ani *et al.* (1998) also isolated the bacteria from nasal cavity of apparently health and diseased camels. Moreover, Younan and Bornstein, (2007), reported isolation of Lancefield group B streptococci from nasopharynx of healthy camels.

With the support of the current findings that this bacteria was associated with respiratory symptoms, Mir *et al.* (2013) isolated *Streptococcus spp* at lower proportion from apparently health equines (18.56%) than from respiratory problem (diseased) equines (46.46%). Gutema *et al.* (2009) also isolated it from nasopharnyx of apparently health, clinically sick donkeys at rate of 28.1% and 36.2% respectively, and considered as primary opportunistic pathogen following viral infections or stressful condition. In cattle, Shehab *et al*. (2001) reported that *Streptococcus spp*. are among the main causative agents of respirat-ory disorders affecting calves.

The current study also isolated *Streptococcus spp* from samples collected from abattoir survey at rates of 64.3% of nasal, 58.2% of tracheal and 56.6% of lung swabs. But with much more lower proportion to the present finding, Azizollah *et al.* (2009) isolated it from nasal, trachea and tonsil at rates of 35%, 28%, and 35% of apparently health camels but not from ;lung. Abubakar *et al.* (2010) and Awol *et al*. (2011) isolated also at lower rate than the present study that they recover it at rate of 9.4% and 1.8% from lung tissues. The difference in isolation rate could be due to the small sample size they have used. It was isolated at much higher rate from lung lesion (78.6%) than normal lung (1.8%). The recovery rate of the bacteria from lung lesion was so higher as about 202 times more than that of from normal lung. This difference might be in agreement with Abubakar *et al.* (2010) findings who isolates *Streptococcus spp* from pneumonic lung (10.9%) more frequent than from normal lungs (6.7%) in camels. It has also been reported among the most frequently isolated bacteria from camel lung lesion (Zubair *et al.,* 2004; El-Tigani *et al.,* 2004; Kane *et al.,* 2005). In addition, Al-Ani *et al* (1998) have recovered it from pneumonic lung of camel at rates of 12.5% as one of the most frequent isolated bacteria.

Despite S*treptococcus spp.* is widely distributed in nature and lives as commensal in the respiratory tract of many species of domestic animals (Azizollah *et al*., 2009), Potentially pathogenic and non-pathogenic species might be present in the upper respiratory tract (Carter and Chengappa, 1991).

Streptococcus pyogen, was recovered at a rate of 14.3% from samples collected at field level with significant association with respiratory diseases symptoms ($p<0.05$). As the result, it was isolated from 25.4% of sick camels but from only 8.6% of the apparently healthy ones. At abattoir level, 24% of nasal, 12.2% of trachea and 15.3% of lung swabs were positive for *S. pyogen* isolation. All of the isolates from the lung samples were only from lung lesion that it was isolated at rates of 21.4% of lesioned lungs. These results were much higher than that of Abo-Elnaga and Osman, (2012), who recovered *Streptococcus pyogens* at rates of 2.9% from condemned lungs of camels. In addition, Mahmoud *et al*. (2005) and Osman *et al*. (2003) have reported *Sterpt. pyogenes* from pneumonic lesions of camel lung's. *Streptococcus pyogenes* (Lancefield group A) is by far the most frequent cause of bacterial pharyngitis and tonsillitis in human (Vandepitte *et al.,* 2003).

In the present study, the isolation rate of *Streptococcus equi subsp equi* from nasal swab samples at residential area *were* 5.7%. With regard to association with respiratory symptoms, it was recovered from 3 (2.6%) of apparently healthy camel and 7(11.9%) of clinically sick camels with significant association to the symptoms so more as 5 times in the sick camels than the healthy ones. As the result of the abattoir samples indicated, it was isolated from all respiratory anatomic sites with rates of 7.1% from nasal, 8.2 from trachea and 7.1 from the lung samples, but was not recovered from normal lungs. With comparison to this *Streptococcus equi* sub-species *equi* was isolated from a sick camels in Ethiopia from respiratory outbreak cases as stated by Yigezu *et al*. (1997). Moreover, *Streptococcus equi* sub-species *equi* have been strongly incriminated as cause of acute camel respiratory disease outbreaks occurred in Ethiopia in 1995 (Bekele, 1999, Roger *et al*., 2001). It is the known in causing a highly contagious and serious infection of horses known as strangles (Radostitis *et al.,* 2007).

Streptococcus equi subsp zooepidemicus was isolated from samples of field level at rate of 5.2% and 16.9% from apparently health and clinically sick camels respectively. It was also found distributed in all anatomical sites of camel respiratory tracts to be isolated with rates of 34.2%, 11.7%, and 17.9% from nasal, tracheal and lung samples respectively. This bacteria was not isolated from normal lung lesion but was recovered from lung lesion at rate of 25%. *Streptococcus equi subsp zooepidemicus* was associated with respiratory symptoms as was with lung lesions. Even though was with higher proportion, with agreement to results of current study in isolating the bacteria at lower rate from health camels than from the sick ones, Mir *et al.* (2013) have isolated it from apparently health at lower rate (9.79%) than respiratory diseased equines (29.13%). In horse, even though, the pathogenicity of different subtypes of *S. zooepidemicus* might vary, it has been determined to cause respiratory diseases independent of prior viral infection (Wood *et al*., 2005). However, Christley *et al.*, 2010 stated that, *S. zooepidemicus* is a significant secondary pathogen associated with viral diseases in horses.

In this research, *S. pneumoniae* was isolated from 4.6% of the nasal swab samples which were collected at field level. It was found significantly associated with respiratory symptoms observed ($p<0.05$). But as reported by Mir *et al.* (2013, in contrast to the current findings, the isolation rate of the bacteria from sick equines (5.51%) was not significant difference with that of apparently healthy ones (5.67%). Regarding to samples from abattoir, they were recovered only from lung lesion samples at rate of 9.3%. This result was in agreement to the finding of Abo-Elnaga and Osman, (2012) who recovered the bacteria from pneumonic lung in camel at rate of 8.6%. Moreover, *S. pneumoniae* has been reported being isolated from pneumonic lungs in camel by other researchers. Mahmoud *et al*.,2005 and Osman *et al*. (2003).

Of the total isolates from samples of field level, it was isolated from 6.9% of camels of three years or younger age group, 4.3% of camels with age between three and eight years and 2.1% of camels older than eight years old. Although, the isolation rates were not statistically significant with age groups in the current study, the result of this study the decrement of the rates with age was, in agreement with the finding of Wood *et al*. (2005)

that S*treptococcus pneumoniae* has been found statistically associated with respiratory infection in horse only that were 2 years old or younger. Mahmoud *et al*. (2005) Osman *et al*. (2003) have also reported the isolation of *S. pneumoniae* from pneumonic lesions of camel lung's. It has been frequently reported as causing inflammatory airway disease in equine (Chapman *et al*., 2000, Christley *et al*., 2001).

Bacillus spp. was the less frequent isolated bacteria in this study. It was isolated at rate of 3.4% from the apparently health and 5.1% from the sick one but not significantly associated with respiratory signs, although, the recovery rate of the isolates from the sick camels were 1.5 times more than those recovered from the health camels. The current study isolated *Bacillus spp* from respiratory tracts of slaughtered camels at rates of 20% of nasal mucosa, 9.2% of trachea and 5.1% of lung swabs. They were isolated both from normal and lung lesion with 3.6% and 5.7% respectively without significant association with the lesion ($p>0.05$). This finding was in agreement with that of Abubakar *et al.* (2010) result who isolated it at rate of 5%. But with higher than the current isolation rates the bacterium has been isolated from condemned lung at a rate of 21.4 % by Abo-Elnaga and Osman, (2012), 16.6%, by Azizollah *et al.* (2009) and 14.66% by Azizollah *et al.* (2009). It was also reported it as the most commonly isolated bacteria from pneumonic lungs in camel. The current isolation of the bacteria regardless of respiratory disease symptoms and the suggestions of the previous studies indicated that *Bacillus spp* is the microflora of upper respiratory tracts of camel, which can involve in pneumonia opportunistically.

The result of nasal swab samples reveled that, *Corynebacterium spp*. was isolated at rate of 9.7% with 5.2% in health camels whereas 18.2% in camels with respiratory problems signs. But it was found associated with respiratory symptoms that it was isolated from sick camels at about 4 time more than being isolated from the health ones ($p<0.05$). Its recovery rate from nasal (3.1%) was lower than that of lung (5.6%) but higher than tracheal (0.0%) swabs from slaughtered camels. It was isolated from lung lesion (7.1) with high proportion than from normal lung (1.8). With higher than this result, Abubakar *et al.* (2010) isolated Corynebacterium *spp* from normal and pneumonic lung in camel in rates

of 2.2% and 10.9% respectively. But it was higher than the findings of Abo-Elnaga and Osman (2012) who isolated *C. pyogenes* in a percentage of 5.7% of pneumonic lesions. The pathogen was involved in pneumonia of camels under condition of stress, poor sanitation and immunosuppression (Kane *et al*., 2005).

Mannhemia hemolytica has also been reported as a cause of primary and secondary pneumonia and a number of non-specific inflammatory lesions in various species of domestic animals (Quinn *et al*. 2004: Radostitis *et al*. 2007) . In this study *M. hemolytica* was isolated at a rate of 12.6% and 14.3% of field and abattoir nasal swabs respectively. The recovery rates of it from apparently normal and clinically sick camels were 8.6% and 20.3% respectively. It was also recovered from 9.2% of trachea and 11.2% of lung swabs from abattoir which was higher than Seddek *et al*. (2002), Abo-Elnaga and Osman (2012) and Al-Tarazi *et al*. (2001) who recovered *M. hemolytica* from camel lung at rates of 1.17%, 1.4% and 6.6% respectively. With similar rate Shemsedin (2002) reported that 8.7% of the total bacterial isolates from lung tissue were *M. haemolytica*. But with much higher rate (56%), *M. hemolytica* was isolated from bacterial pneumonic lungs of slaughtered camels (Al-Rawashdeh *et al*., 2000). Seroprevalence study on camels with respiratory signs conducted by Kebede and Gelaye (2010) in North Gondar and North Wollo in Ethiopia reveled that, *M. haemolytica* was positive in 56.5% and 8.7% respectively. Camel pasteurellosis, due to *M. haemolytica* has long been reported from lung and whole blood of febrile camels in Shinnelle zone of Somali National Regional State of Ethiopia by Bekele (1999). In Ethiopia few works have been done on camel respiratory diseases and all of them were focused on pathological and bacteriological examination in abattoir samples and that is why the isolates of some bacteria as *M. haemolytica* in the lung tissue seems to be low in number. However, in most active respiratory disease problem *M. haemolytica* can be the predominant isolates (Bekele, 2008).

The study isolated also *Pasteurella maltocida* at a rate 22.3% of field and 8.2 % of abattoir nasal swabs. It was also isolated from 11.2% of tracheal and 15.3% of lung swabs. The recovery rates from camels with and without respiratory problems were 20.3 and 8.6% respectively in which, the isolation rates of the bacteria from sick camels was 3.5

times more than that of from health camels. The results of abattoir samples were not in agreement with nasal swabs results from field and previous results of Abo-Elnaga and Osman (2012) and Mahmoud *et al*. (2005) who recovered it from pneumonic lungs of camels at rates of 2.9 and 7.4% respectively . Pasteurella species are commensals residing in the nasopharyngeal microflora and are all capable of causing infection when the body defense mechanism are impaired (Abo-Elnaga and Osman, 2012). Eventhough both *P. multocida* and *M.haemolytica* are considered as important contributory pathogens in enzootic or primary pneumonia in different animals, *M. haemolytica* assumes greater prominence than *Pasteurella spp*., in the same environment both in terms of infection intensity and pathogenicity (Omer *et al.*, 2012).

It has also been isolated from the deep pulmonary tissues of the examined cases. This may be an indication of the presence of suitable conditions in the lungs facilitating the invasion of pasteurlosis into deep tissues (Abo-Elnaga and Osman, 2012). Since pasteurellosis usually related with various debilitating factors, it was appeared as a econdary bacterial complication following parainfluenza exposure. The association of parainfluenza with pasteurellosis causes serious respiratory disease complication and even death in camels, if they were not treated in early time of infection (Kebede and Gelaye, 2010).

Actinomyces spp was isolated, in the present study, at a rate of 5.1 from nasal swabs at residential area and 6.1 from camels at abattoir level. This was in comparable with the previous results of Al-Tarazi *et al*.(2001) from camel respiratory tracts at rate of 6.6%. The current study did not recover this bacterium from lung samples. But Abo-Elnaga and Osman (2012) isolated it from camel lung lesion at rate of 1.4%. Moreover, *Actinomyces pyogenes* has been reported as one of primary causes of pneumonia in sheep (Omer *et al*.,2012).

Escherichia coli was isolated at a rate of 20.6% in this study of which were recovered from healthy camels at rates of 13.8 % and from camels with pulmonary disease symptoms at rate of 33.9%. The isolation rate of *E.coli* from sick camels was about 3 times greater than from the health ones. *E.coli* was isolated also isolated from 15% of nasal,

and 2% of lung swabs in abattoir samples but was not from trachea. It was not isolated from normal lung but from 2.9% of lesioned lungs. The isolation rate was lower than the report of Al-Tazari *et al*.(2001), Mahmoud *et al*. (2005, Awol *et al.* (2011) and Abo-Elnaga and Osman, (2012) who recovered *E.coli* at a rates of 26.7% , 11.1%, 17.5% and 8.6% respectively from camel lung lesions but in comparable with result of Zubair *et al*. (2004) who recovered it as 3% from camel lung lesion. *E.coli* has been reported as predominant isolates from sheep respiratory tracts) commensals, residing in the nasopharyngeal microflora and are all capable of causing infection when the body defense mechanisms are impaired (Zamri-Saad *et al*., 2006 and Yimer and Asseged, 2007). Their presence is mainly confined to ruminants with most adequately characterized strains originating from cattle, sheep and goats (Biberstein and Hirsh, 1999).

*Proteus spp*was isolated at higher rate from abattoir nasal swabs (7.1%) than that of camels sampled at field (2.9%). It was not found associated significantly with respiratory symptoms that the isolation rate from health camel was 3.4% whereas it was 1.7% insick camels ($p>0.05$). It was recovered from all threesites of camel respiratory tracts with small proportion from lung (0.5%). However, the isolates from lung samples were only from lesion. This finding is contrary to Abubakar *et al.* (2010) that they isolated *Proteus spp* more frequently from milder pneumonic lungs than normal ones in camels. But Al-Tarazi *et al*.(2001) isolated it from 29 pneumonic lungs of camels at rates of 2.66%. With agreement to the result of nasal swab collected at the field, Mir *et al.* (2013) and Gutema *et al*. (2009) isolated *Proteus vulgaris* from upper respiratory tracts of equine at rate of 3.42% and 2.1% respectively. *Bacillus* and *Proteus* isolates were collected from mixed cultures of pneumonic lungs and were previously considered less pathogenic in camel respiratory tracts (Al-Doughaym *et al*., 1999).

Klebsiella spp was only isolated from abattoir samples at rates of 7.1% and 2% respectively from nasal and lung swabs. All of the isolates from lung swabs were from lesion which could be the indicative for the pathogenic nature of this bacteria. This finding was lower than the result of Abo-Elnaga and Osman, (2012) who recovered it from pneumonic lungs of camel at rate of 10% of 70 cases. Higher isolation rates have also recorded in

previous reports that Al-Tarazi *et al*.(2001) isolated it at rate of 14.66%, Abubakar *et al.* (2011) in 8.1% of pneumonic lungs. But lower records has been reported by Azizollah *et al.* (2009) who recovered *Klebsiella spp* from 1.27% of pneumonic lungs in camel. In addition,in agreement with the current finding, Azizi *et al.* (2013) isolated *Klebsiella pneumoniae* from pulmonary abscesses of sheep at rate of 1.89%. The isolation rate of Gutema *et al.* (2009) from nasopharnyx of donkey was less than this finding that they isolated *K.pneumoniae* as 5.8%. The current result was also in accordance with the previous results of Zubair *et al*. (2004) and Kane *et al*. (2005) who reported that *Klebsiella spp* was commonly isolated from pneumonic lesions in camels. The involvement of the bacteria in respiratory diseases of animal was shown by Mir *et al*. (2013) who isolated this bacteria at more rate (5.51%) from respiratory diseased equine than the normal ones (2.06). Since *K. pneumoniae* inhabits the intestinal tracts of animals, fecal contamination of the environment may account for the wide distribution of the organism, and contributes to the occurrence of opportunistic infections (Quinn*et al.,* 2004). However, *Klebsiella spp* has been isolated less prominent and so that considered as secondary invader in camel respiratory diseases (Abo-Elnaga and Osman, 2012).

5.3. Drug sensitivity

The drug sensitivity pattern of the isolates that represent is shown in Table 8 and 9. It was found that most of the *M.hemolytica*isolateswere highly resistant to Streptomycine (83.3%) and Cloxacillin (75%). Even the rest proportion of the isolates were (16.7 and 25%) susceptible intermediately. But they were relatively susceptible to Chloramphenicol (41.7%) and Trimatoprim-salphomethaxazole (58.3%). With similarity to *M. hemolytica*, *P. maltocida* isolates were found resistant to Streptomycine and Cloxacillin at rates of 100% to each. But with high susceptibility rate than *M.hemolytica*, they were susceptible to chloramphenicol (71.4%) and trimatoprim-salphomethaxazole (87.5%).

In the current study, *Streptococcus spp.* wererelatively susceptibleto Vancomycine (35%) and Naldixic acid (36%). Sixty four percent of them were intermediately susceptible to vancomycin but no one of them were found susceptible to it. But they were resistant to

streptomycin (91.4%), Doxacillin (51%), Penicillin (90.9%) and Ampcillin (97.6%). This agrees with the report of Muhammad *et al.*, (2012) who determined that *Streptococcus spp.*isolates from mastitic camel were resistant to penicillin and ampcillin.

The susceptibility of *Staphylococcus spp.*isolates was at rates of 45.5% and 54.5% to Doxacillin and Ampicillin respectively. But they were found more resistant to Penicillin (90.5%) and Cloxacillin (86.4). They were also found intermediately susceptible to Nal-dixic acid at rate of 43.5%.

The high resistance rate of the pathogens to commonly used antibiotics could be aroused from uncontrolled drug usage by non professional pastoralists. This can lead to the emergence of drug resistant strains of pathogenic bacteria which threat not only camel population of the study area, but also other animal and human which share the same environment.

5.4. Viral Isolation

The study showed that the overall proportion of virally suspected camels was 20% (35/175). The study conducted by Ayelet *et al*. (2013) to investigate camel respiratory problems associated with viral agents reported 8.9% of slaughtered camel in. With duration of cytopathic effect (CPE) development from 7 to 14 days ranges, the CPE were observed with characteristics such as many scattered, rounded, refractory cells and syncytia formation.Out of 35 samples inoculated on VERO cell culture, 15 (42.9%) showed more than 50% CPE. Similarly, Shaker (2003) reported about isolation of PIV3 from camel lungs in Egypt. Intisar *et al*. (2009) isolated PIV3 from camel lung specimens in MDBK cell cultures observing the typical CPE of PIV3: rounded retractile cells, cell elongation and sloughing with some syncytia formation, which was similar with early descrpiton givenby Henrickson (2003).

Traditionally, the isolation of the respiratory viruses has been performed with a variety of different cell types by using tube cultures. This method is considered the "gold standard" for the diagnosis of viral respiratory tract infections. Typically, tube cultures are held for

up to 14 days and observed for the appearance of cytopathic effect (CPE). In addition, cultures can be screened by hemadsorption for influenza and parainfluenza viruses or by indirect immunofluorescence to detect all of the common respiratory viruses. The disadvantages of tube cultures include the long period before results are available, the many labor-intensive steps during the time that the cultures are held, and the need for highly trained technologists to observe the cultures for CPE (Olsen *et al.*, 1993).

In the current study, five of CPE positive samples tested for *Parainfluenza virus* gave visible positive PCR results as *Parainfluenza virus 3 (PIV3)* which showed that *Parainfluenza virus 3* plays a role in camel respiratory disease syndrome. The finding of the present study is the first isolates of *Parainfluenza virus 3* from Ethiopian camels. *Parainfluenza virus* 3 is one of the viruses known to cause respiratory infection. Ayelet *et al.* (2013) did not find PIV3 in CPE positive samples characterized by initial rounding of VERO cells, elongation and syncytia formation of infected cells by PCR amplified using PIV3 specific primers. At the time they suggest that the absence of a PCR product could be due to the use of VERO cell lines for virus culture in the study instead of the commonly used MDBK cell by other researchers which is more susceptible for *parainfluenza viruses*.

Parainfluenza type 3 (PI3) with other viruses such as *Bovine viral diarrhea* (BVD) and *Bovine herpesvirus type 1 (BHV-1) viruses* has been incriminated to cause acute respiratory diseases in (Elbayoumy *et al*., 2013). Although, *Parainfluenza virus (PIV)* is the viruses known to cause respiratory infection in camel, geographical distribution of the virus in camels have not been well studied so far. However, there have been reports from many camel rearing countries. Serological findings reported by Olaleye *et al.* (1989) in Nigeria and Schwartz and Dioli (1992) in Ethiopia were the early indicative of the common occurrence of *Parainfluenza* 1 and 2 virus infection in camel rearing areas. Olaleye *et al*., (1989), who investigated camel respiratory disease in Northeastern Nigeria, determined that out of 150 camel sera samples, tested by complement fixation test, 22.3% were positive to parainfluenza-1, 18.5% to parainfulenza-3 virus and 12.7% to influenza virus B. Schwartz (1992) reported that parainfluenza 1, 2 and 3 were common and wide-

ly distributed in most camel rearing areas. In addition, Dioli and Stimmelmayr (1992) reported that viruses associated with respiratory infections in camels are parainfluenza 3 (PI3), influenza virus A and B, adenovirus, respiratory syncytial virus (RSV) and infectious bovine rhinotracheitis (IBR).

The detection and isolation of PIV3 using haemo-agglutination inhibition (HI) method from imported Djiboutian camels in Egypt was reported for the first time by Nawal *et al*. (2003). Moreover, Intisar *et al.* (2009) isolated PIV3 from camel lung specimens on MDBK cell cultures observing the typical CPE of PIV3: such as rounded retractile cells, cell elongation and sloughing with some syncytia formation, which was similar with early described by Henrickson (2003).

In Ethiopia, the evidence of a study conducted by Kebede and Gelaye (2010) was suggestive for Parainfluenza virus-3 being the primary pathogenic agent in the camel respiratory disease outbreak in the country. During the study they have determined that there was significant difference in positive proportion of sera from outbreak area and surveyed area without outbreak as 70.5% and 6.8% respectively. There was also high antibody titer level in outbreak area than the surveyed area without outbreak.

In the current study, some bacteria were isolated concurrenttly with virus. The isolation of *E.coli* and *Staphylococcus spp* from viral positive camels was about 8 and 2 respectively times more than those from which virus was not isolated. Other bacteria found associated with virus infection were *Manhemia hemolytica, Pasteurella maltocida*, *Actinomyces spp* and*Corynebacterium spp*. This is supportive for the fact that bacteria residing in upper respiratory tracts of animals can cause diseases as secondary invaders in viral infection. Kebede and Gelaye (2010) stated that a number of microbial agents involved as a primary or secondary infection of camel respiratory disease. *Parainfluenza virus* concurrently occurs with *Pasteurella spp* as secondary invaders was the primary infectious in camel pulmonary diseases. The association of *parainfluenza* with pasteurellosis causes serious respiratory disease complication and even death in camels if they were not treated in early time of infection. This is because pasteurellosis usually related with various debi-

litatingfactors, it appeared as a secondary bacterial complicationfollowing parainfluenza exposure.

*Parainfluenza virus*es are important respiratory tract pathogens of human as well as mammals and birds (Loney *et al.,*2009). They are are non segmented, negative sense ssRNA viruses with polymorphic nature. The classification of the virus is in the subfamily *Paramyxovirina*e, family *Paramyxoviridae* order *Mononegavirales*. Virions are bounded by a lipid bilayer membrane bearing spikelike surface projections composed of the tetrameric hemagglutinin-neuraminidase (HN) or trimeric F glycoprotein (Loney *et al.,* 2009). They are related antigenically, except that, of the avian viruses, only *Newcastle disease virus (NDV)* and possibly avian parainfluenza virus type 2 exhibit antigenic relatedness to the other para influenza viruses (Kusagawa, *et al.,*1994). The *PIV*s replicate in the epithelial cells that line the respiratory tract, causing rhinitis, pharyngitis, laryngitis, tracheobronchitis, bronchiolitis, and pneumonia. Early during parainfluenza virus infection, the mucous membranes of the nose and throat are involved (Iorio *et al.,* 1992). Transmission of *parainfluenza viruses* is by direct animal-to-animal contact or by large-droplet spread. However, the viruses do not persist long in the environment . The high rate of infection early in life, coupled with the frequency of reinfection, suggests that these viruses spread readily from animal to animal (Bando *et al*., 1990).

6. CONCLUSION AND RECOMMENDATION

In the present study, various types of aerobic bacteria were isolated from respiratory tracts of apparently healthy and sick camels of Borana. Some of these isolates are pathogenicnot only to camel but also to other animals and human. They include the bacteria which have been incriminated to cause camel sudden death (CSD). Most of the bacteria were isolated from both healthy and clinically sick camels with higher rate from the sick ones which is suggestive of opportunistic nature of the isolates.

The antibiotic susceptibility of some pathogenic bacteria to commonly used antibiotic drugs indicated that great proportion of them were resistant to the antibiotic disc. Compounding the problems associated with the use of antimicrobial agents is the fact that many camel owners administer treatment to their animals and that inappropriate dose may be used, as the amount given are often merely extrapolated from other large animals and this assumption might not be accurate.

The isolation of pneumotropic virus, *Parainfluenza virus 3* from clinical cases concurrent with opportunistic bacteria is indicative for the distribution of the virus in study area that favors the involvement of microflora in camel respiratory disease outbreaks.

Based onthe above conclusion, the following recommendations are forwarded.

- Detailed molecular based investigation of bacterial isolates from camel respiratory tracts should be conducted in order to characterize the types of bacteria involved in respiratory diseases;
- Controlled drug administration should be practiced and adequate veterinary service which is compatible with movable nature of the pastoralists should be designed;
- The distribution of *parainfluenza virus* and other camel respiratory disease causing viruses should be studied and prevention and control methods should be designed; and

- Further epidemiological study to identify risk factors for camel respiratory disease and seasonal dynamicity should be conducted.

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8. APPENDICES

Annex **A:** Questionnaire to collect related information

1. General Information:
   - **a.** Name _____
   - **b.** District _____ PA _____
   - **c.** Herd size (total all) _____(Hrd.S.)
   - **d.** Total adult female (above puberty) in the herd____(F.Abv.P)
   - **e.** Total male in the herd___(M.Hrd)
   - **f.** Adult male camel (above puberty) in the herd_____(M.Abv.P)
   - **g.** Heifer_____
   - **h.** Bull_____
   - **i.** Calf_____
   - **j.** ID No. of camels sampled_____(S.ID.No)
2. Level of education of camel herder/owner (Edu.L.)

   Illiterate

   Primary school (≤ grade8) ☐

   Secondary school ☐

   Tertiary /university ☐
3. Do you own other domestic animals? Yes ☐ No ☐
4. If yes, mentions the species of the animals:

   1.Cattle 2.Goats 3.sheep 4.Horse 5.Mule 6.Donkeys 7. Dog 8.Cat
5. Do you keep (herd) camels together with other livestock? Yes ☐ No ☐
6. If yes, with which type of domestic animal? 1.Cattle 2.Goats 3.sheep 4.Horse 5.Mule 6.Donkeys 7. Dog 8.Cat
7. Do you keep camels at night in the same kraal (night house) with other domestic animal?

   Yes ☐ No ☐

8. If yes, with which type of livestock?

   1. Cattle 2.Goats 3.sheep 4.Horse 5.Mule 6.Donkeys

9. Have you ever seen camels eating soil? Yes ☐ No ☐

10. Have you ever seen camels grazing close to the ground like cattle, sheep and goats?

    Yes ☐ No ☐

11. Do you give mineral supplements (salt) to your camels? Yes ☐ No ☐

12. Indicate the water supply for your camel

    - ✓ Tap (municipality) ☐
    - ✓ Wells ☐
    - ✓ Pond ☐
    - ✓ River ☐
    - ✓ Other,(specify)_____

13. Are there wild felids (lion, leopard, cheetah, jaguar, and puma) in the grazing area of your camel or along your migration route? Yes ☐ No ☐

14. What are major livestock diseases commonly known in the area?

    _____

15. What are major camel diseases commonly known since earlier in the area?

    _____

16. Is there any new camel disease introduced recently? Yes ☐ No ☐

17. If yes, what name has been given to it? _____.

    What are major symptoms observed? _____

    _____ _____

18. Which group is more commonly affected?

    Young ☐ Adult ☐

19. According to your thought, where did the disease come from?

_____

20. Are camel respiratory diseases common in this area? Yes ☐ No ☐

If 'yes' what major symptoms are observed?

_____

21. Are there traditional medicines/treatments given to camels suffered from respiratory tract diseases? Yes ☐ No ☐

If 'yes' what are they? _____

What are the results of the medication? Aggravation of the symptoms?_____.

Cure?____. Describe if other_____.

22. Were your camel(s) vaccinated against any disease? Yes ☐ No ☐

If yes when was/were they vaccinated? _____

For what type of disease have they been vaccinated? _____

23. Are you familiar with taking sick camel to veterinary clinic? Yes ☐ No ☐

24. How long does it take to reach at the nearby veterinary clinic?_____

If its distance is known in km. please specify it._____.

25. Are chemical drug injections given to sick camels by local farmers? Yes ☐

No ☐

Annex **B:** Composition and preparation of media used in the study

Stuart Transport Medium

Formula in grams per liter:

Agar Nº 2.....3.00

Sodium Thioglycollate 1.00

Sodium Glycerophosphate.....10.00

Methylene Blue.....0.002

Preparation

Suspend 14.1 grams of the medium in one litre of distilled water. Heat with frequent agitation and boil for one minute. Dispense in screw-capped tubes and sterilize in an autoclave at 121°C (15 lbs. sp.) for 15 minutes.

Source: Fortuna (2012)

Brain Heart Infusion Broth

Formula in grams per liter:

Gelatin peptone.....10.00

Beef Heart Infusion 10.00

Calf Brain Infusion.....7.50

Sodium Chloride 5.00

Disodium Phosphate.....2.50 Dextrose..... 2.00

Preparation

Suspend 37 grams of the medium in one litre of distilled water. Heat slightly if needed until complete dissolution. For blood cultures add 0.5 to 1.0 grams of agar per liter of rehydrated medium. Boil for one minute. Dispense and sterilize in autoclave at 121°C (15 lbs. of pressure) for 15 to 20 minutes. For best results the medium should be used on the same day, or boiled or heated for a few minutes, then left to cool before using.

Source: Fortuna (2012)

Blood Agar

Formula in grams per liter:

Heart Infusion.....10.00

Meat Peptone..... 10.00

Sodium Chloride.....5.00

Bacteriological Agar..... 15.00

Preparation

Suspend 40 grams of the medium in one litre of distilled water. Leave to stand for 5 minutes and mix well until a uniform suspension is obtained. Heat with gentle agitation and boil for one minute. Sterilize to 121°C (15 lbs. sp.) for 15 minutes. Cool to 45-50°C, and add 5 -10% sterile defibrinated blood, homogenize and pour into Petri plates.

Source: Fortuna (2012)

Macconkey Agar

Formula in grams per liter

Pancreatic Digest of Gelatin..... 17.00

Lactosemonohydrate.....10.00

Sodium Chloride.....5.00

Peptone Mixture 3.00

Bile Salts nº 3.....1.50

Neutral Red 0.03

Crystal Violet.....0.001

Bacteriological Agar 13.50

Preparation

Suspend 50 grams of the medium in one liter of distilled water. Mix well until a uniform suspension is obtained. Heat with frequent gentle agitation and boil for one minute. Sterilize in autoclave at 121º C (15 lbs. sp) for 15 minutes. Cool to 45 °C, and pour into Petri dishes. Allow the plates to solidify and place them upside down to avoid excessive moisture in the surface of de medium.

Source: Fortuna (2012)

Edwards Medium

Formula in grams per liter

Peptic digest of animal tissue…… 10.000

Beef extract ……10.000

Esculin ……1.000

Sodium chloride…….. 5.000

Crystal violet ……..0.0013

Thallous sulphate…… 0.330

Agar…….. 15.000

Final pH 7.4±0.2 at 25°C

Preparation

Suspend 41.33 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at D 115°C for 20 minutes. Cool to 50°C and aseptically add 5 to 7% v/v sterile sheep blood. Mix well and pour into sterile Petri plates.

(D corresponds to 10lbs pressure)

Source: HiMedia Laboratories (2011)

Mannitol Salt Agar

Formula in grams per liter:

Sodium Chloride.....75.00

Peptone Mixture..... 10.00

D-Mannitol 10.00

Beef Extract..... 1.00

Phenol Red.....0.025

Bacteriological Agar..... 15.00

Final pH 7.4 ± 0.2 at 25ºC

Preparation

Suspend 111 grams of the medium in one litre of distilled water. Mix well and heat with frequent agitation until complete dissolution. Boil for one minute. Sterilize in autoclave at 121°C (15 lbs. of steam pressure) for 15 minutes. Pour into Petri dishes.

Source: Fortuna (2012)

Mueller Hinton Agar

Formula in grams per liter

Beef Infusion..... 2,00

Casein Peptone H 17,50

Starch..... 1,50

Bacteriological Agar 17,00

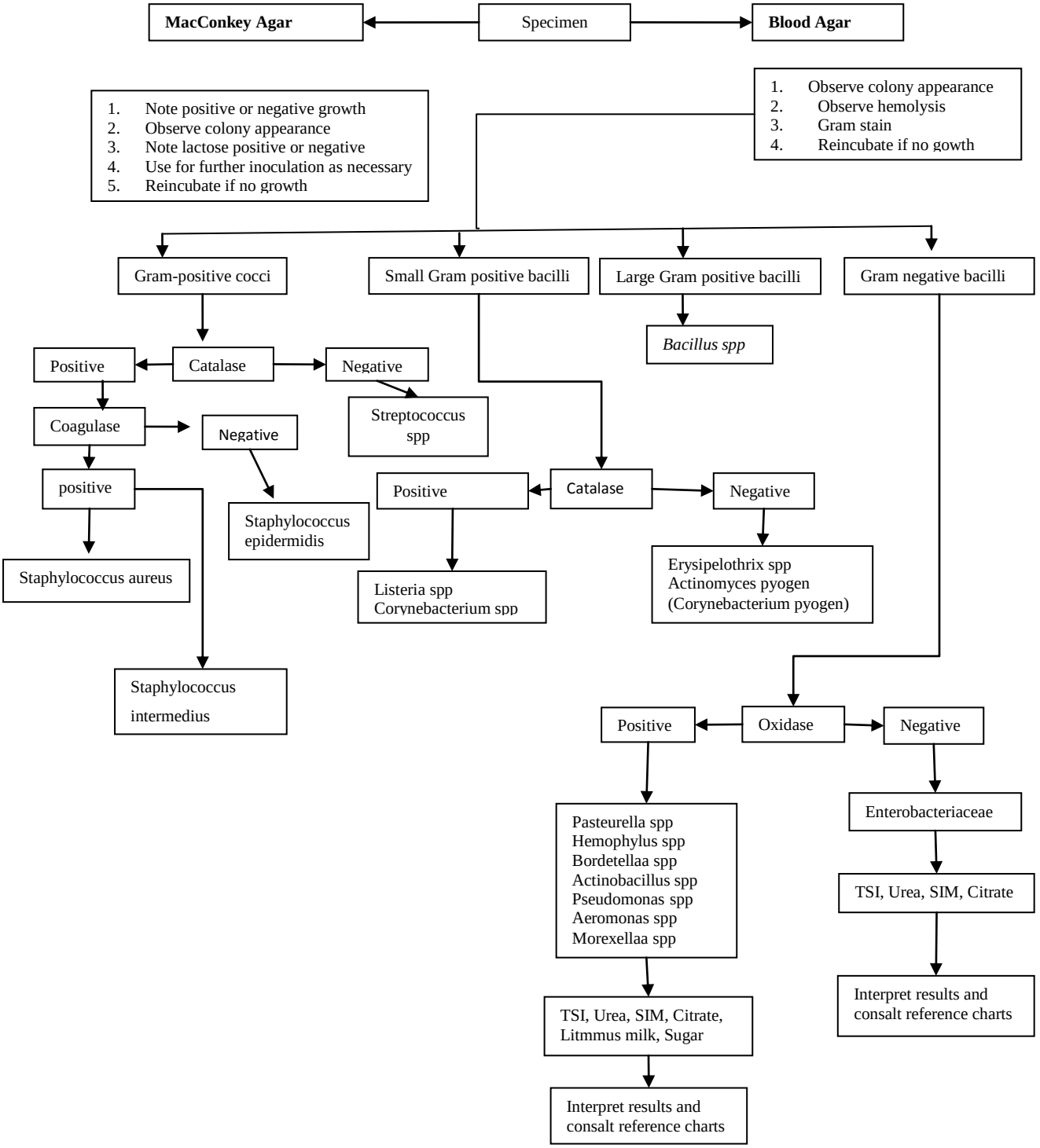
Final pH 7,4 ± 0,2 at 25ºC

Preparation

Suspend 38 grams of medium in one liter of distilled water. Mix well. Heat agitating frequently and boil for about one minute. Dispense and sterilize in autoclave at 116 - 121°C (15 lbs.sp) for 15 minutes. Cool to 45° or 50° C and add defibrinated blood if desired. The blood mixture should be chocolated by heating to 80° C for 10 minutes if Neisseria development is desired. DO NOT OVERHEAT. To remelt the cold medium, heat as briefly as possible.

Source: Fortuna (2012).

Annex **C:** Flow charts for isolation and identification of bacteria



Source: Ikran (2007**)**

Annex **D:** The composition of stain and other solutions used, and procedure followed in Gram's staining

Composition and preparation of Stain and other solutions

1. Ammonium oxalate crystal violet solution

Composition

Solution I

Crystal violet……2gm

Ethyl alcohol (95%)…….20ml

Solution II

Ammonium Oxalate…….0.8gm

Distilled water……80ml

Preparation

Mix solution I and II and filter through whatman filter paper No. 1

2. Lugol's iodine solution (mordant)

Composition

Iodine…….....1gm

Potasium iodide…….2gm

Distilled water……..300ml

Preparation

Dissolve the ingredients and filter

3. Ethyl alcohol (95%) to be used as decolorizer

4. Safranin (counter stain

Composition

Safranin (2.5%) solution in 95% ethyl alcohol…… 10ml

Distilled Water……..100ml

Preparation

Mix and filter

Procedure

1. Make bacterial smear and fix over the flame

2. Pour ammonium oxalate crystal violet stain and allow it act for 1minut

3. Rinse with tap water

4. Add Lugol's iodine and allow it to react for one minute

5. Rinse with tap water

6. Decolorize with 95% ethyl alcohol for about 30seconds till no color comes out from the smear

7. Counter stain with safranin solution for 1 minute

8. Wash with tap water, dry and examine under the microscope with oil immersion objective

Interpretation

Gram positive bacteria appear deep violet while Gram Negative bacteria appear red.

Source: Quinn *et al.* (2004).

Annex **E:** Biochemical tests to identify *Streptococcus spp, Staphylococcus spp* and *Pasteurella spp*

Biochemical reactions and other characteristics of *Streptococcus spp*

	Acid formation							**Aescullin hydro-lysis**	**Sodium hypurate**	**Growth in 6.5%**
	Inulin	**Lactose**	**Manitol**	**Raffinose**	**Sallicin**	**Sorbitol**	**Trehalose**			
S. pyogens	-	+	V	-	+	-	+	-	-	-
S. agalactiae	-	+	-	-	(+)	-	+	-	+	-
S. dysgalactiae	-	+	-	-	-	-	+	-	-	-
S.dysgalactiae subsp equisimilis	-	v	-	-	(+)	-	+	-	-	-
S. equi subsp equi	-	-	-	-	+	-	-	-	-	-
S. equi subsp zooepidemicus	-	+	-	-	+	+	-	-	-	-
Enterococcus faecalis	-	+	+	-	+	+	+	+	v	+
S. bovis	+	+	v	+	+	-	v	+	-	-
S.equinus	+	-	-	+	(+)	-	v	+	-	-
S.porcinus	-	(+)	+	-	+	+	+	+	-	+
S. canis	-	(+)	-	-	n	-	(+)	v	-	-
E. avium	-	+	+	-	+	+	+	+	v	(+)
S. suis type 2	(+)	+	-	(+)	+	-	+	+	-	-
S. suis type 1	+	+	-	-	n	-	+	-	-	-
S.uberis	+	+	+	-	+	+	+	+	+	(+)
S.pneumoniae	+	+	-	+	v	-	+	(+)	-	-

(+)= majority of strains positive; v= variable reaction; n= information not available

Biochemical reactions and other characteristics of some *Staphylococcus spp.*

Species	**Coagulase**	**Hemolysis**	**Pigment productio**	**Urease**	**Manitol fermented (Manitol Salt agar**	**Maltose fermented Purple agar + 1% maltose**	**Novobiocin (5µg) disc**	**Polymyxin B 300 unit disc**
S.aureus	+	+	+	d	+	+	S	R
S.intermedius	+	+	-	+	d	±	S	S
S.hyicus	d	-	-	d	-	-	S	R
S. epidermidis	-	d	-	+	-	-	S	R
S.saprophyticus	-	-	d	+	+	-	R	S
S.equorum	-	d	-	-	d	d	R	n
S.simulans	-	d	-	+	±	-	S	S
S.chromogenes	-	-	+	d	d	-	S	R

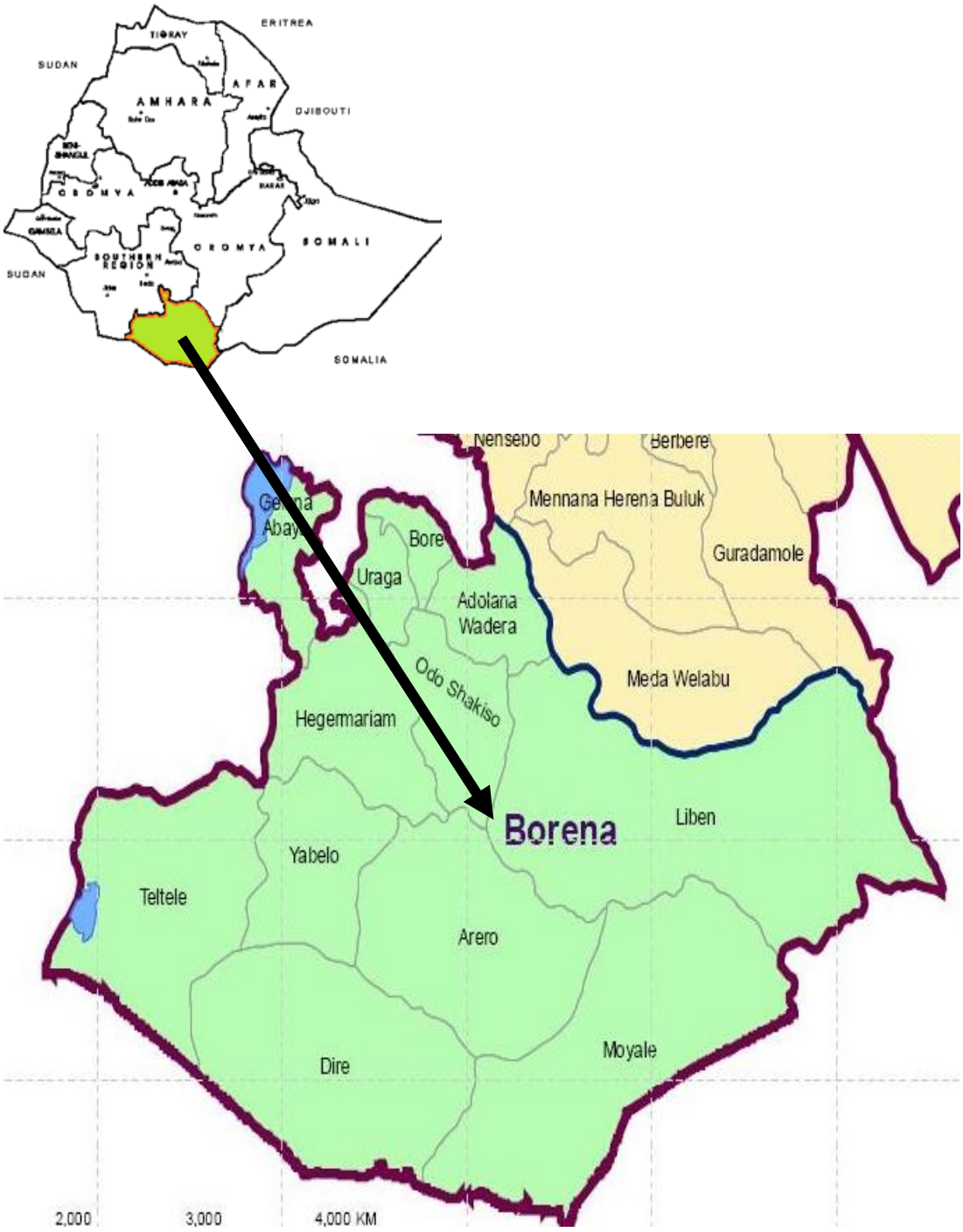
+=90% or more strain positive; ± = 90% or more weakly positive; d=11-89% positive; -=90% or more strains negative; () =delayed reaction; n=not known; R=resistant; S= susceptible

Biochemical reactions and other characteristics of important *Pasteurolla spp*

Species	**Beta hemolysis**	**Growth on MacCon-key agar**	**Indole**	**Urase**	**Ornithin decarbox-ylase**	**Acid (24-48hrs) from**				
						Glucose	**Lactose**	**Sucrose**	**Maltose**	**Manitol**
P. maltocida	-	-	+	-	(+)	+	(-)	+	(-)	(+)
P. haemolytica	+	(+)	-	-	(-)	+	(+)	+	+	+
P. pneumotropica	-	(-)	(+)	+	+	+	(+)	+	+	-
P. canis	-	-	±	-	+	+	n	n	-	-
P.dagmatis	-	-	+	+	-	+*	-	-	+	-
P.caballi	-	-	-	-	±	+*	+	n	(+)	+
P.aerogenes	-	+	-	+	(+)	+*	(-)	+	+	-
P. gallinarum	-	(-)	-	-	±	+	-	+	+	-

(+)= most positive; (-)= most negative; ± =positive and negative strains; n= data not available; +*=gas and acid from glucose

Annex **F:** Map of the study area



Annex **G:** Local name and corresponding scientific names of some camel diseases in Borana

	Diseases
Local name	Representative Scientific name
Salessa	Abortion
Marareb	PPR
Luka-dhig	Arthritis
Silisa	Pasteurollosis
Buta	Hind limb Paralysis
Nyanye	Rabies
Harka	Black leg
Sirgo	Circling Diseases
Dhidhissi (Dhido)	Paraflaria
Oyale	FMD
Dhuguda	Chronic respiratory disease
Sombessa Re'ee	CCPP
Sombessa Looni	CBPP
Mila-Mura	Lameness
Fino	Pox
Dhula	Puss
Mata-dhab	Stiffness of the neck
Furi	Acute Respiratory problem
Dhukuba Tiru	Jaundice
Awarsa	Trypanomosis
Tuma	Systemic diseases
Simphirti?	Madiness
Butale	Stiffness of the extremities
Qandho	Lymphadenitis
Baga	LSD

Annex **H:** Young camels with respiratory disease remain laid down