

**EPIDEMIOLOGICAL AND SOCIO-ECONOMIC INVESTIGATION OF CAMEL
SUDDEN DEATH (CSD) AND ITS RELATIONSHIP TO PESTE DES PETITS
RUMINANTS (PPR) IN AWASH FENTALE, AFAR REGIONAL STATE, ETHIOPIA**

FITSUM ALEMAYEHU

**A thesis submitted to the school of Graduate Studies of Addis Ababa University in partial
fulfillment of the requirements for the degree of joint masters in Transboundary Animal
Disease Management**

**ADDIS ABABA UNIVERSITY
SCHOOL OF VETERINARY MEDICINE
JOINT MASTERS PROGRAM IN TRANSBOUNDARY ANIMAL DISEASE
MANAGEMENT**

**NOVEMBER 2011
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MANAGEMENT**

ADVISORS

**Dr. TEFAYE SISAY (SCHOOL OF VETERINARY MEDICINE, ADDIS ABABA
UNIVERSITY)**

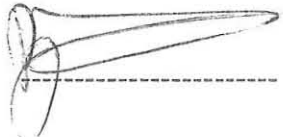
**Dr. MAXIMILIAN BAUMANN (INTERNATIONAL ANIMAL HEALTH, FREIE
UNIVERSITÄT)**

Dr. GELAGAY AYELET (NATIONAL VETERINARY INSTITUTE)

NOVEMBER 2011, DEBRE ZEIT

APPROVAL BY THE BOARED OF EXAMINERS

The thesis is hereby approved by the following members of board of examiners

Name and Title	Signature	Date
Dr Maximilian P.O. Baumann (Dr. med.vet., MPVM, DipECVPH), Co-ordinator of postgraguade studies in International Animal Health, Faculty panel Veterinary Public Health, Faculty of Veterinary Medicine, Freie Universitaet, Berlin/Germany.	-----	-----
Dr. Tesfaye Sisay (Assistant Professor, DVM,MSc,PhD), Head of department of Microbiology, Immunology, Epidemiology and Public health, Addis Ababa University, School of Veterinary Medicine, Debre zeit		<u>16/01/2012</u>
Dr Khitma Malik (Associate professor, BVSc, MSc, PhD) Head of veterinary preventive and public health department Khartoum University, Sudan	-----	-----

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LIST OF ABBREVIATIONS

AGID	Agar Gel Immuno Diffusion
CD150	Cluster of differentiation 150
cELISA	competitive Enzyme Linked Immuno Sorbent Assay
CI	Confidence Interval
CIRAD	Centre de Cooperation Internationale en Recherche Agronomique pour le Development
CSA	Central Statistics Agency
CSD	Camel Sudden Death
CVRL	Central Veterinary Research Laboratory
df	Degrees of Freedom
EIs	Emerging Infections
EMVT	Elevage et Medecine Veterinaire des pays Tropicaux
FADDL	Foreign Animal Disease Diagnostic Laboratory
H-Protein	Haemagglutinin Protein
MAb	Monoclonal Antibody
mm	millimeter
NVI	National Veterinary Institute
NAHDIC	National Animal Health Diagnostic and Investigation Centre
N-Protein	Neuraminidase Protein
NCBI	National Center for Biotechnology Information
OD	Optical Density
OIE	Office Internationale des Epizooties
OR	Odds Ratio
PA	Participatory Appraisal
Pa	Pastoral association
PCR	Polymerase Chain Reaction
PE	Participatory Epidemiology

PI	Percent Inhibition
PSU	Primary Sampling Unit
PPR	Peste des Petits Ruminants
PPRV	Peste des Petits Ruminants Virus
RNA	Ribo Nucleic Acid
RP	Rinderpest
RPV	Rinderpest Virus
RT-PCR	Reverse Transcriptase- Polymerase Chain Reaction
SLAM	Signaling Lymphocyte Activation Molecule
SSUs	Second stage Sampling Units
TFR	Task Force Report
TLU	Tropical Livestock Unit
VNT	Virus Neutralization Test
W	Kendal's coefficient of concordance
χ^2	Chi-square

ABSTRACT

A cross-sectional survey using serological and participatory appraisal investigation methods was applied between March 2011 and November 2011 to determine the seroprevalence of peste des petits ruminants (PPR) in camel and its associations with major risk factors like age, sex, pastoral association (Pa) and female lactation status, and to assess the community knowledge about causes and the socio-economic impact of a camel sudden death outbreak in 2006 in Awash Fentale of Afar Regional state, Ethiopia. Seroepidemiological investigations were performed using the competitive ELISA technique. The overall seroprevalence of PPR in camel in the study area was 13.7% (n= 446). Higher seroprevalences were recorded in camel of Doho Pa (19.1%) being significantly different from the other Pas, in female camel (14.1%) compared to male (6.7%); in lactating camels (14.9%) compared to dry camels (11.7%) and in adults >4years (14.2%) compared to young camels (12%). From the risk factors analyzed in the univariate and multivariate logistic regression analysis including Pa, sex, age and female lactation status, only Pa remained to be significantly associated ($P=0.01$) with seroprevalence with an odds ratio of 3 (95% CI: 1.012-9.417) when Doho was compared to Sabure Pa (2.4%). The participatory methods used were pair-wise ranking and comparison, matrix scoring, and proportional piling. The result of pair-wise ranking indicated strong agreement among the 5 informant groups ($W=0.883$; $p=0.00$) where camel sudden death (*Galireba*) was ranked first followed by pneumonia (*Mesengele*). Matrix scoring for the disease indicators/disease signs showed strong levels of agreement among the 5 informant groups ($W= 0.672$ -1.000; $p< 0.05$) for the Awash Fentale Afar pastoralist's descriptions of camel sudden death with at least some overlap with modern veterinary descriptions. The results of proportional piling indicated that about 49.3% of the camel population suffered from various diseases and 35.1% died during the 2006 camel sudden death outbreak period. For camel sudden death (*Galireba*) the highest mean annual incidence and mortality of 23.3% and 22.8%, respectively were reported with the highest mean annual incidence in adults (45.4%), being significantly ($p<0.05$) different from calves (10%); similar findings were noted for mortality in adults compared to calves. The participatory

appraisal results indicated camel sudden death is an important camel disease in Awash Fentale Afar pastoral production system to warrant the institution of an appropriate control strategy. The semi-structured questionnaires revealed further that camel is a very important animal to the Awash Fentale Afar pastoralists in terms of food security and social benefits such as transport, source of income, and ceremonial purposes. All of the respondents indicated the importance of camel milk for children and for its clan based consumption. The mobility of the camel herds for many reasons is likely to contribute in particular to the transmission of infectious diseases. The reason that camel sudden death in 2006 affected camel herds which were not sick, clearly indicates its peracute nature and thus only preventive measures can be applied. The high mean number of camels dead (15) per herd and a market price of 4,134.7 Birr due to CSD for mostly adult, productive camels did have a direct and serious impact on the (re)building-up of camel herds using replacement stock. Most of the signs shortly before and at death (69%) were inconclusive including recumbency, dyspnoea, arching of neck, oozing of blood from anus (81.1%) and nasal discharge (12.6%). The post-mortem results revealed black, enlarged lungs, blackish effusion from the thoracic cavity, extended and black liver, hypertrophied heart, airway passages full of white foamy gaseous fluid, distended carcass, and no rigor mortis. Even though the absence of rigor mortis and blood clotting are pathognomonic signs of anthrax, vaccination against anthrax and even mass treatment with broad-spectrum antibiotics did not stop the mortality, although a reduction in mortality was observed. No etiological agent such as *Bacillus anthracis* was isolated.

Although camel movement control is impractical (at least at present) in the Awash Fentale Afar pastoral system, the determined level of seropositivity of PPRV and the reported loss due to morbidity and mortality attributed to camel sudden death justifies the launching of a disease identification and control, prevention and emergency rehabilitation programmes. Thus PPR and its effects in camels and small ruminants and possible interactions should be further studied.

Key: Camel sudden death, PPR, seroprevalence, cELISA, Participatory Appraisal, Awash Fentale Afar

1. INTRODUCTION

Camels (*Camelus dromedarius*) are the livestock species uniquely adapted to hot and arid environments providing milk, meat, wool, hair, hides, serve for riding and are used as draft animals for transport (Bekele, 1999; Schwartz and Dioli, 1992; Kadim *et al.*, 2008). Camels are known to have particular physiological features by which they regulate body temperature to changes in ambient temperatures enabling them to survive and adapt to such harsh conditions (Wilson, 1984; Yagil, 1985; Higgins *et al.*, 1992). Camels are suitable for optimized utilization of previously marginalized resources such as semi-arid and arid rangelands (Schwartz and Dioli, 1992). Camel's ability to survive and even produce in drought prone areas is the reason for the steady increase in numbers while the recurring drought is causing huge losses in other livestock species in the Horn of Africa (Bekele, 1999).

An estimated 11.5 million camels are living in the eastern part of Africa (Djibouti, Eritrea, Ethiopia, Kenya, Somalia and Sudan) in the arid and semi-arid range lands and this figure represents over 80% of the African and two thirds of the world population (Hartley, 1979; Bekele, 1999). These data also explain the importance of camels for the region and the camel herders as camels do also play a significant socio-cultural role in the live of pastoralists (Hartley, 1979).

Ethiopia hosts about 2.3 million heads of camel, which are found in the arid and semi-arid regions of the country (CSA, 2009). The importance of camel as a means of survival in this country cannot be overemphasized as camels serve particularly during drought for better milk production compared to other livestock species (Bekele, 1999).

Although camels seem to suffer from fewer diseases than other domestic animals and epidemics are rare, the major diseases which affect camels in Ethiopia include trypanosomiasis, helminthiasis, mange, and camel pox (Bekele, 2002).

Of the many diseases of camels that are rampant in the Horn of Africa the recently emerged camel disease “Camel Sudden Death (CSD)” which causes are still unknown is an important disease with high mortality which commenced in the mid-90s with the last well reported outbreak in 2007(Roger *et al.*, 2001; Dawo, 2010). The 1995 camel disease outbreak in Ethiopia with severe respiratory involvement had a highly contagious nature with high morbidity rates of over 90% and variable mortality rates (Fekadu *et al.*, 2003; Roger *et al.*, 2001). The multifactorial speculated causes include *Mannheimia haemolytica*, *Pasteurella multocida*, *Parainfluenza 3* virus, PPR (Peste des Petits Ruminants) and *Morbilli*-like viruses (Bekele, 1999; Fekadu *et al.*, 2003; Roger *et al.*, 2001).

In 2005 an outbreak of suspected “CSD” had occurred in Afar and East Shoa zone causing the sudden death of around 530 camels (Getachew *et al.*, 2005). The outbreak was investigated by a team from the National Animal Health Diagnostic and Investigation Centre (NAHDIC-Sebata), National Veterinary Institute (NVI-Debre Zeit), the Central Veterinary Research Laboratory Dubai (CVRL-Dubai) and the Foreign Animal Disease Diagnostic Laboratory (FADDL-APHIS-USA). The investigation team came up with different findings which included trypanosomiasis, hydatid cyst disease, infestation with tapeworms, *Trichuris* spp., sarcocysts, haemonchosis, intoxication resulting from gossypol, from euphorbia, and from clostridial as well as undescribed bacterial diseases. However, the researchers pointed out that the above listed health problems may not be the primary cause of the problem but could be secondary or may even not be associated at all with the problem. Thus, the team highly recommended detailed epidemiological survey and further laboratory investigations to be carried out to come up with the primary causes of the problem. Therefore, identification and characterization of the primary causative agents which is most probably of viral agent/s is highly required (Wernery *et al.*, 2006).

A similar unspecified disease, suspected as “CSD” occurred in 2007 in the Somali regional state and the Borena and Guji Zones of Oromia Regional State of Ethiopia, and was also observed in Somalia and Kenya during the same time. The disease was characterized by sudden death without any overt clinical signs and or symptoms. It was reported that lactating and pregnant camels were more affected than other female and male camels. According to Daniel and Solomon (2007) herds

with animals in good body condition were more affected than poor ones. Very recently, Dawo (2010) reported that the 2007 outbreak of sudden camel mortality in the southern pastoral areas of Ethiopia targeted particularly productive age groups like pregnant, lactating and breeding male and female camels. Fortunately, there were no reports of zoonotic consequences of this disease while the pastoralist used to eat meat and drink milk of diseased as well as of apparently healthy camels (Daniel and Solomon, 2007; Dawo, 2010). Earlier in mid-August 2004, in Sudan an outbreak of a previously unknown fatal disease of camels was reported with the similar clinical signs as reported in Ethiopia but also including colic, difficulty in respiration and sudden death which reportedly linked to PPR with Agar gel diffusion test, reverse transcriptase polymerase chain reaction and virus isolation in cell culture (Khalafalla *et al.*, 2010).

This “camel sudden death” syndrome is still not understood clearly and becoming mysterious for the herders, animal health and production professionals and policy makers. It is hypothesized that the “CSD” disease occurs cyclically, thus by eventually killing a significant number of camels threatening the livelihood of the East African pastoral community, and that of Ethiopia in particular. Because of the unknown nature and etiology of the mortality, it has been difficult to take appropriate prevention and control measures at a national level. Hence, any investigation into possible causes of the “camel sudden death” syndrome is expected to have a significant contribution to the design of cost efficient control and/or preventive measures and, therefore contribute to food self-sufficiency of the people inhabiting the affected areas.

Thus, the objectives of this study are:

- to determine the seroprevalence of PPR and its statistical association with major risk factors like age, sex, pastoral association, female reproductive status in sudden camel death exposed area of the pastoral association of Awash Fentale of Afar regional state and
- to assess the community knowledge about causes of the “camel sudden death” and to assess the socio-economic impact of sudden camel death in outbreak hit pastoral associations of Awash Fentale of Afar Regional State.

2. LITRATURE REVIEW

2.1. The Camel

The one-humped camel (*Camelus dromdarius*) can live in areas that are inhospitable to other domestic animals and are therefore an important factor in the capacity of humans to survive in and make use of these drier regions (Yagil, 1985; Schwartz and Dioli, 1992; Al-Eknah, 2000). Camels are known to have particular physiological features by which they regulate body temperature to changes in ambient temperatures enabling them to survive and adapt to such hard conditions (Wilson, 1984).

Camels provide milk, meat, wool, hides, leather, and their dung is used as fuel for fires. They are used for riding and transport, are a means of investment and long-term savings, and are a source of prestige for their owners. Sales of surplus milk, livestock or livestock products are sources of cash income for pastoral families (Kaufmann, 2005). There is a large market for exportation of live camels. In addition, camels are slaughtered for consumption and during ritual occasions (Wilson, 1984; Higgins *et al.*, 1992).

2.2. The “Camel Sudden Death (CSD)” syndrome and its socio-economic impact in Ethiopia

Camels have been formerly considered as hardy animals and less susceptible to most of the diseases that affect other livestock in the same ecological zones. However, as more research was conducted camels were found to be susceptible to a large number of pathogenic agents. They may contract many other diseases, some of which are still unknown (Megersa, 2010) such as recently emerged camel disease “Camel Sudden Death (CSD)”. The 1995 camel disease outbreak in Ethiopia with severe respiratory involvement drew the nation’s and international agencies attention and has affected almost the whole camel population in less than a year (Bekele, 1999).

By then this not well defined disease had a highly contagious nature with high morbidity rate of over 90% and a variable mortality rate (Fekadu *et al.*, 2003; Roger *et al.*, 2001) killing 365 camels in Shinile, Somali region. Besides the mortality, loss of productions including milk from reduced yield (36.2%) to complete agalactia (63.8%) and the costs of antibiotics for treatment and vaccination costs were the rough monetary costs of the outbreak (Bekele, 1999). In 2004, CSD was observed in Fentale district of Oromiya Region killing 148 camels (TFR, 2007).

In 2005 an outbreak of “CSD” had occurred in Afar and East Shoa Zone causing the sudden death of around 530 camels (Getachew *et al.*, 2005). In January, 2006, the outbreak was investigated by a team from different laboratories including National Animal Health Diagnostic and Investigation Centre (NAHDIC-Sebata), National Veterinary Institute (NVI-Debre Zeit), Central Veterinary Research Laboratory Dubai (CVRL-Dubai) and Foreign Animal Disease Diagnostic Laboratory (FADDL-APHIS-USA). The investigation team came up with different findings, however, the researchers pointed out that the findings may not be the primary cause of the problem. Thus, the team highly recommended detailed epidemiological survey and further laboratory investigations to be carried out to come up with the primary causes of the problem (TFR, 2007). Therefore, identification and characterization of the primary causative agents which is most probably of viral agent/s is highly required (Wernery *et al.*, 2006). In December 2006, CSD claimed 144 camels in Afder zone of Chereti wereda which brought the fall of price of live camels and frustration in the consumption of camel meat and milk in the local residents (TFR, 2007).

In June–July 1992, a contagious acute disease characterized by pneumonia (named camel contagious pneumonia), lacrimation, and difficulty in respiration caused a mortality of about 5% affected camels in southern Butana, southern Gedarif and extended to Kassala provinces. An unusual high number of deaths were reported in camels in Somalia in 2006 and Kenya in 2007. Mainly adult camels were reported to be dropping dead without any prior symptoms. Herd mortality in affected herds was 3.7% and 6.6 % in Puntland and Kenya, respectively. In addition the mean number of cases per herd varied between the two countries. While the mean was 2.0 in Puntland, pastoralists in Kenya reported a mean of 6.8 cases per herd. Mainly females (74% in Puntland and 88% in Kenya) were affected within the herds and out of those all were dry,

pregnant or lactating. The results were comparable in Puntland and Kenya of the females affected 59% were dry and pregnant and 41% lactating females. The average age of affected camels was 9.7 years in Puntland and 9.5 years in Kenya (Gluecks and Younan, 2010).

A similar unspecified disease, suspected as “CSD” occurred in 2007 in the Somali regional state and Borena and Guji Zones of Oromia regional state of Ethiopia, and was also observed in Somalia and Kenya during the same time. The disease was characterized by sudden death without any overt clinical signs and or symptoms. It was reported that lactating and pregnant camels were more affected than other female and male camels. According to Daniel and Solomon (2007) herds with animals in good body condition were more affected than poor ones. Very recently, Dawo (2010) reported that the 2007 outbreak of sudden camel mortality in the southern pastoral areas of Ethiopia targeted productive age groups like pregnant, lactating and breeding male and female camels and killed 187 camels. In addition to loss of milk in lactating camels and loss of the calf in the pregnant individuals, the average value of the dead camel was 2,377.6 birr (US\$264). Fortunately, there were no reports of zoonotic consequences of this disease while the pastoralist used to eat meat and milk of diseased as well as of apparently healthy camels (Daniel and Solomon, 2007; Dawo, 2010).

2.3. Emergence of viral infection

Generally, emerging infections (EIs) can be defined as “infections that have newly appeared in a population or have existed previously but are rapidly increasing in incidence or geographic range (Brown, 1997; NCBI, 2004) or recently been discovered or caused by newly-evolved pathogens” (Daszak *et al.*, 2001). The reasons underlying the general increase in emerging diseases are multiple and often overlapping (Domingo, 2010) and include physical movement to a susceptible population, crossing of species boundaries (Trei *et al.*, 2010) environmental disruption and the alteration of ecosystems (Domingo, 2010) and socio-economic activity changes including human population increase, abnormal antibiotic drug use, increased transportation (Jones *et al.*, 2008).

The role of the laboratory in the creation of emerging diseases, i.e. genetic variation of viruses should also not be ignored (Hoelzer and Parrish, 2010).

Emerging viral diseases are nowadays common both in humans as well as animals. About 25.4% of emerging pathogens are viruses or prions in emerging infectious diseases recorded between 1940-2004 (Jones *et al.*, 2008). Viruses with RNA as their genetic material can quickly adapt to and exploit the varying conditions because of the high error rates of the virus enzymes (polymerases) that replicate their genomes. In addition to virus genetic variation such as mutation, recombination, and reassortment, environmental factors including ecological, social, health care, and behavioral influences coupled with enormous increases in the human and animal population, with the advances in the speed and volume of global transportation create further increased opportunity for emergence and re-emergence of viral diseases (Domingo, 2010).

Within the genus of *Morbillivirus* and related viruses, several emerging diseases have been recently described. The existence of mild strains of rinderpest in domestic cattle has had devastating effects in African buffaloes, eland and lesser kudu in Africa (Parker, 1997; Kock *et al.*, 1999) also could cause disease in goats, sheep, and rarely in pigs (Murphy *et al.*, 1999). Peste des Petits Ruminants Virus (PPRV) was isolated during an outbreak in the mid 1990s in Indian buffaloes (Govindarajan *et al.*, 1997) and isolated from camels affected by CSD outbreak in Sudan (khalafalla *et al.*, 2010). New morbilliviruses with devastating effects have been described in marine mammals (Mah *et al.*, 1988), while canine distemper virus caused mortalities in lions of East Africa (Roelke-Parker *et al.*, 1996), in leopards and other large cats in zoos (Appel *et al.*, 1994) have underscored the ability of canine distemper virus to invade new host species. Viruses belonging to the *Paramyxoviridae* family, initially classified as *morbillivirus*, were recently described in diseases declared in horses (O'Sullivan *et al.*, 1997), pigs and human (Chua *et al.*, 2000). It was hypothesized that the cause of the "CSD" in camel is associated with a "new" morbillivirus which could result from an interspecies transfer of PPRV or Rinderpest-Virus (RPV) strains from cattle or goats and sheep to camel or through the emerging of a new variant *morbillivirus* serologically related to PRV and/or RPV (Roger *et al.*, 2001).

Other examples of emerging and re-emerging RNA virus are described for parvoviruses (Hoelzer and Parrish, 2010), bluetounge virus, African horse sickness virus, Ebola virus, adenovirus, and Nipah virus, Hendra virus (MacLachlan and Guthrie, 2010; Trei *et al.*, 2010).

2. 4. Peste des Petits Ruminants(PPR) and its associations with camel health

2. 4. 1. PPR (Peste des petits ruminants)

Peste des petits ruminants (PPR) is a highly contagious and infectious viral disease of domestic and wild small ruminants (Furley *et al.*, 1987). PPR Virus belongs to the *Morbillivirus* genus of the *Paramyxoviridae* family (Gibbs *et al.*, 1979). There is close antigenic relationship with rinderpest virus (RPV), canine distemper virus, measles virus, dolphin distemper virus, phocine distemper virus and porpoise distemper virus (Murphy *et al.*, 1995; Albayrak and Gur, 2010). PPR-virus isolates can be grouped into four distinct lineages on the basis of partial sequence analysis of the fusion (F) protein genes; lineage III is the lineage found in Eastern Africa (Ethiopia) (Abraham *et al.*, 2005; OIE 2008a).

It is an economically significant disease of small ruminants such as sheep and goats (Dhar *et al.*, 2002). It was first described in Côte d'Ivoire in West Africa (Gargadennec and Lalanne, 1942) where it used to be named as Kata, pseudorinderpest, pneumoenteritis complex and stomatitis-pneumenteritis syndrome (Braide, 1981). Transmission requires close contact between infected animals being in the febrile stage and susceptible animals (Braide, 1981) because of the liability of the virus outside the living host. Discharges from eyes, nose and mouth, as well as loose faeces contain large amounts of the virus. Fine infective droplets are released into the air from these secretions and excretions, particularly when affected animals cough and sneeze (Taylor, 1984). Animals in close contact inhale the droplets and are likely to become infected. Although close contact is the most important way of transmitting the disease, it is suspected that infectious

materials can also contaminate water and feed troughs and bedding, turning them into additional sources of infection (Lefèvre and Diallo, 1990).

The incubation period is about four days. The disease particularly affects young animals. Affected goats exhibit fever, dry muzzle and a serous nasal discharge which become mucopurulent. Erosions on the mucous membrane of the buccal cavity are accompanied by marked salivation. Ulcers develop in the mucosae of the alimentary, respiratory and urinary tracts. Conjunctivitis with ocular discharge is a feature of the disease. A profuse diarrhea, which results in dehydration, develops within days of infection. Signs of tracheitis and pneumonia are common. There is a severe leucopenia which facilitates secondary bacterial infection. Pulmonary infections caused by *Pasteurella* species are common in the later stages of the disease. Pregnant animals may abort. Mortality rates in severe outbreaks often exceed 70 % and actually affected goats may die within ten days of exposure to the virus (Forsyth and Barret, 1995).

The pathology caused by PPR is dominated by necrotizing and ulcerative lesions in the mouth and the gastro-intestinal tract (Roeder *et al.*, 1994). In the respiratory system, small erosion and petechiae may be visible on the nasal mucosa, turbinates, larynx and trachea. Bronchopneumonia may be present, usually confined to the anteroventral areas, and is characterized by consolidation and atelectasis.

Definitive diagnosis of PPRV infection relies on laboratory techniques such as virus isolation, demonstration of PPRV antigen, viral nucleic acid and specific antibodies. The serological tests like AGID (Agar Gel Immuno Diffusion Test), CIEP (Counter Immuno Electro Phoresis) etc. often fail to differentiate PPR from RP. cELISA (competitive Enzyme Linked Immuno Sorbent Assay) is rapid, sensitive and specific and the most commonly used technique. RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) test can be used as routine diagnostic tool for PPR diagnosis, which has good correlation with virus isolation (Brindha *et al.*, 2001). PCR (Polymerase Chain Reaction) in combination with nucleotide sequencing has made it method of choice for molecular characterization of viruses (Diallo, 1990).

There is no specific treatment against the disease. Control of PPR in non infected countries may be achieved using classical measures such as restriction of importation of sheep and goats from affected areas, quarantine, slaughter and proper disposal of carcasses and contact fomites and decontamination of affected premises in case of introduction. Control of PPR outbreaks can also rely on movement control (quarantine) combined with the use of focused ("ring") vaccination and prophylactic immunization in high-risk populations (Braide, 1981).

Epidemics in sheep and goats cause mortality rates of 50-80 % in naïve populations. Antelope and other small wild ruminant species can also be severely affected (Abu Elzein *et al.*, 2004). A case of clinical disease has been reported in wildlife resulting in deaths of gazelles (*Gazella dorcus*), ibex (*Capra ibex nubiana*), gemsbok (*Oryx gazelle*) and Laristan sheep (*Ovis orientalis laristanica*). Cattle, buffaloes, camels and pigs can become infected but there is little or no evidence of disease associated with their infection (Gibbs *et al.*, 1979; Taylor, 1984). PPRV antigen has been detected in an outbreak of respiratory disease in camel and sick domestic buffaloes (Roger *et al.*, 2000; Govindrajan *et al.*, 1997). In addition, PPRV is considered as a serious predisposing factor for respiratory disease complex in sheep and particularly in goats. (Taylor *et al.*, 1990; Bailey *et al.*, 2005). Considering the immunosuppressive effect of PPRV as all other morbilliviruses, it may therefore be possible, depending on the age and physical state of the host animal, that PPRV can occasionally overcome the innate resistance of large ruminants and lead to the development of clinical signs similar to Rinderpest (Diallo, 2007). This may explain the disease signs that had occurred in buffalo and camels following PPRV infection. This ability of PPRV to infect large ruminants could pose a serious threat to cattle populations in PPR endemic areas which, with the success of the global Rinderpest eradication programme, are no longer vaccinated against Rinderpest and so do not possess cross protective immunity against this virus.

2. 4. 2. PPR (Peste des petits ruminants) and camels

PPR has been thought to be a disease of only domestic (sheep and goats) and wild small ruminants. However, PPRV antibodies detected in cattle in Mali (1.7%), Cameroon (4.5%), 0.3%

in Sudan (Intisar *et al.*, 2010), and 9% and 20.2% in small ruminants and also 3% in camels in Ethiopia (Abraham *et al.*, 2005) are of great concern. PPRV antigen has been detected in an outbreak of respiratory disease in camel and sick domestic buffaloes (Roger *et al.*, 2000; Govindarajan *et al.*, 1997; Khalafalla *et al.*, 2010). PPRV was also suspected to be involved in the epizootic disease that affected one humped camels in Ethiopia in 1995-1996 where 7.8% antibody seroprevalence was found (Roger *et al.*, 2001). Within the *Morbillivirus* genus and related viruses, several emerging diseases have been described (Govindarajan *et al.*, 1997; Parker, 1997). In that context, new diseases could result from an interspecies transfer of PPRV or RPV strains from cattle or goats and sheep to camel or through the emerging of a new morbilliviruses, serologically related to PPRV and/or RPV (Roger *et al.*, 2001). It is, however, unknown if seroconverted camels serve as carriers and whether they have potential to spread PPR virus or can be affected clinically (Singh *et al.*, 2009).

This difference may result from a difference in cell susceptibility to the virus. The cell susceptibility can be affected by the rate of infection, the affinity of the virus for its cell membrane receptor, the efficiency of intercellular spreading of the virus and capacity to induce damages in infected cells. However, the factors which determine the virulence remain largely unknown and have not been related to a single event. Low virulence outcome may result from a lesser infection of the cells or a lower replication of the virus in the cells, both resulting in a lower viral antigen distribution through different organs and tissues (Wohlsein *et al.*, 1995). Such characteristics may account for the milder clinical disease and lower mortality. The capability of cells to be infected and support active virus replication has important implications on the pathogenesis of the disease.

Cellular receptors are one of the major determinants of the host range and tissue tropism of a virus (Baron *et al.*, 1996). Human Signaling Lymphocyte Activation Molecule (SLAM; also known as CD150), a membrane glycoprotein expressed on some lymphocytes and dendritic cells (Cocks *et al.*, 1995), is a cellular receptor for most of the morbilliviruses (Tatsuo *et al.*, 2000). The tissue distribution of human SLAM can explain the pathology of measles. Selective infection and destruction of SLAM positive cells may be the principal mechanism underlining

the immunosuppressive effect of morbilliviruses in general (Tatsuo *et al.*, 2000). Morbilliviruses use SLAMs of their respective host species as cellular receptors (Tatsuo *et al.*, 2001). However, Measles virus, Canine distemper virus, and Rinderpest virus strains could use SLAMs of non host species as receptors, albeit at reduced efficiencies (Tatsuo *et al.*, 2001). Thus, the finding that these three morbilliviruses use SLAMs as cellular receptors suggests that the usage of SLAM as a receptor has been maintained from the ancestral virus, accounting for an essential part of the pathogenesis of morbillivirus infections. Genetically, it was assumed that when cattle were domesticated, they passed a morbillivirus, a progenitor of modern Rinderpest virus, to humans, which eventually evolved into Measles virus. Similarly, carnivores could have contracted a morbillivirus infection from their ruminant prey, which then evolved into Canine distemper virus (Barrett and Rossiter, 1999). PPRV has also probably arisen from a Rinderpest virus ancestor through adaptation to small ruminants. Interestingly, adaptation of Rinderpest virus to another species seems to have resulted in a reduction of the host range (basically primates for Measles virus, carnivores for Canine distemper virus and small ruminants for PPRV).

2.5 Serological and Participatory investigations

2.5.1 Serological investigation of PPR

Many tests have been used for the demonstration of PPR antibodies in serum including virus neutralization test, agar gel diffusion test, immunoelectrophoresis and recently blocking and cELISA. The virus neutralisation test (VNT) is sensitive and specific, but time-consuming and expensive. The standard neutralisation test is carried out in roller-tube cultures of primary lamb kidney cells or Vero cells when primary cells are not available. VNT is the most reliable test for detection of morbillivirus antibodies (Rossiter, 1994). Serum against either PPR or RP may neutralise both viruses, but would neutralize the homologous virus at a higher titer than the heterologous virus. Therefore for differentiation purpose reciprocal cross neutralization is used (Taylor and Abegunde, 1979).

Competitive and blocking ELISA based on monoclonal antibodies specific for N-protein (Neuraminidase protein) (Libeau *et al.*, 1995) and H-protein (Haemagglutinin protein) (Anderson and McKay, 1994; Saliki *et al.*, 1993; Singh *et al.*, 2004) were developed for detection of antibodies in animal sera. These tests either used gradient purified virus or expressed antigens. In the N-protein cELISA, the serum antibodies and the monoclonal antibody (MAb) compete on specific epitope on nucleoprotein obtained from recombinant baculovirus. Though no cross reaction in N-protein cELISA was reported, a high level of competition up to 45% was observed among the negative (Libeau *et al.*, 1995). Despite the fact that neutralizing antibodies are not directed against the N-protein, but the H-protein (Diallo *et al.*, 1995), a correlation of 0.94% between VNT and cELISA was observed suggesting that the former was more sensitive (Libeau *et al.*, 1995). The relative sensitivity of this cELISA to VNT was 94.5, while the specificity was 99.4%. Both blocking ELISA and cELISA detecting anti-H antibodies are based on competition between an anti-H monoclonal antibody (MAb) and serum antibodies, but in case of blocking ELISA the test sera are preincubated with antigen and then incubated with the MAb (Saliki *et al.*, 1993). The sensitivity and specificity of the H-blocking ELISA were found to be 90.4% and 98.9% respectively (Saliki *et al.*, 1993). PPR cELISA using MAb directed against the H-protein cross reacted to some extent with rinderpest, while RP cELISA is specific, therefore an animal was assumed to have experienced RP if it is positive in both PPR and RP ELISA (Anderson and McKay, 1994). The absorbance in PPR ELISA is converted to percentage of inhibition (PI) using the formula: $PI = 100 - (\text{absorbance of the test wells} / \text{absorbance of the mAb control wells}) \times 100$. Sera showing PI greater than 50% are scored positive. The overall specificity of c-ELISA test was 98.4% with a sensitivity of 92.2% when compared with VNT.

2.5.2 Participatory Appraisal

A major constraint to veterinary surveillance and consequent disease control in developing countries has been the lack of information on disease morbidity. Data collection may be thwarted by poor laboratory diagnostic support, insufficient manpower, and difficult terrain (Broadbent,

1979). There also may be a general failure of conventional veterinary services (Swift *et al.*, 1990). This has given impetus to the elaboration of alternative methods of data collection as part of the wider goal of improving the delivery of veterinary services in the context of closer participation in disease control by rural communities. The need for methods of data collection and analysis that are powerful, quick, careful and cheap led to a range of rapid rural appraisal techniques, using multidisciplinary teams, both in the agricultural sector generally (McCracken *et al.*, 1988) and specifically in relation to animal health issues (Ghirotti, 1992). Involvement of communities in data-gathering resulted in the term participatory appraisal being applied to this mainly qualitative approach (Pretty, 1995). More recently, participatory epidemiology has been coined to describe community participation in data collection and disease control (Mariner, 1996; Catley and Mariner, 2001). Participatory epidemiology relies heavily on indigenous knowledge and terminology. This is known to be extensive. Moreover, local diagnosis often compares favorably with conventional scientific investigation (Baumann, 1990; Edelsten, 1995; Mariner and Roeder, 2003; Catley *et al.*, 2004). It therefore has a useful role to play in the timely identification of disease in the field, and to confirm the absence of clinical disease.

There is substantial evidence that Participatory Appraisal (PA) methods do generate information that precisely describes local people perceptions on animal health problems (Leyland, 1991). The methods are relatively resource friendly and are easily flexible according to given circumstances and information needs (Catley, 1999). In addition, there is an increasing interest by research centers to involve rural communities in the definition of research problems and identification of their solutions (Catley and Irungu, 2000), since community-based programs look promising with regard to sustainability (Budd, 1999). PA has been used to good effect by community based animal projects and consequently, there is now potential for incorporating this method into conventional animal health data collection system (Catley, 1997).

A wide range of participatory epidemiology (PE) methods are available and these methods can be categorized into three main groups: informal interviews; visualization methods; and ranking and scoring methods. Information derived from different methods is cross-checked or 'triangulated' in a similar way that a clinician combines information from different sources to reach a diagnosis.

Indeed, the use of conventional veterinary diagnostic tools is an integral part of, and in some cases overlap with PE methods. These tools include direct observation, livestock owner interviews, and clinical and pathological examination (Catley, 2005). Compared to the conventional methods, participatory appraisal methods are used to generate qualitative data which can define local people's problems and solutions and hence bottom up approach. The Participatory appraisal approaches are holistic in nature using multiple methods in a process called triangulation and the communities also have a greater access, control, understanding, analysis of information and decision making which makes the project sustainable. Typically, quantitative data driven approaches are attempted, but prove to be untenable in large pastoral areas with relatively small and mobile human population, limited modern infrastructure, and insecurity. Other problems include lack of baseline data to perform random sampling procedures and the difficulty of following the herd during longitudinal studies (Catley and Mariner, 2002). The use of standardized PA tools, which produce quantitative data, is another option for veterinary epidemiologist for pastoral areas.

3 .MATERIALS AND METHODS

3.1. Study area

The cross-sectional study using a serological and participatory appraisal survey was carried out in Awash Fentale weredas of Afar regional state in the CSD exposed kebeles, Dudub, Doho, Sabure, Boloita and Kebena (Fig 1).

The Afar region is one of the four major pastoral regions in Ethiopia located in north eastern part of the country bordering Oromiya region in the south, Tigray region and Eritrea in the north, Djibouti and Somalia region in the east, and Amhara region in the west. The region is divided in to five administrative zones, which are further subdivided into 29 weredas.

The majority of the land is rocky and the annual precipitation is low (150-500 mm/annum) which makes crop cultivation unsuitable. The altitude of the region ranges from 1500 meters above sea level in the western highlands to -120 meters below sea level in the Danakil/Dallol depression. Afar is characterized by an arid and semi-arid climate with low and erratic rainfall. Temperatures vary from 20°C in higher elevations to 48°C in lower elevations. Rainfall is bi-modal throughout the region with a mean annual rainfall below 500 mm in the semi-arid western escarpments decreasing to 150 mm in the arid zones to the east. Afar is increasingly drought prone. The region receives three rainy seasons. The main rains, *karma* account for 60% of the annual rainfall and lasts from mid-June to mid-September followed by rainy showers in mid-December called *dadaa* and a minor rainy season during March – April called *sugum*. Disruptions on the performance of any rainy season will impact on the availability of pasture and water as well as the overall food security situation of the pastoral and agro-pastoral communities (Joanne *et al.*, 2005).

The livestock population is estimated at about 4 Million. Commonly kept livestock include cattle, goats, sheep, camels and donkeys. Cattle and camel milk is an important component of the children's diet. Sheep and goats are kept for meat and cash income. Donkeys are kept in limited numbers for transportation purposes. The livestock population in the region is estimated at 703,424 cattle, 1,003,000 heads of sheep, 2,014,418 heads of goats, 301,733 camels and 16,976 donkeys. Assuming the pastoral population to be 1.2 million, the per capita livestock holding is only 1.09. This is far lower than the recommended 3-4 Tropical Livestock Unit (TLU) per capita required for pastoralists to lead a sustained and active life (Joanne *et al.*, 2005).

Livestock are central to the Afar economy. Ninety percent of Afar inhabitants derive their livelihoods from subsistence livestock production with 90% being pastoralists and 10% agropastoralists. Pastoralists are predominantly nomadic with approximately 80 % practicing transhumant pastoralism (Joanne *et al.*, 2005).

Awash Fentale is one of the wereda located in the north eastern part of Ethiopia, 225km from Addis Ababa. It is a lowland area with an altitude of 931 meters above sea level and with an annual average rainfall of 450mm. The average maximum and minimum temperatures are 38°C and 21°C, respectively. The Awash National Park is situated in this wereda (Gizaw, 2003). Most of the forest areas of the Awash Fentale are found along the Awash River banks and in the Awash Park. The dominant tree species of the forest is Acacia. The wereda has 52,000 Cattle, 35,000 Sheep, 47,192 Goats and 3,230 Camels (CSA, 2009). The camels in the Awash Fentale wereda are the study population.

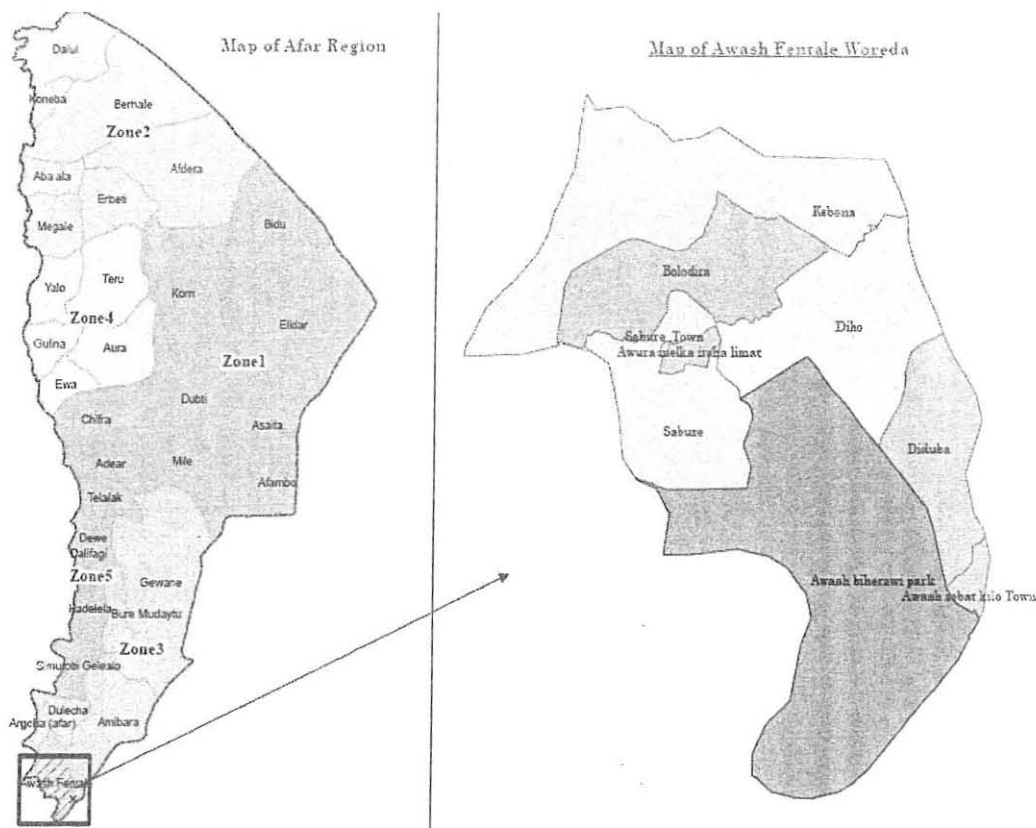


Figure 1: Map of Afar regional state showing location of the study area, Awash Fentale wereda and its respective pastoral kebeles, Ethiopia

3.2 Samples and data collection procedures

For the serological investigation blood samples are collected using multistage cluster sampling.

A two stage cluster sampling design was used with primary sampling units (PSUs) or clusters. The PSUs are randomly selected pastoral villages in the purposively selected kebele and second stage sampling units (SSUs) are randomly selected camels within these selected pastoral villages.

A pastoral village is defined as a group of households residing in an area (encampment) and which keeps their large ruminants (cattle and camels) in a mixed single herd sharing the same rangelands and water resources.

A household may be either;

- a) A one person household, that is a person who owns a herd of animals, keeps his/her large ruminants in a communal rangeland and share common water resources with the pastoral village and provides for his own family.
- b) A multi person household, that is a group of two or more persons living together, having their own herd of animals managed in common and keep their large ruminants in a communal rangeland and share common water resources with the pastoral village.

Determination of sample size and number of clusters (Pastoral village)

The number of camels to be sampled from each wereda is calculated with an expected prevalence (P_{exp}) of 10 % taken from a previous study (Abraham *et al.*, 2005) at an individual animal level at 95% confidence level using the formula stated by Thursfield (2007).

$$n = \frac{1.96^2 P_{exp} (1 - P_{exp})}{d^2} = \frac{3.8416 \times 0.1 \times 0.9}{0.0025} = 138$$

where: n = required sample size;

P_{exp} = expected prevalence;

d = desired absolute precision (0.05).

To increase the precision of the sampling method due to small sample size calculated and to account for multistage cluster sampling, the current sample determined was inflated by multiplying by 3 resulting in a total sample size of 414. However, finally 446 camels were included in the sample.

The number of clusters to be sampled is determined after estimating the in-between cluster variance, V_c (Thrusfield, 2007).

However, this estimation requires previous cluster sample data which are not available for this study. Thus, the between-cluster variance component was estimated by guessing the standard deviation and taking an assumption that the outbreak resulted in a similar scenario between clusters hence a very low variation is expected between the clusters (i.e., the average difference expected between an individual cluster prevalence and the overall mean cluster prevalence), and then squaring it to give the between-cluster variance component (Thrusfield, 2007 and Abraham *et al.*, 2005).

$$V_c = (P - p)^2 = (0.004)^2 = 0.000016$$

Where: P = mean overall cluster prevalence

p = individual cluster prevalence

V_c = between-cluster variance

Then, the number of clusters to be sampled can be calculated using the formula

$$g = \frac{1.96^2 TV_c}{d^2 T - 1.96^2 P_{exp}(1 - P_{exp})}$$

$$= \frac{3.8416 \times 139 \times 0.000016}{0.05^2 \times 139 - 3.8416 \times 0.1 \times 0.9} = \frac{0.0085437184}{0.001756} = 4.56 \sim 5 \text{ clusters}$$

Where: g = number of clusters to be sampled;

P_{exp} = expected prevalence

T = total number of animals to be sampled

V_c = between-cluster variance

Then, from each of the 5 clusters, 89 animals should be sampled ($446/5=89$).

3.3 Study methods

3.3.1 Serological investigation of field samples

Blood samples were collected from the camels from selected clusters included in the sample. The sera were collected by jugular vein puncture using plain vacutainer tubes and kept overnight to clot, then sera were separated by simple decantation. Samples which clot insufficiently were centrifuged. The cELISA developed by CIRAD-EMVT, (Libeau *et al.*, 1995) was used to detect specific PPR antibodies (the materials used and procedures followed are presented in Annex 1 and 2).

3.3.2 Participatory Appraisal

Participatory methods including semi-structured interviews, pair-wise comparison and ranking, matrix scoring and proportional piling were used to generate information on the knowledge of camel owners about the major diseases and risk factors that challenged their livestock production, with particular reference to CSD. The first stage of the participatory disease appraisal involved greeting, introduction and checking whether it was a convenient time for pastoralists to undertake the appraisal. Local translators, key informants and checklists (semi-structured questionnaires) were used to generate information on the knowledge of herders about camel diseases and other production constraints as described in (Catley and Mohammed, 1996) and (Catley and Admassu, 2003). A total of 5 key informant groups with group size varying from six to eight were interviewed during the study period and 95 camel owners were interviewed individually.

Pair-wise comparison and ranking

The key informant groups were asked to list diseases that affect their camel herds first and compare these diseases in pairs and decide which was most important. The results were then presented in simple matrix and total scores for each disease were counted. Diseases were introduced into discussion with their local names and represented with easily memorable objects with of course frequent check of memory and understanding. Mortality, loss of production, rate of transmission, difficulty to control and frequency of occurrence were some of the ranking criteria used by herders. Informants were asked frequently why they gave priority to the selected disease and hence in addition to confirming their decision, this process was also used to generate indicators for matrix scoring.

Matrix scoring

The five top ranked diseases, based on the pair-wise ranking exercise, were scored against a list of 15 clinical and epidemiological features (indicators) listed along the y-axis of the matrix (Table 6). The 5 informant groups were briefed every time and checked whether they correctly understand and correlate the objects with the intended cattle diseases to be scored before every scoring process. A group was then given 25 stones and asked to score each disease using stones. The quantity of 25 stones was chosen because it was assumed that five stones per disease would be sufficient to show differences between diseases (Catley and Mohammed 1996; Catley 2006). When the informants had scored each disease, the facilitator checked the scoring with the group and advised them that they could make adjustments to the scoring if they wished and then the number of stones allocated to each disease was counted and recorded.

The level of agreement between the five informant groups was assessed using the Kendal's coefficient of concordance (W) with P value and Median scores (with minimum and maximum scores) calculated using the Statistical Package for the Social Sciences (SPSS version 17). W values vary from 0 to 1; the higher the value the higher the level of agreement between informants groups. Evidence of agreement between informant groups was categorized as 'weak', 'moderate' and 'strong' according to published guidelines on the interpretation of W (Siegal and Castellan, 1988) and the p values assigned to W by SPSS software. Accordingly, agreement was

termed weak for $W < 0.26$, $P > 0.05$; moderate for $W = 0.26 - 0.38$, $P < 0.05$ and strong for $W > 0.38$, $P < 0.01$.

Proportional piling

Before performing the proportional piling to estimate the relative incidence and mortality caused by five most important camel diseases during the outbreak, informants were asked to classify the animals into different age groups according to their production systems. Discussion with Afar livestock keepers indicated that their herds were categorized by age groups as calves (less than or equal to six month), young (between six month and less than or equal to two years) and adult (greater than 2 years). Each informant group was allowed to maintain a pile of 100 stones for each age group and splitting the piles into two relative numbers of sick and healthy stocks during the outbreak. The pile of stones representing the sick camel was then subdivided into the five top ranked diseases and others diseases to show the relative numbers of animals affected by different diseases during the outbreak year. Subsequently, each informant was asked to remove some of the already allocated stones representing the sick to indicate the number of dead animals during the year for each five prioritized and other diseases of camel. Mean age-specific incidence and mortality rates and their 95% confidence intervals were calculated for each disease and correlations between age groups and diseases were assessed using Pearson's correlation coefficients using SPSS 17.

Semi-structured questionnaire survey

The semi-structured questionnaire survey comprising a general socioeconomic impact assessment of CSD, individual camel livestock histories and information about dead camel (Annex 3 and 4) was administered to 95 camel owners.

For the socio-economic impact assessment the interviewees from each pastoral village cluster were interviewed with regard to baseline demographic informations, about their livestock composition, income source, use of camel, and knowledge on the common diseases of camel

besides the detailed information including the mortality and clinical signs of CSD in their camel herd (Annex 3). Information about dead camels was used to obtain general and detailed important information about the dead camels in the herd during the specific outbreak times, i.e. age, use, market price, clinical signs and symptoms before and after death and post mortem findings if done (Annex 3). Individual camel livestock histories were used to assess any event of interest (notable from birth with the season) until the outbreak happened, thus, allowing for timelines of major events in the camel herds in the past years such as animal movements, camel disease outbreaks and PPR like syndromes of a dam camel and its offspring which were present in the herd during the outbreak time (Annex 4).

3.4. Data analysis

Data are entered in Microsoft Excel spreadsheets and Statistical Package for Social Sciences (SPSS) 17 was used for descriptive statistics (proportions) to summarize the data from both the laboratory and the participatory investigation results; chi-square test and logistic regression techniques were used to determine associations of PPR seroprevalence with the risk factors age, sex, location, and lactation status. In all the analyses, confidence level was at 95% and $p \leq 0.05$ was set for significance.

4. RESULTS

4.1 Serological investigation

Five pastoral associations/kebeles namely Dudub, Doho, Sabure, Boloita and Kebena were selected from Awash Fentale Wereda, Afar Regional State. The details of the demographics of the camels sampled are presented in Table 1.

Table 1. Demographic parameters of the camels sampled (n=446) from Awash Fentale, Afar Regional State, 2011

		Number of camels sampled	Percentage(%)
Pastoral Association	Dudub	59	13.2
	Doho	131	29.4
	Sabure	82	18.4
	Boloita	54	12.1
	Kebena	120	26.9
Sex	Male	30	6.7
	Female	416	93.3
Lactation status	Dry	94	22.4
	Lactating	322	77.6
Age	Calves(≤ 6 month)	40	9
	Young camel(>6 month-- ≤ 4 year)	83	18.6
	Adult camel (>4 year)	323	72.4

The seroprevalence of PPR with a breakdown for the different risk factors is presented in Table 2. The overall sero-prevalence of PPR in all the study areas was 13.7%. The result of the chi-

square test revealed that there is a significantly higher seroprevalence in Doho (19.1%) compared to the other pastoral kebeles ($P=0.002$). For the other risk factors like age, sex, lactation status indicated in Table 2, no statistically significant differences were found.

Table 2. Frequency and distribution of camel samples by Pastoral Association, sex, lactation status, age and c-Elisa seropositivity in Awash Fentale wereda, 2011

Risk factors		Number of camels sampled and percentage (%)	Number and Percentage(%) c-Elisa positives for PPR	95%CI for percentage(%) of positives in c-Elisa	Degree of freedom	Chi-square value	P-value
Pastoral Association	Dudub	59 (13.2)	4 (6.8)	0.83-13.22	4	16.655 5	$P=0.002$
	Doho	131 (29.4)	25 (19.1)	12.37-25.83			
	Sabure	82 (18.4)	2 (2.4)	0.91-5.71			
	Boloita	54 (12.1)	8 (14.8)	5.33-24.27			
	Kebena	120 (26.9)	22 (18.3)	11.38-25.22			
Sex	Male	30 (6.7)	2 (6.7)	2.25-15.65	1	1.399	$P=0.247$
	Female	416 (93.3)	59 (14.1)	10.76-17.44			
Lactation status	Dry	94 (22.4)	11 (11.7)	5.2-18.2	1	0.614	$P=0.433$
	Lactating	322 (77.4)	48 (14.9)	11.01-18.79			
Age	Calves(≤ 6 month)	40 (9)	5 (12.5)	2.25-22.75	2	0.321	$P=0.852$
	Young camel(>6 month-- ≤ 4 year)	83 (18.6)	10 (12)	5.01-18.99			
	Adult camel (>4 year)	323 (72.4)	46 (14.2)	10.39-18.01			

Univariate logistic regression was used to assess any association between the seropositivity for PPR and the risk factors indicated (Table 3). The Pastoral association is the only risk factor which is significantly associated with seropositivity where Doho with the highest seroprevalence

($p=0.012$, odds ratio=3.087, being three times at risk of being seropositive compared to the reference group Sabure.

Table 3. Results of univariate logistic regression analysis of risk factors and seropositivity for c-Elisa for Peste des Petits Ruminants in Awash Fentale wereda, 2011

Risk factors		Number of camels sampled and percentage (%)	Number and Percentage(%) of positives in c-Elisa	p Value	Odds ratio(OR)	95% CI for OR
Pastoral Association	Sabure	82 (18.4)	2 (2.4)			
	Doho	131 (29.4)	25 (19.1)	0.012	3.087	1.012-9.417
	Kebena	120 (26.9)	22 (18.3)	0.037	3.243	1.075-9.787
	Boloita	54 (12.1)	8 (14.8)	0.017	2.391	0.677-8.451
	Dudub	59 (13.2)	4 (6.8)	0.227	0.344	0.61-1.942
Sex	Female	416 (93.3)	59 (14.1)			
	Male	30 (6.7)	2 (6.7)	0.260	0.26	0.537-9.970
Lactation status	Dry	94 (22.4)	11 (11.7)			
	Lactating	322 (77.4)	48 (14.9)	0.434	0.757	0.376-1.523
Age	Calves(≤ 6 month)	40 (9)	5 (12.5)			
	Young camel(>6 month-- ≤ 4 year)	83 (18.6)	10 (12)	0.606	1.212	0.584-2.518
	Adult camel (>4 year)	323 (72.4)	46 (14.2)	0.943	1.043	0.331-3.283

In the multivariable logistic regression (Table 4), only three risk factors are considered excluding the risk factor sex. There is only one risk factor i.e. pastoral association which still showed a significant association with seropositivity in cELISA for PPR ($P=0.033$, Odds ratio=3.382) where as age ($P>0.05$, OR for calves=1.215, OR for young=1.110) and lactation status ($P>0.05$, OR=0.827) are not significantly associated with seropositivity with cELISA for PPR.

Table 4. Results of multivariable logistic regression analysis of risk factors positive to c-Elisa for Peste des Petits Ruminants in Awash Fentale wereda, 2011

Risk factors		Number of camels sampled and percentage%	Number and Percentage(%) of positives in c-Elisa	P Value	Odds ratio	95% CI
Pastoral Association	Sabure	82 (18.4)	2 (2.4)	0.198		
	Doho	131 (29.4)	25 (19.1)	0.033	3.382	1.101-10.385
	Kebena	120 (26.9)	22 (18.3)	0.012	2.297	0.647-8.159
	Boloita	54 (12.1)	8 (14.8)	0.055	2.985	0.975-9.141
	Dudub	59 (13.2)	4 (6.8)	0.230	0.346	0.061-1.960
Lactation status	Dry	94 (22.4)	11 (11.7)			
	Lactating	322 (77.4)	48 (14.9)	0.672	0.827	0.343-1.992
Age	Calf(≤ 6 month)	40 (9)	5 (12.5)	0.900		
	Young(>6 month- ≤ 4 year)	83 (18.6)	10 (12)	0.646	1.215	0.529-2.790
	Adult(>4 year)	323 (72.4)	46 (14.2)	0.889	1.110	0.257-4.784

4.2 Participatory appraisal investigation

4.2.1 Pair-wise comparison and ranking

CSD ('Galirebe') was ranked top for its overall importance in four out of five informant groups. Pneumonia ('Mesengele') was ranked first in Doho pastoral kebele informant group and second and third in the other two informant groups. Coughing ('Kahu') ranked last in four of the five informant groups besides Anthrax ('Fira') which ranked last in only one informant group. The Kendall's coefficient of concordance (W) for all informant groups for the above diseases

indicated strong agreement between the different informant groups (W=0.883; P=0.000; n=5) (Table 5).

Table 5. Summary of the camel diseases ranked by Awash Fentale pastoralists for their overall importance in the CSD outbreak year 2006.

Local name of disease	Equivalent English name	Disease rank
<i>Galirebe</i>	Camel sudden death	1
<i>Mesengele</i>	Pneumonia	2
<i>Anberaruke</i>	Camel Pox	3
<i>Geremole</i>	Trypanosomosis	4
<i>Rebreba</i>	Plant poisoning	5
<i>Fira</i>	Anthrax	6
<i>Kahu</i>	Coughing	7

4.2.2 Matrix scoring of camel diseases

The analysis of matrix scoring showed good levels of agreement among 5 informant groups (W= 0.672-1.000; p< 0.05). The groups of informants also indicated that loss of rigor mortis, bellowing before death, sudden death, coughing and loss of productive animals are the most important signs of CSD. Additional signs of CSD as mentioned by the pastoralists in the semi-structured interview were nasal discharge, dyspnoea, oozing of blood from external orifices, diarrhoea and nervous signs. The informants agreed on most of the clinical signs and epidemiological features listed in Table 6.

Table 6. Matrix scoring of indicators/signs for camel diseases by 5 camel herder informant groups from the pastoral association from Awash Fentale wereda, 2006.

Indicators/ disease signs	Kendall's Coefficient of concordance (W) & p value	Diseases				
		Plant poisoning Median (Max, Min)	Camel Sudden Death Median(Ma x, Min)	Camel pox Median (Max, Min)	Camel Trypanosomosis Median (Max,Min)	Pneumonia Median (Max,Min)
Sudden death	W=0.960,P=0.001	1(0,2)	22(19,24)	0(0,0)	0(0,0)	2(1,4)
Lacrimation	W=0.672,P=0.009	2(0,4)	20(16,22)	0(0,3)	0(0,3)	1(0,5)
Nasal discharge	W=0.924,P=0.001	1(1,5)	9(3,14)	0(0,2)	0(0,0)	13(10,17)
Coughing	W=0.971,P=0.001	0(0,2)	8(7,11)	0(0,0)	0(0,0)	15(14,18)
Dyspnoea	W=0.914,P=0.001	0(0,2)	14(11,19)	0(0,0)	0(0,1)	11(6,12)
Mouth lesion	W=1.000,P=0.000	0(0,0)	0(0,0)	25(25,25)	0(0,0)	0(0,0)
Diarrhoea	W=0.890,P=0.001	15(8,19)	5(5,15)	1(0,2)	0(0,3)	2(1,3)
Oozing of blood from external orifices	W=0.914,P=0.001	0(0,1)	25(24,25)	0(0,0)	0(0,0)	0(0,0)
High mortality	W=0.784,P=0.003	1(0,4)	20(19,22)	0(0,0)	0(0,1)	4(0,5)
Loss productive animals	W=0.958,P=0.001	0(0,1)	21(20,24)	0(0,0)	0(0,0)	3(1,4)
Bellowing before death	W=1.000,P=0.000	0(0,0)	25(25,25)	0(0,0)	0(0,0)	0(0,0)
loss of rigor mortis	W=1.000,P=0.000	0(0,0)	25(25,25)	0(0,0)	0(0,0)	0(0,0)
Nervous signs	W=0.874,P=0.002	10(9,14)	12(8,13)	0(0,0)	2(0,4)	0(0,3)

4.2.3 Proportional piling

Disease incidence as estimated by proportional piling

The Awash Afar pastoralists categorize camel into three age groups: calves, young and adult camel. The disease incidence in specific age groups as estimated by the pastoralists is illustrated in Fig. 2-7. In the outbreak year, according to the result of the proportional piling 49.3% of the camel population suffered from various diseases where the highest mean annual incidence was caused by CSD (*Galirebe*)(23.3%) followed by pneumonia (*Mesengele*)(10.4%). According to proportional piling (Fig. 2-7), the incidences of acute plant poisoning (*Rebreba*) and camel pox (*Anberaruke*) were high in young stock (Fig. 2 and 4). On the other hand, the incidence of CSD (*Galireba*) and trypanosomosis (*Geremole*) increased with age (Figs 3, 5, and 6).

Conversely, compared to other important diseases such as Pneumonia (*Mesengele*), trypanosomosis (*Geremole*) and camel pox (*Anberaruke*), CSD (*Galireba*) was the most commonly observed disease during the outbreak year with an annual mean incidence percentages of 23.3% (Fig. 8).

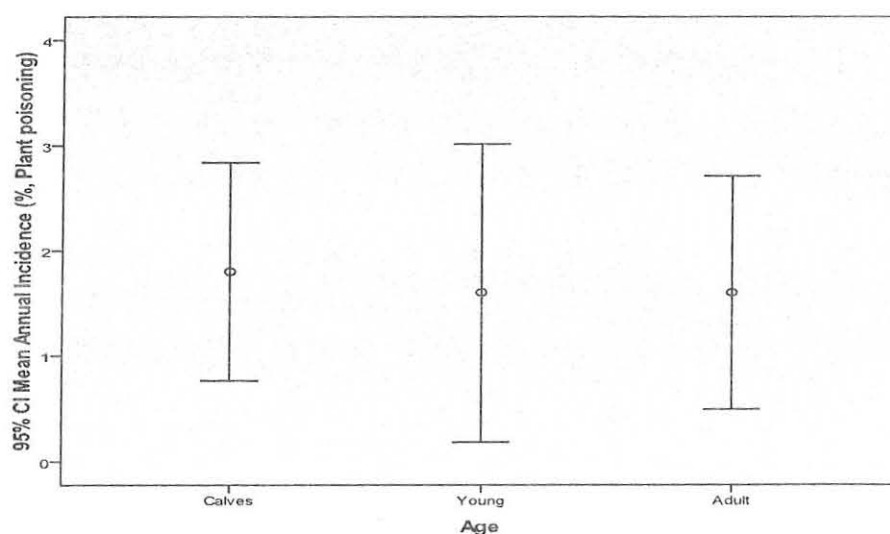


Figure 2. Mean annual incidence estimates with 95% CI for plant poisoning (*Rebreba*; n=5) in five pastoral kebeles of Awash Fentale wereda in the CSD outbreak year 2006 by age group

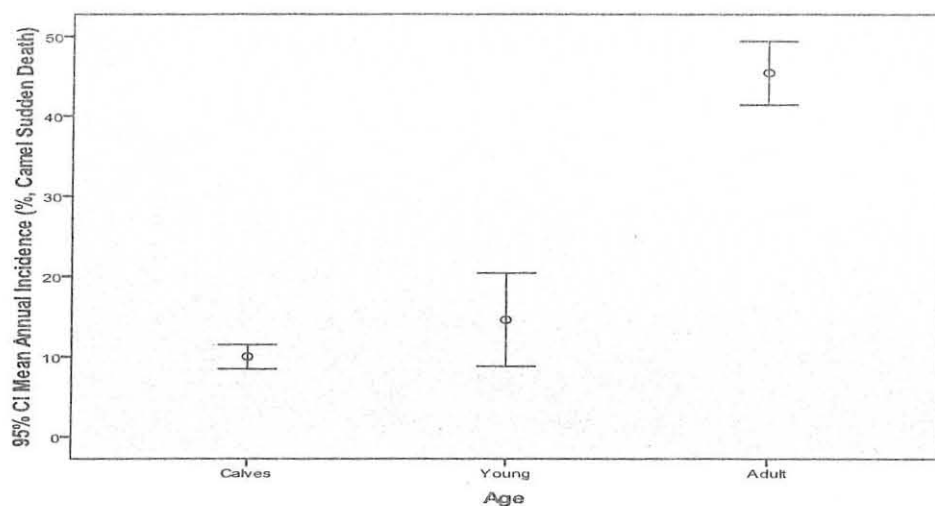


Figure 3. Mean annual incidence estimates with 95% CI for camel sudden death (*Galireba*, n=5) in five pastoral kebeles of Awash Fentale wereda in the CSD outbreak year 2006 by age group

According to Fig.3, significant ($p < 0.05$) differences in the incidence of CSD between adult camel and the other two age categories is indicated by the highest incidence (45.4%) in adults followed by young (14.6%).

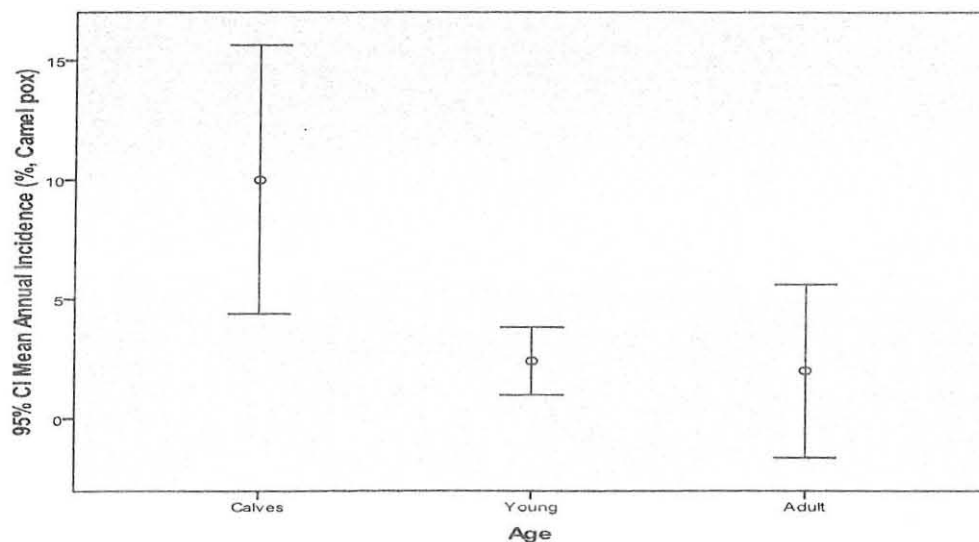


Figure 4. Mean annual incidence estimates with 95% CI for camel pox (*Anberaruke*, n=5) in five pastoral kebeles of Awash Fentale wereda in the CSD outbreak year 2006 by age group

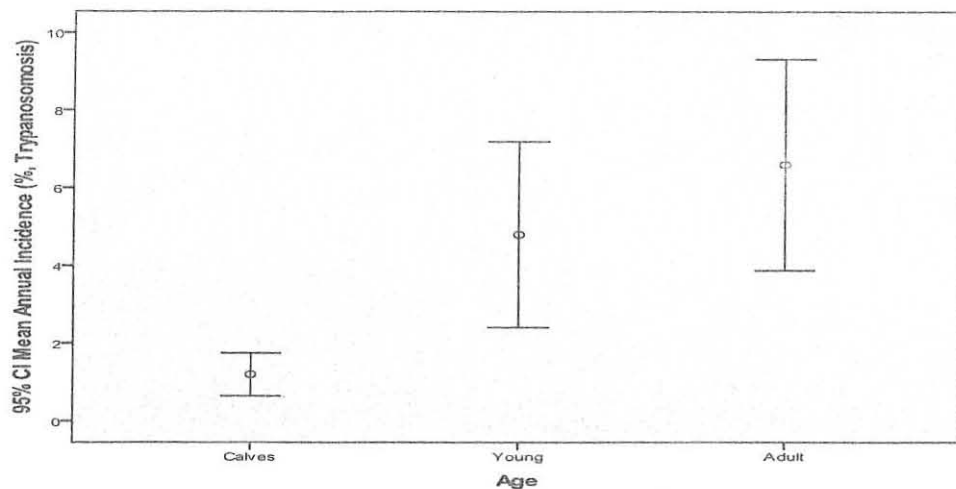


Figure 5. Mean annual incidence estimates with 95% CI for trypanosomosis (*Geremole*, n=5) in five pastoral kebeles of Awash Fentale wereda in the CSD outbreak year 2006 by age group

Although there was no significant ($p>0.05$) difference between the young and adult age categories, the difference in the incidences of camel pox (*Anberaruke*) and trypanosomosis (*Geremole*) were significantly different ($p<0.05$) between calves and the other age categories (Fig.4 and 5).

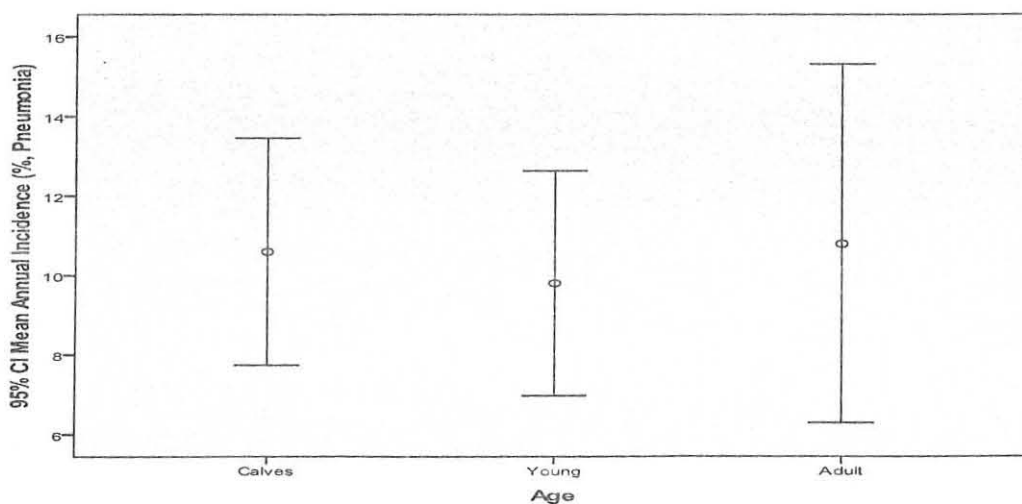


Figure 6. Mean annual incidence estimates with 95% CI for pneumonia (*Mesengele*, n=5) in five pastoral kebeles of Awash Fentale wereda in the CSD outbreak year 2006 by age group

The mean annual incidence of pneumonia (*Mesengele*) varied from 9.8 % in young to 10.8% in adults basically not showing differences ($P>0.05$) (Fig.6).

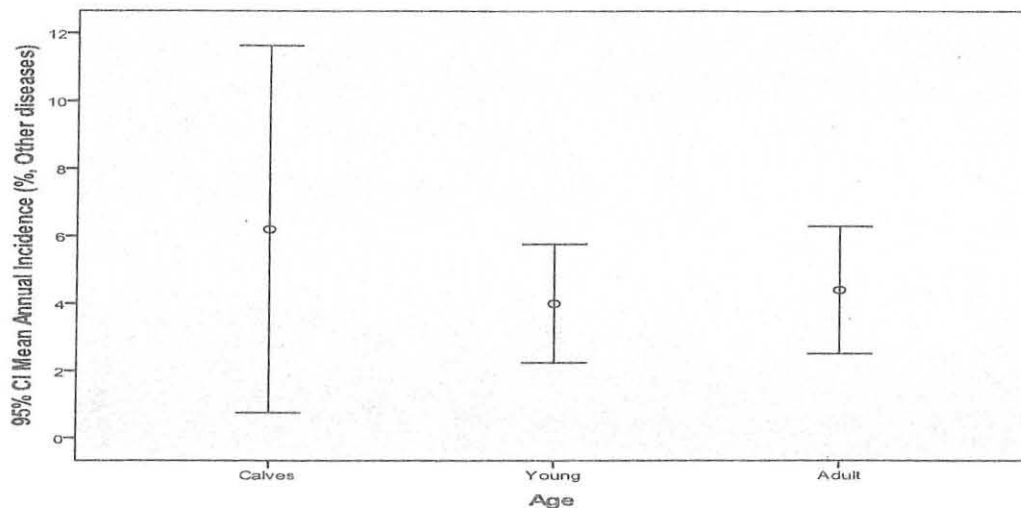


Figure 7. Mean annual incidence estimates with 95% CI for other diseases (n=5) in five pastoral kebeles of Awash Fentale wereda in the CSD outbreak year 2006 by age group

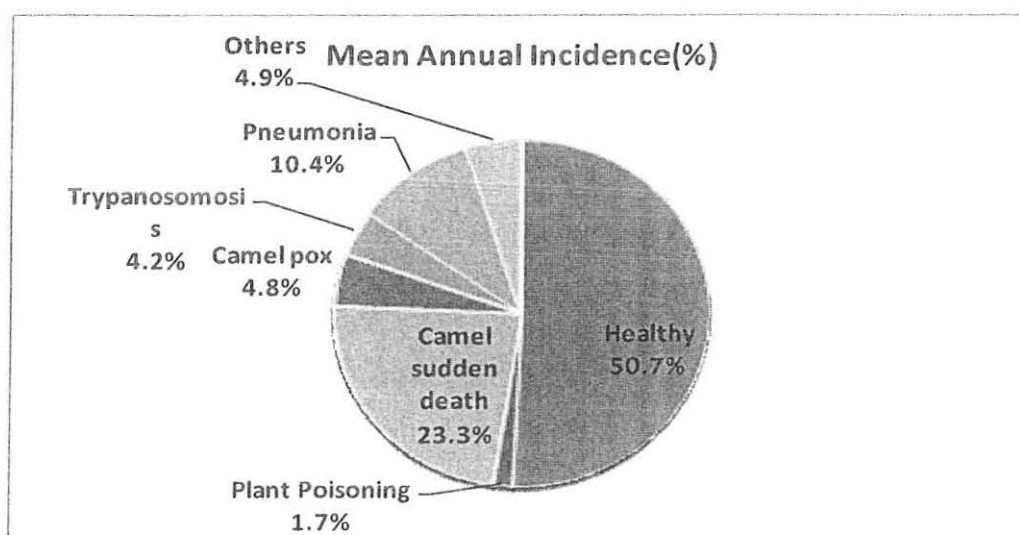


Figure 8. Mean incidence of camel diseases as determined by proportional piling, in relation to healthy camel of all age groups in Awash Fentale wereda in 2006/ camel sudden death outbreak.

The result of mean incidence of camel diseases as determined by proportional piling (Fig.8) was for the year 2006/CSD outbreak in the study area by the recalled information from the five informant groups.

Disease mortality as estimated by proportional piling

The estimated mortality rates attributed to important camel diseases in different age groups are shown in Fig. 9-14. Of the total 35.1% deaths reported in the 2006 CSD outbreak period, 1.1%, 22.8%, 1.4%, 0.9%, 6.5% and 2.3% were due to plant poisoning, CSD, Camel pox, trypanosomosis, Pneumonia and other diseases, respectively. Case fatality rates of 68%, 98%, 29%, 23%, 63% and 48% were seen in association with plant poisoning, CSD, Camel pox, trypanosomosis, pneumonia and other diseases, respectively. The correlation coefficients for disease mortality by age groups were 0.920 ($P<0.05$) for CSD (Galirebe) and 0.23($P>0.05$) for pneumonia (*Mesengele*).

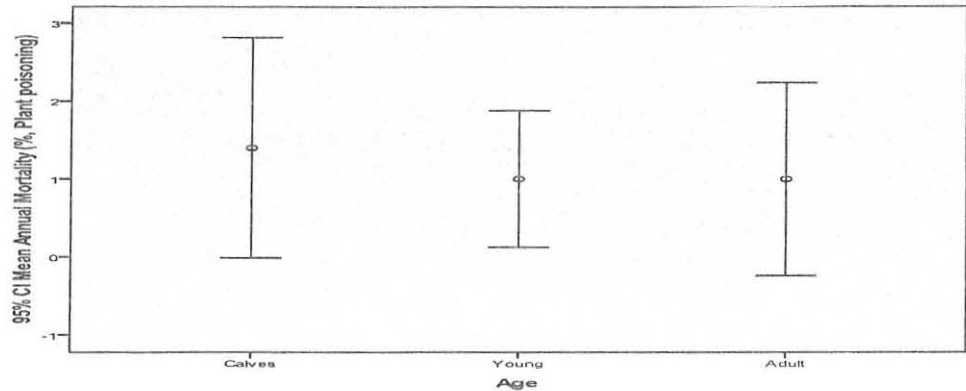


Figure 9. Mean case specific annual mortality estimates with 95% CI for plant poisoning (*Rebreba*; n=5) in the five pastoral kebeles in Awash Fentale wereda in the CSD outbreak year 2006 by age group.

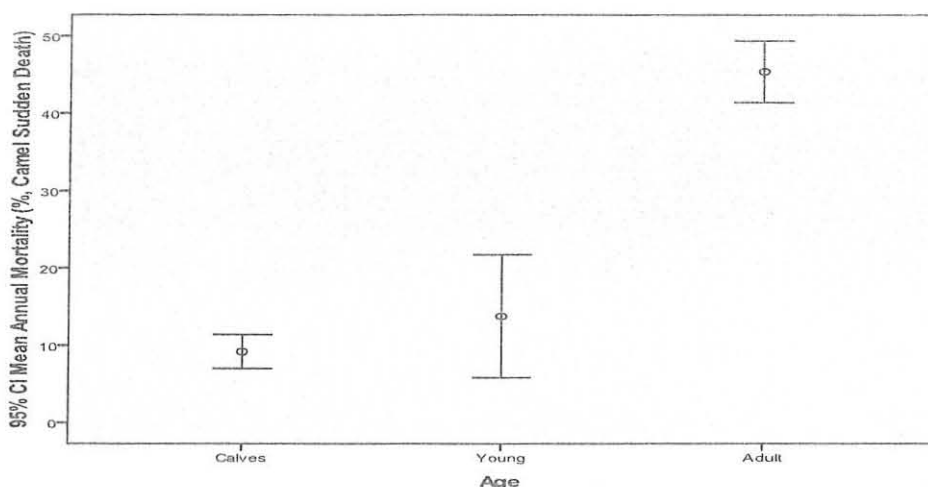


Figure 10. Mean case specific annual mortality estimates with 95% CI for camel sudden death (*Galireba*, n=5) in the five pastoral kebeles in Awash Fentale wereda in the CSD outbreak year 2006 by age group.

The mean annual mortality due to CSD was significantly ($p < 0.05$) higher in adults than the other age groups. The CSD (*Galirebe*) had the highest mortality (45.4 %) in adults and lowest (9.2%) in calves (Fig. 10).

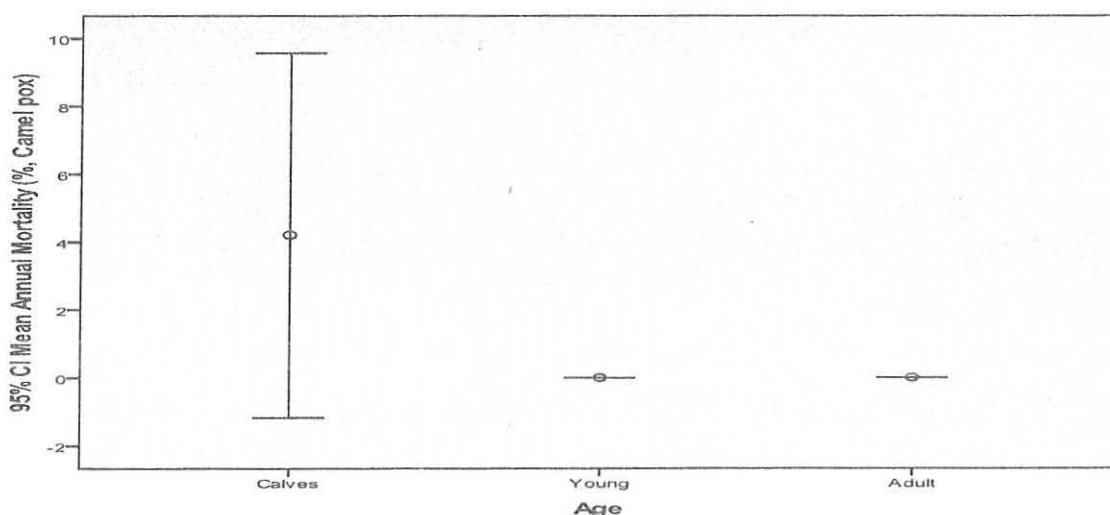


Figure 11. Mean case specific annual mortality estimates with 95% CI for camel pox (*Anberaruke*, n=5) in the five pastoral kebeles in Awash Fentale wereda in the CSD outbreak year 2006 by age group

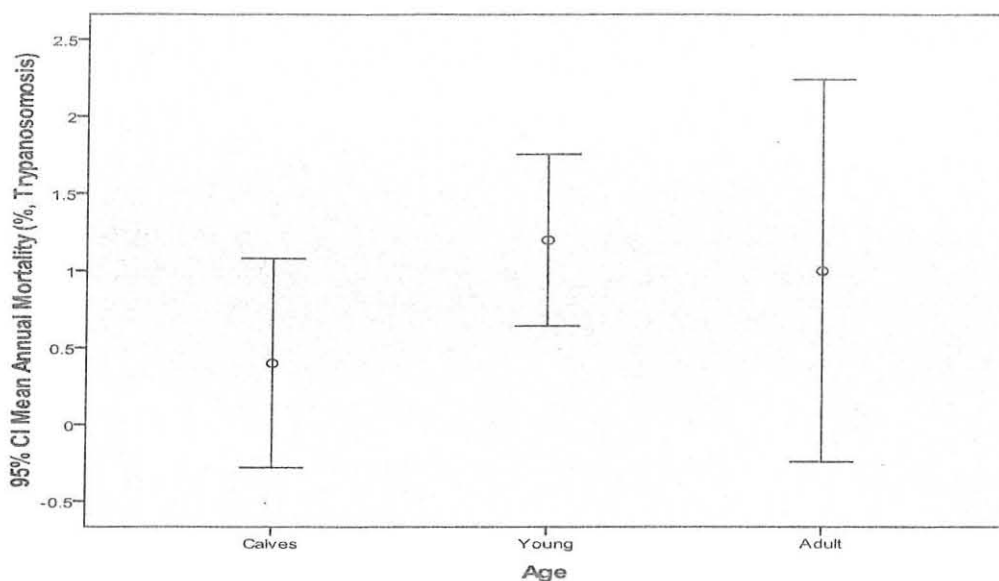


Figure 12. Mean case specific annual mortality estimates with 95% CI for trypanosomosis (*Geremole*, n=5) in the five pastoral kebeles in Awash Fentale wereda in the CSD outbreak year 2006 by age group

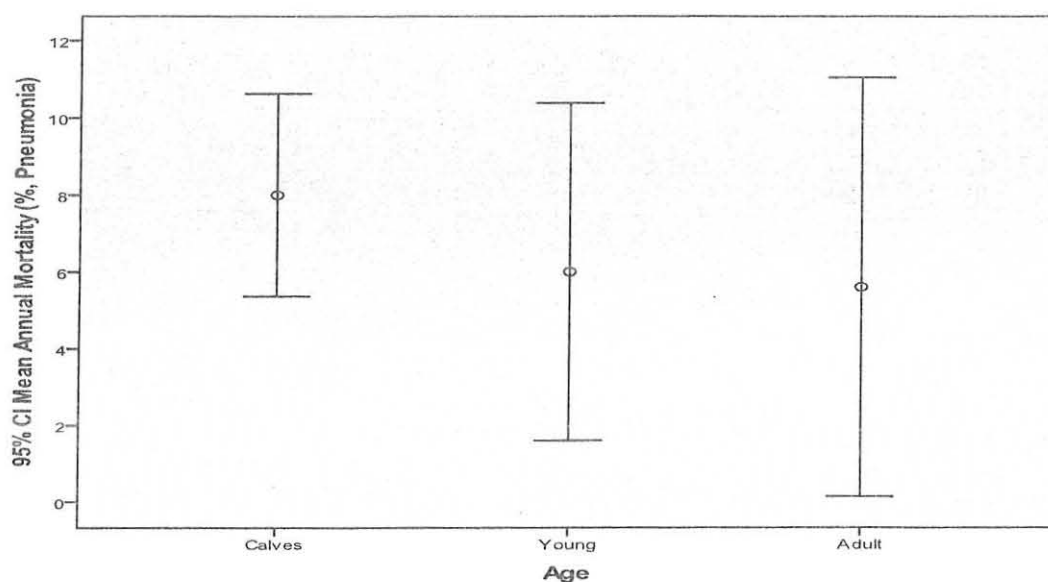


Figure 13. Mean case specific annual mortality estimates with 95% CI for pneumonia (*Mesengele*, n=5) in the five pastoral kebeles in Awash Fentale wereda in the CSD outbreak year 2006 by age group

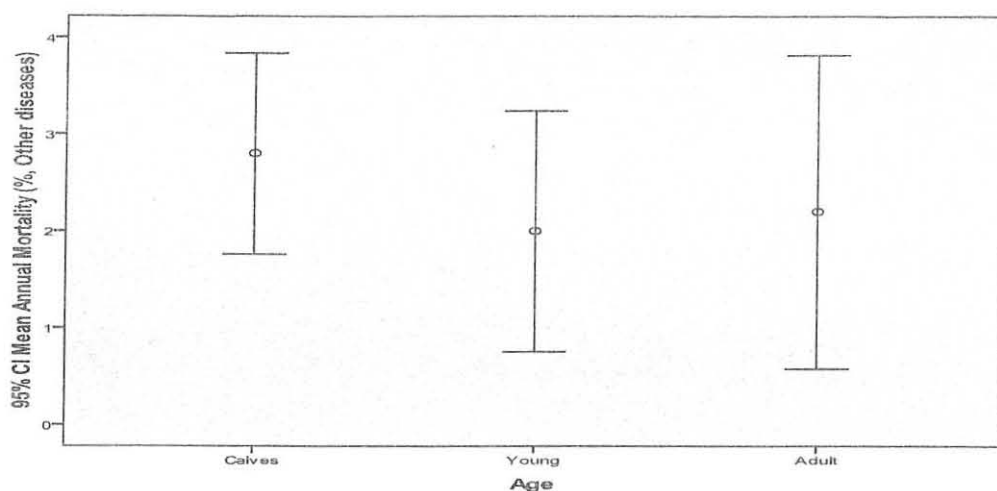


Figure 14. Mean case specific annual mortality estimates with 95% CI for other diseases (n=5) in the five pastoral kebeles in Awash Fentale wereda in the CSD outbreak year 2006 by age group

Overall, there were no significant differences ($P>0.05$) in all age groups in all diseases but CSD (Fig 9,11,12,13 and 14).

4.2.4 Semi-structured questionnaires

General socio-economic impact assessment of CSD

According to the response of the interviewees, the average camel holding is 20 camels (SD ± 7.3 , minimum 9, maximum 35). 58 of the 95 interviewees (61.1%) disposed their camels for holidays, marriage ceremony or dowry and sales (Fig. 15). All of the respondents revealed that camel milk for children and camel shepherds, whereas goat and cattle milk is for the whole community and some cereals are household food sources. The consumption pattern of camel milk is clan based where any member of the clan is entitled for its consumption. All respondents mentioned that they use their camels as live asset or insurance at the time of disaster. Most the respondent (98.9%) incurred their income by selling animals including camels. All respondents use their camels as pack animals. Grazing, watering, trade, cultural and ceremonial activities

were ranked first for the reason of camel herd mobility. Almost half of the interviewees (47.4%) mentioned *Geremole*(trypanosomosis), *Rebreba*(plant poisoning) , *Mesengele*(pneumonia), *Galireba*(CSD), *Boru* (coughing), *Anberaruke*(camel pox) as major camel diseases.

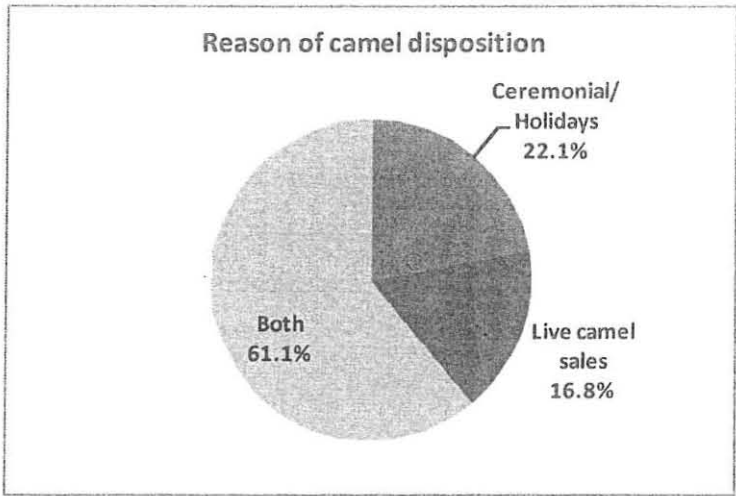


Figure 15. Reasons of camel disposition in Awash Fentale Wereda in 2006.

As mentioned by all camels were at their own pastoral association at the time of the first deaths due to CSD outbreak before four years and they also disclosed that they mobilized their camel herds following the first death. All of the interviewees responded that the camels were not sick prior to the outbreak. The mean camels dead numbers were 15 (SD±7.4, minimum 6, maximum 32) per camel holding. All respondents mentioned that they know PPR like syndrome in their herd including camels and 62 of them (65.3%) associated nasal discharge and coughing with the PPR like syndrome in their camels.

Individual camel livestock history

All respondents disclosed that the only notable change they noticed in all female camels and their calves were the routine migration to common grazing sites in Awash park and watering sites near Doho. Besides 4.2% of the respondents mentioned that camel cows were bought from the Amibara live camel market.

Information from dead camel form

From 24.2% of the respondents we learned that the highest number of dead camels were reported from Doho and all of them were adult lactating female camels which were used as pack animals.

The mean market price for the dead camels is 4,134.7 birr ($SD \pm 366.6$, range from 3700-5000 birr).

Ninety point five percent (90.5%) (86) of the dead camels were at the grazing area whereas the rest were found dead not having been sick before death. All the interviewees noted that there was good pasture at the time of outbreak and indicated the local name of the CSD as *Galireba*. 69.5% of respondents mentioned recumbency, dyspnoea, arching of neck as signs seen at the time of death of the camels followed by recumbency, dyspnoea, dullness, (9.5%), arching of neck (7.4%), recumbency, dyspnoea, nasal discharge and arching of neck, (6.3%). The highest number of respondents 81.1% indicated oozing of blood from anus whereas 12.6% mentioned nasal discharge as the most common sign after death of the camels.

Among the dead camels 14(14.7%) were examined post mortem by the local veterinary officers revealed black, enlarged lung, blackish effusion from the thoracic cavity, extended and black liver, hypertrophied heart, airway passages full of white foamy gaseous fluid, distended carcass, and no rigor mortis.

5. DISCUSSION

The serological findings for PPRV the present study shows an overall prevalence rate of 13.7% in the study areas. The overall seroprevalence rate reported in this study is higher than the previous findings from Ethiopia where seropositivity of 7.8 % (Roger *et al.*, 2001), 3% (Abraham *et al.*, 2005) and 1.5% (Megersa, 2010) were reported. The result is also higher than other findings reported from Egypt with 4.2 % seroprevalence (Ismail *et al.*, 1992) and from Turkey (Albayrak and Gur, 2010) where all the camels tested were seronegative. The result of the current study is similar to the finding from Sudan with 14% seroprevalence (Haroun *et al.*, 2002) but much higher than the recent investigation done in Sudan with 0.3% (Intisar *et al.*, 2010). Differences in the seroprevalence rates in this study compared to the other studies may be due to difference in study sites especially in Ethiopia, camel herd size, sample size, camel breed type, and management system especially whether camel herds having contact with small ruminants where PPR is endemic in most of the goat herds (Abraham and Berhan, 2001).

Among the risk factors considered pastoral association was significantly associated with the seropositivity in cELISA for PPR. The highest pastoral kebele level seroprevalence recorded in Doho (19.1%) as compared to Dudub (6.8%) and Sabure (2.4%), probably reflects the availability of watering sites making this pastoral association to be visited more frequently by other pastoral associations as personally observed; the camel herds in the latter kebele were not in frequent contact with the small ruminants thus having little exposure. A breakdown for age specific seroprevalences revealed an increasing prevalence with age with the highest records in adults (14.2%) although not significant. This result is in contrast to PPR in small ruminants where the susceptibility of young animals aged 3 to 18 months was proved to be very high, thus, being more severely affected than adults or unweaned animals (Taylor *et al.*, 1990). This may indicate the cumulative experience of the population with the agent (Murphy *et al.*, 1999). Therefore, those camels aged greater than four years, might have acquired the infection from multiple exposure to small ruminants where the disease is endemic. PPR seropositivity appeared to have no significant associations with sex and female lactation status.

The presence of antibodies for PPRV in camels has raised a reasonable doubt and, thus, gave way to different explanation. Roger *et al* (2001) suggested that their presence might have been due to an immune humoral response that expressed only the passage of the virus and an immune receptivity of the camel, but without any clinical or sub-clinical expressions. Abraham *et al* (2005) and Haider *et al* (2008) share the same view that antibody seroprevalence detected in camels, cattle, goats and sheep may have confirmed natural transmission of PPR virus under field conditions.

The other suggestion by Roger *et al* (2001) was that it may be due to susceptibility to a morbillivirus closely related to RPV or PPRV, which is virulent for the camels. This virulence could be foreseen taking into account that morbilliviruses have an immunosuppressive effect (Hass and Barrett, 1996) that led to secondary bacterial infections effectively treated by the antibiotics. Indeed, during the epizootic in 2007, *Streptococcus equi* subspecies *equi* has been isolated from sick camels in Afar region (Yigezu *et al.*, 1997) and *Mannheimia haemolytica* from camels in Somali region (Bekele, 1999). Within the morbillivirus genus and related viruses, several emerging diseases have been recently described. The existence of mild strains of rinderpest in domestic cattle has had devastating effects in African buffaloes, eland and lesser kudu in Africa (Anderson, 1995; Kock *et al.*, 1999; Parker, 1997). PPRV has been isolated during an outbreak in Indian buffaloes (Govindarajan *et al.*, 1997). New morbilliviruses with devastating effects have been described in marine mammals (Mah *et al.*, 1988) and canine distemper virus caused mortalities in lions of East Africa (Roelke-Parker *et al.*, 1996). Viruses belonging to the *Paramyxoviridae* family, initially classified as morbillivirus, were recently described in diseases declared in horses and human (O'sullivan *et al.*, 1997) and pigs and human (Chua *et al.*, 2000). In that context, Roger *et al* (2001) presupposed that this new disease could result from an interspecies transfer of PPRV or RPV strains from cattle or goats and sheep to camel or through the emerging of a new morbillivirus, serologically related to PRV and/or RPV. This was in agreement with Barrett and Rossiter (1999) where, genetically, it was assumed that when cattle were domesticated, they passed a morbillivirus, a progenitor of modern Rinderpest virus, to humans, which eventually evolved into the measles virus. Similarly, carnivores could

have contracted a morbillivirus infection from their ruminant prey, which then evolved into Canine distemper virus. This could be explained because morbilliviruses use human signaling lymphocyte activation molecule (SLAM; also known as CD150), a membrane glycoprotein expressed on some lymphocytes and dendritic cells (Cocks *et al.*, 1995), of their respective host species as cellular receptors (Tatsuo *et al.*, 2001) even including camels. A similar suggestion was made by Khalafalla *et al* (2010) in Sudan where besides a seroprevalence of up to 14% (Haroun *et al.*, 2002), viral antigen and virus isolation confirmed PPRV from outbreak samples of 2006. It was suggested that this phenomenon must be linked to the emergence of a virulent viral strain in this part of Africa, maybe having a selective advantage over the previous strains that were originally present in Sudan resulting in waves of outbreaks in small ruminants and camels as well (Kwiatek *et al.*, 2007). The occurrence over a period of a few years of strains with increased virulence in association with large outbreaks is a phenomenon already observed for measles virus (Santibanez *et al.*, 2002). Molecular typing characterized the strains of PPR discovered in camels in 2004 as lineage IV. Phylogenetic analysis of a wider sampling of PPRV strains isolated between 2000 and 2009 in Sudan evidently showed the emerging of PPRV strains of lineage IV in this country, a lineage present in Asia and in the Middle East. This study also reported on genetic relationship between the PPRV strains circulating in sheep goats and camels and of a possible genetic bias of PPRV according to the host (Kwiatek *et al.*, submitted in Khalafalla *et al.*, 2010).

Another speculation by Chauhan *et al.*, (2009), Diallo (2007) and Albayrak and Gür (2010) was that cattle, buffaloes and dromedaries also may be infected with PPRV but being less vulnerable than sheep and goats. Considering the immunosuppressive effect of PPRV as all other morbilliviruses, it may therefore be possible, depending on the age and physical state of the host animal, that PPRV can occasionally overcome the innate resistance of large ruminants and lead to the development of clinical signs similar to Rinderpest.

Singh *et al* (2009) states that, it is, however, unknown whether these animals serve as carriers and whether they have the potential to spread PPR virus. Thus, there is need to address this issue particularly with regards to PPR control and eradication programmes.

The present study has indicated that CSD was the first priority disease followed by pneumonia, in Awash Fentale wereda based on the prioritization and ranking of camel diseases by the Afar pastoralists due to its severity, morbidity, high mortality and its negative economic impacts. The strong agreement between the informant groups implied that the prioritized diseases were common problems to all the participating pastoral association. This was in agreement with findings of Eshetu (2003) in Somalia pastoral areas, Tadesse (2003) and Eshete (2006) in the Afar pastoral area, and Rufael *et al* (2008) in Borana pastoral area of Ethiopia. As reported in this study, pneumonia was the second priority camel disease. Despite low mortality and morbidity rates, the recovery period is quite long having negative impact on the overall productivity. Agab and Abbas (1999) reported pneumonia as the ninth most common disease problem with a peak incidence during autumn. Several risk factors including sudden climatic changes, stress of migration, crowding around limited watering points were reported to be associated with pneumonia (Megersa, 2010). In the Afar pastoral area daily conversation tends to focus on the condition and availability of pasture, the health of animals, the availability of water and local security issues. Daily meetings are also held among elders to discuss livestock issues and these discussions centered on the exchange of information, locally called '*dagu*', related to disease outbreaks. These aspects of pastoralist behavior explained the strong agreement among informant groups when relating livestock diseases to clinical and epidemiological indicators. Although it was thought that proportional piling was subjected to recall bias, there are a number of reports of pastoralists' ability to provide very detailed verbal accounts of past disease events, covering the entire lifespan of their stock (McDermott *et al.* 1987; Akabwai 1992; Iles 1994; Catley 2006). The present study has also shown that pastoralists could remember and quantify past events in such a way that veterinarians could use and interpret it biologically.

The results of disease sign matrix scoring indicated that pastoralists were able to characterize most of the overt clinical characteristics of livestock diseases in general. The indicators such as sudden death, oozing of blood from anus, dyspnoea used for the specific camel diseases and most of the clinical presentations of the camel diseases were well described by the group of informants; indeed most of the signs listed for CSD were consistent with the case definition in Somali and Kenya given by Gluecks and Younan (2010). As indicated by the community, 49.3%

of the camel population suffered from various diseases where the highest mean annual period prevalence was due to CSD (*Galirebe*) (23.3%) followed by pneumonia *Mesengele*, (10.4%). This reported highest incidence for CSD might have resulted from interest in period between the CSD outbreak period in 2006 and the study period interested in for the participatory investigations. The incidence of CSD (*Galireba*) increases with age being highest in adults (45.4%) followed by young camel (14.6%) reflecting what was observed in the present serological investigation for PPRV.

Out of the total 35.1% deaths reported in 2006 CSD outbreak period, CSD (*Galireba*) scored the highest mean annual mortality 22.8% with Case fatality rate of 98%. The incidence and case fatality rates were based only on the clinical signs just before death. The mean annual mortality due to CSD was significantly higher in adults than the other age groups with the highest mortality in adults (45.4 %) and the lowest (9.2%) in calves. This result is substantially higher than the CSD crude mortality of 15.9% recorded in Borana (Dawo, 2010). The low mortality rate in calves recorded in this study for most of the diseases except for plant poisoning and camel pox is even lower than the crude mortalities reported from other parts of Ethiopia 30% by Tuffa and Baars (1998) and 45% by Getahun and Kassa (2002) from Eastern Ethiopia, and 15 to 20% by Megersa *et al* (2008) from Borana area under non-outbreak conditions. Similarly, Kaufmann (2005) also reported mortality rates of 25%, 22% and 27% in Rendille, Gabra and Somali camel calves of Northern Kenya, respectively, and again under non-outbreak conditions. Dawo (2010) suggests that the disease showed clear selection for mature and reproductively active camels. Coupled with the high mortality rate in adults, empirical knowledge of informants this conforms well to the survey done on CSD in Kenya and Somalia (Gluecks and Younan, 2010) that reported high mortality rates in adults with herd mortality in affected herds of 3.7% and 6.6 % in Puntland and Kenya, respectively. In addition, the mean number of cases per herd varied between the two countries. While the mean was 2.0 in Puntland, pastoralists in Kenya reported a mean of 6.8 cases per herd with an average age of 9.7 years in Puntland and 9.5 years in Kenya. However, the average age of a dead camel in Borana was lower (6.5 ± 2.5) (Dawo, 2010). The highest mortality in adults with 93.3% females of which 77.4% were lactating indicates that the disease targets reproductive age groups, suggesting a serious impact on the establishment of new

camel herds using replacement stock. The high mortality in both pregnant and lactating camels has a direct negative effect on the livelihood of pastoralists because they depend on milk from their animals. In a study carried out in Sudan, It seems also that that the severity of PPR in camels was much higher in adult animals since, more than 50% of deaths were reported in adult animals (mostly recently-delivered and pregnant females) (Khalafalla *et al.*, 2010). The differences between the current and other studies can be due to different camel camel management systems, camel populations and especially the methodology where results of the current study of where based on PE methods unlike the other studies.

The mixing of the herds in Doho watering sites and Awash park vicinity grazing sites could lead to an easy way of transmission of contagious diseases such as PPR as most of the camels routinely migrate to these sites which is similar to the conditions of acquiring infection in Borana camels (Megersa, 2010; Melaku and Feseha, 1986).

The average camel holding of 20 ± 7.3 camel recorded in this study is in between the records given for apparently large sized herds (25.2 ± 2.2) of Gabras and of Borana (18.4 ± 1.8) pastoralists (Megersa, 2010), but much higher than records for Nigeria of 9 camels (Hassan *et al.*, 1988) and lower than both medium size herds averaging 38 heads and large herds averaging 92 heads in Saudi Arabia (Al-Khamis and Young, 2006). Typical camel herd composition figures are hard to derive, but often more animals are raised than are needed for family subsistence. In part this is an attempt to guard against theft and natural disasters such as droughts, epidemics and predators (Mukasa-mugerwa, 1981). The difference in the average camel herd can be due to reluctance of herders to give exact herd sizes (Hussien, 1989) and the management system of camels. Most of the camel holder 61.1% disposed their camels for holidays, marriage ceremony or dowry and sales. The reasons of disposition are the same for Gabras which do not commonly slaughter except for cultural ceremonies and festivals. Moreover, camel slaughtering is uncommon except for emergency slaughtering and its meat is not preferable among Boranas (Megersa, 2010).

The results revealed about the use of camel milk for children and camel shepherds and its clan-based consumption pattern was also reported in different studies (Schwartz and Dioli, 1992; Joanne *et al.*, 2005; Megersa, 2010; Hartley, 1979; Hussien, 1989; Farah *et al.*, 2004; Bett *et al.*, 2008). The use of camels as live asset or insurance at the time of disaster revealed in this study was exemplified in the 1973/75 drought in the Horn of Africa (Hussien, 1989; Farah *et al.*, 2004). As the study disclosed most of the Awash Fentale wereda pastoralists incurred their income by selling animals including camels. Other studies revealed the same (Joanne *et al.*, 2005; Megersa 2010; Farah *et al.*, 2004) but unlike the Borana pastoralist, milk is for household consumption only – culturally Afars do not sell milk. The use of camels as pack animals by Awash Fentale Afar pastoralists is also reported for Borana pastoralists (Megersa, 2010), Kababish pastoralists of Kordofan, northern Sudan (Asad, 1970), and Somali pastoralists (Farah *et al.*, 2004).

Grazing, watering, trade, cultural and ceremonial activities were the reasons for camel herd mobility in the current study similar to pastoralist in Kenya, Somali, Djibouti (Joanne *et al.*, 2005; Hussien, 1989) even though the most common reasons were movement for the search of pastures and water for their camels. The majority (47.4%) of the interviewees mentioned *Geremole* (trypanosomosis), *Rebreba* (plant poisoning), *Mesengele* (pneumonia), *Galireba* (CSD), *Boru* (coughing), and *Anberaruke* (camel pox) as major camel diseases. The pastoralist have their own local names for the camel diseases reported. Most camel diseases are known by herders' local vernacular names, but not in medical terms or by English names with which most health workers are familiar (Megersa, 2010). The findings reported are similar to some studies done in Ethiopia (Demeke, 1998, Megersa, 2010), in Turkana, Kenya particularly mentioning trypanosomosis (Mochabo *et al.*, 2005), in Sudan (Agab and Abbas, 1999) and in Kenya and Somalia where CSD was also mentioned by the pastoralists (Gluecks and Younan, 2010). All camels were at their own pastoral associations at the time of the first death due to CSD before four years in 2006. The continued mobilization of camel herds preceding the first death might have facilitated the infection (Megersa, 2010; Melaku and Feseha, 1988).

The no signs of disease were exhibited before the outbreak was similar to the scenarios seen in Borana (Dawo, 2010), Kenya and Somalia (Gluecks and Younan, 2010). The mean number of dead camels per pastoralist household were 15 (SD±7.4, minimum 6, maximum 32) is higher than the mean number of cases per herd of 2 and 6.6 cases in Puntland and Kenya, respectively. The figure can be higher if the results of the latter were given per pastoralist household. The highest mortality in adults where 93.3% were females among which 77.4% lactating, indicates that the disease targets reproductive age groups suggests a serious impact on the establishment of camel herds using replacement stock since the youngest age of camels at puberty is four years as reported from Eastern pastoral areas of Ethiopia (Melaku and Feseha, 1988). The high mortality in both pregnant and lactating camels has a direct negative effect on the livelihood of pastoralists because they depend on milk from their animals as also the present study confirms. This result is also in agreement with the study done in Sudan where it seems that the severity of PPR in camels was much higher in adult animals; more than 50% of deaths were reported in adult animals, mostly recently-delivered and pregnant females (Khalafalla *et al.*, 2010). The association of the PPR like syndrome in camels is similar to PPR in their small ruminant herds with predominant respiratory signs being similar to some of the studies done on the 1995 outbreak (Bekele, 1999; Fekadu *et al.*, 2003; Roger *et al.*, 2001). Rufael (1996) reported 'furro', a disease condition of unknown etiology which is manifested by discharge from the eye and mucopurulent nasal discharge in camels. These clinical signs reported are similar to typical signs of PPR in small ruminants; however, a clinical disease in camels due to PPRV had not yet been established (Roger *et al.*, 2001). Recently apparently similar outbreaks in camels with signs including dyspnoea, bilateral nasal discharge and coughing were reported from zone one of Elidar (Derayitu, Edeyita, Bugi and Egida pastoral kebeles) and Dubti (Saha and Hankis pastoral kebeles) weredas of Afar Regional state with inconclusive etiology (Personal communication, Afar National Regional State, Pastoral Agriculture and Rural Development Bureau, Semera Regional Veterinary Laboratory). So due to the inconclusive nature of the clinical signs and the non-outbreak conditions during the serological investigation, the clinical signs can not necessarily be linked to PPR under non-outbreak conditions.

The highest number of dead camels (24.2%) were reported from Doho and all of them were adult lactating female camels which were used as pack animals. The mean market price for dead camels is 4,134.7 birr in the study area is higher than reported from Borana and Guji of 2,377.60 birr (Dawo, 2010). This difference might be due the difference in market price of the areas and also the outbreak year.

Ninety point five percent (90.5%) of the dead camels were at the grazing area whereas the rest were found dead which is higher than the proportion of camels found dead in their enclosure (45%) in Kenya and Somalia (Gluecks and Younan, 2010). All the interviewees noted that there was good pasture at the time of outbreak which is similar to the case in Borana indicating plant poisoning is unlikely because enough feed was available (Dawo, 2010). The pastoralists gave *Galireba* as the local name of the CSD. Usually, the camels were not sick before death. Most respondents (69.5%) reported recumbency, dyspnoea and arching of neck as the signs seen at the time of death. Again high number of respondents (81.1%) revealed oozing of blood from anus as the most encountered sign unlike the 12.6% who mentioned nasal discharge as the most common sign after death of the camels. The same observations were recorded in Borana (Dawo, 2010), Ethiopia, Sudan and Kenya (Gluecks and Younan, 2010). 14.7% of the dead camels were examined post mortem by the local veterinary officers and revealed black, enlarged lung, blackish effusion from the thoracic cavity, extended and black liver, hyperthrophied heart, airway passages full of white foamy gaseous fluid, distended carcass and no rigor mortis being similar to the report of Dawo (2010). Dawo (2010) further stated the absence of rigor mortis and absence of blood clotting were some of the findings that were signs common between the outbreaks that occurred in 2006 and 2007, in addition to mortality of pregnant and lactating camels. However, the absence of rigor mortis and absence of blood clotting are pathognomonic signs of anthrax. Accordingly, anthrax was suspected but vaccination against anthrax and even mass treatment with broad-spectrum antibiotics did not stop the mortality, although a reduction in mortality was observed (Personal communication, Afar National Regional State, Awash Fentale Pastoral Agriculture and Rural Development Bureau). No etiological agent such as *Bacillus anthracis* was isolated (TFR, 2007, Dawo, 2010).

6. CONCLUSIONS AND RECOMMENDATIONS

The outbreak of camel mortality noticed in Afar pastoral area of Ethiopia in 2006 is a true challenge for field veterinarians, animal health researchers and academics as well as the country at large because it targeted the reproductive age groups of camels and had unknown etiology. This study elucidated higher overall seroprevalences of PPR in camels compared to most of the records in outbreak times in Afar region and elsewhere. The seroprevalence was higher in adult, lactating, females. Even though PPR is not established as a disease of camels, it can signify further assessment. The observed agreement between informants groups and case definitions of veterinary literature regarding most of the clinical signs of important camel diseases has proven that the Awash Fentale Afar pastoralists have an enormous wealth of knowledge on diagnosis of camel diseases. Therefore, participatory appraisal methods are useful to complement conventional methods in the control of complex camel diseases such as CSD. The analysis of pair wise ranking and proportional piling singled out CSD as a very important disease of camels in the study area. And the highest mean annual incidence and mortality of CSD among different camel diseases and among the productive mostly lactating and breeding adult camels demonstrated that CSD is a very serious disease which can affect the livelihood of the pastoralists negatively since the Awash Fentale Afar pastoralists depend on camel for food and many other social purposes.

The semi-structured questionnaires revealed that camels are very important animals in terms of food security and social benefits such as transport, income source, and ceremonial purposes. The importance of camel milk for children and its clan based consumption was also an important finding which further magnifies the importance of camel for the community. The mobility of the camel herds for many reasons could have resulted favourably in transmission of infectious diseases including CSD. The reason that affected camel herds were not sick except for few clinical signs just before death can clearly show the peracute nature of the disease and only preventive measures can be applied. The high mean number of dead camels and lower market price due to CSD for the mostly adult, productive camels can have a direct serious impact on the

establishment of camel herds using replacement stock. Most of the signs just before death were inconclusive including recumbency, dyspnoea, arching of neck. Oozing of blood from anus and nasal discharge were common findings in dead camels. The post mortem revealed black, enlarged lung, blackish effusion from the thoracic cavity, extended and black liver, hypertrophied heart, airway passages full of white foamy gaseous fluid, distended carcass, and no rigor mortis. Even though the absence of rigor mortis and blood clotting are pathognomonic signs of anthrax, vaccination against anthrax and even mass treatment with broad-spectrum antibiotics did not stop the mortality, although a reduction in mortality was observed. No etiological agent such as *Bacillus anthracis* was isolated. Although camel movement control is impractical (at least at present) in Awash Fentale Afar pastoral system, the determined level of seropositivity of PPRV and losses due to CSD morbidity and mortality, as investigated through participatory appraisal methods justify the launching of disease identification as well as, preventive and emergency rehabilitation programmes. These finding suggests that PPR in camels should be studied further so that justified control options can be used. Therefore, based on these findings, the following is recommended:

- The participatory epidemiological and conventional veterinary methods are complementary; therefore, they should be used side by side during animal health research, especially in pastoral areas, to make use of the rich experience of the pastoral community.
- Intensive participatory socio-economic investigations should be conducted to further assess the impact of CSD in pastoral areas to develop emergency systems and policies for rehabilitation of affected regions.
- Participatory disease search and the investigation of possible signs of disease immediately, and if possible during an outbreak, by teams of veterinary professionals using detailed participatory epidemiological techniques, should be conducted.
- Epidemiological investigations on multiple risk factors and causative agents (despite the fact that the causative agent(s) have not been confirmed by laboratory analysis) to collect epidemiological data before, during and after an outbreak of mortality, which could help to control the outbreaks, are warrented.

- Intensive research on PPR and other suspected agents in camels using serological surveys, virus isolation and cell line propagations from camels, experimental infections and molecular studies using random primers should be conducted to assess the susceptibility of camels to PPR, their role in the epidemiology of PPR in small ruminants and possible control and preventive measures in case of camel sudden death syndromes.
- Investigation of all possible causes of sudden deaths in camels with particular emphasis on viruses, toxins produced by bacteria and fungi, and mineral deficiency, especially of selenium, are further needed the temporal outbreak pattern of has not been identified

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8. ANNEXES

Annex 1. Material used for cELISA

- Spectrophometer
- Micro-ELISA plate (Nunc immuno I maxisorp), multichannel pipettes and tips.
- Incubator with shaker.
- Recombinant N- PPR antigen.
- Anti-N PPR virus monoclonal antibody.
- Anti-mouse conjugate.
- Control sera/ strong positive, weak positive and negative sera/.
- Substrate- chromogen solution/ OPD-H₂O₂/.
- Phosphate buffered saline (PBS).
- Blocking buffer /PBS+ 0.05% Tween 20+0.5% negative lamb serum.
- Washing buffer /dissolved PBS 1/5 in distilled water+ 0.05% Tween 20/.
- Stop solution /sulfuric acid, 1M/.

Annex 2. Procedure of cELISA

1. The plate was coated with PPR antigen by adding 50µl it diluted in PBS in 1/1300 dilution rate and incubated for 1 hr at 37°C.
2. Washed the plates three times in washing buffer and blotted dry.
3. 45µl of blocking buffer was added to all wells, then added a further:
 - 5µl of blocking buffer to the monoclonal control wells.
 - 55 µl of blocking buffer to conjugate control wells.
 - 5µl of test sera to test wells.
 - 5µl of strong positive, weak positive and negative sera to control wells
 - 50µl of monoclonal antibody diluted 1/ 100 in blocking buffer to all wells except the conjugate control wells and incubated 1 hr at 37°C.
4. Washed the plates three times and blotted dry.
5. Added 50 µl of anti-mouse conjugate diluted 1/1000 in blocking buffer.
6. Incubated at 37°C for 1 hr.
7. Washed the plate three times with washing buffer.
8. Added 50 µl substrate/ chromogen solution and kept for 10 min. in dark place.
9. Finally stop solution was added and read with an ELISA reader at 492 nm.

Percentage inhibition (PI) was calculated using the formula:

$PI = 100 - [(OD \text{ of the test wells} / OD \text{ of the monoclonal wells}) \times 100]$. The samples with $PI > 50\%$ (cut-off) were considered as positives. The antibody seroprevalence results were apparent prevalence (AP) and true prevalences will be calculated (Rogan and Gladen, 1978): $\text{true prevalence} = (AP + Sp - 1) / (Se + Sp - 1)$, where Sp is specificity and Se is sensitivity.

Plate layout of C-ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cc	Cc	1									
B	C++	C++	2									
C	C++	C++	3									
D	C+	C+	4									
E	C+	C+	5									
F	Cm	Cm										
G	Cm	Cm										
H	C-	C-										

Where:

Cc: Conjugate control

C++: Strong positive control

C+: Weak positive control

Cm: MAb control

C-: Negative control

1,2,3...Serum sample

Annex 3: Camel general socio-economic impact assessment format

Recorder:

Date:

Information about the herd

Owner Name:

Wereda:

Peasant association kebele:

Season: Wet: ☐ Dry: ☐

Income Source:

Type and number of livestock species raised by the individual owner

Cattle:

Sheep:

Goat:

Camel:

Reasons of camel disposition: Live animal sales : ☐

Ceremonial or Holidays: ☐

Both:

Household food source(milk or meat): _____

Milk consumption pattern: Household based: _____

Clan based: _____

Live Assets or insurance in time of disasters: _____

Date of 1st death (suspected as Camel Sudden Death)

Location of the herd at the time of the first death:

Migration of the herd during the last months preceding the first death:

Camel movement factors

Grazing and watering: ☐

Trade: ☐

Ceremonial/cultural purpose: ☐

All: ☐

Number of camels sick:

Number of camels dead:
 Within a specified time period
 Total number of camels in the herd during that time period:
 Income source;
 Milk sales: _____
 Live animal sales: _____
 Other: _____
 Use as pack animals: _____
 Other specify; _____

List major camels diseases in the area during the outbreak year

Local name	Clinical signs	Scientific name
-----	-----	-----
-----	-----	-----
-----	-----	-----
-----	-----	-----
-----	-----	-----
-----	-----	-----
-----	-----	-----

Do you know about “PPR like syndrome” and symptoms(Explaining the symptoms of PPR in small ruminants) in the herd (your herd, the herds you know)?
 No. of yes: ☐
 No. of no: ☐

If yes, had camels been involved?

No. of yes: ☐

No. of no: ☐

If yes, list the clinical symptoms observed in affected camels

Nasal discharge & coughing: ☐

Nasal and lachrymal discharge: ☐

Severe diarrhea: ☐

Severe diarrhea with coughing: ☐

Oral lesions, severe diarrhea and coughing with nasal discharge: ☐

If yes, list the clinical symptoms observed in affected small ruminants

When was the last outbreak with “PPR like syndrome” observed?

Information about a dead camel

Age of the camel:

Sex:

Market price:

Lactation status:

Lactating:

Dry/Non-lactating:

Place of death:

Date of death:

Circumstances at the time of the death:

Notable changes in the environment:

Has the animal been sick before death?:

Local name of the case:

If yes, how long? :

Symptoms before death:

☐ Fever

☐ Anorexia

☐ Dullness

☐ Nervous symptoms

☐ Swollen glands

☐ Diarrhoea

☐ Respiratory symptoms

☐ Haemorrhage

(from where:.....colour of blood:)

Ocular discharge: ☐

Other:...

Signs after death:.....

Post mortem findings (If there is):

Annex 4. Individual camel livestock history recording

Herd No.: _____ Camel recording No. _____

Camel Dam name: _____ Dam no.: _____

Camel Age: _____

No. of offsprings: _____

Event of interest includes any activities which do have notable importance in the life of the camel.(Migration, disease, date of purchase or birth etc)

Last year 2010 till birth

i.e. from same season last year to same season this year: give name of season Season when event happened

Event of interest 1

Event of interest 2

Event of interest 3

Camel Offspring recording no: _____

Camel Offspring name: _____

Camel age: _____

Last year 2010 till birth

i.e. from same season last year to same season this year: give name of season Season when event happened

Event of interest 1	Event of interest 2	Event of interest 3

9. CURRICULUM VITAE

PERSONAL DATA

Name: Fitsum Alemayehu Lemma
Date of Birth: 05 May, 1983
Place of Birth: Addis Ababa
Marital Status: Married with one child
Languages: Amharic and English
Address: Haramaya University, School of Veterinary Medicine
P.O.Box 138, Dire Dawa, Ethiopia.
Mobile Phone: +251-911-646165, Fax: +251-25-5530325/31
E-mail: fitsumalemayehu@yahoo.com

EDUCATIONAL BACK GROUND AND QULIFCATION

- Doctorate Degree in Veterinary Medicine from Addis University Faculty of Veterinary Medicine, Debre Zeit, 2006
- Achieved Senior secondary school Certificate at Bole High school, Addis Ababa, 1997-2000
- Achieved Junior secondary school Certificate at Atse Tewodros Junior Secondary School, Addis Ababa, 1993-1996
- Achieved primary secondary school Certificate at Abunel nderias Elementary School, Dire Dawa, 1989-1992

WORKSHOPS AND SEMINARS

- Certificate of attendance in Bioinformatics as tool for gene amplification, cloning and molecular modeling seminar in a training held by the Biotechnology department, IVRI,
- Certificate of attendance in annual meeting of Indian veterinary microbiologists, Immunologists and specialists in infectious diseases under the heading of concepts and strategies for disease diagnostics and immunoprophylactics.
- Certificate of attendance in non-credit course of German as foreign language, Addis Ababa University.
- Certificate of attendance in teaching methods, Haramaya university

WORK EXPERIENCE

2006 -to date

- Offering lectures (Vet. Physiology, Microbiology etc),
- Involving in research activities of the department (Ethno veterinary remedies for bovine mastitis etc),
- Advisorship (Current status of *Maedi-Visna* virus in sheep, Debre birhan, Ethiopia, Seroprevalence and serotype identification of FMD virus in Cattle, Moyale, Ethiopia, Pig Fasciolosis in Addis Ababa municipality abattoir, Addis Ababa, Ethiopia, Seasonal Dynamics of ticks and tick borne diseases in Jijiga, Ethiopia etc.),

RESEARCH WORK

- 2005 Senior Seminar: The problem of drug resistance in trypanosomosis, FVM, AAU.
- 2006 - Thesis Dissertation of DVM: Seroprevalence Study of PPR in Northshoa, Ethiopia and Abstract accepted for Society for Tropical Veterinary Medicine as a poster for 9th Biennial Conference, 03/06/07, oliver.sparagano@newcastle.ac.uk

- Seroprevalence Study of PPR in animals for slaughter in Luna Abattoir, Modjo, Ethiopia 2007
- Current status of *Maedi-Visna* virus in and around Debre Birhan, Ethiopia
- Pig *Fasciolosis* in pigs slaughtered in Addis Ababa Municipality Abattoir
- Seroprevalence and serotype identification of FMD virus in Moyale woreda

REFERENCES

Dr Maximilian P.O. Baumann (Dr. med.vet., MPVM, DipECVPH), Co-ordinator of postgraduate studies in International Animal Health, Faculty panel Veterinary Public Health, Faculty of Veterinary Medicine, Freie Universitaet, Berlin/Germany.

Dr. Tesfaye Sisay (Assistant Professor, DVM,MSc,PhD), Head of department of Microbiology, Immunology, Epidemiology and Public health, Addis Ababa University, School of Veterinary Medicine, Debre zeit.(Research Advisor). E-mail: Tesfu74@yahoo.com

Dr. Gelagay Ayelet, (DVM,MSc), Vaccine production and sales process owner, National Veterinary Institute, Debre Zeit, Ethiopia. E-mail: gelagayayelet@yahoo.com

Dr. Shiferwe Jenbere (DVM, MSc), Vaccine Research and development team leader, National Veterinary Institute, Debre Zeit, Ethiopia. E-mail: shife2000jem@yahoo.com

Dr. Berhe G\egizehabiher(DVM,MSc, PhD), Director of Animal Production and Health division of FAO head quarters, UN (Under graduate Research Advisor) E-mail: berheg@gmail.com

Dr. Tapas Kumar Goswami ,Ph.D (immunology).Principal Scientist, Immunology, of Indian veterinary research Institute, Bareilly, India

E-mail: goswami.tapas@gmail.com, tapasgoswami_ivri@yahoo.com

Mobile Phone: +91-9458269399

10. SIGNED DECLARATION SHEET

I, the undersigned, declare that the thesis is my original work and has not been presented for a degree in any University and that all sources of material used for the thesis have been duly acknowledged.

Name Fitsum Alemayehu Lemma.

Signature _____

Date of submission _____

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

Dr. Tesfaye Sisay _____

Dr. Max. Baumann-----

Dr. Gelagay Ayelet-----