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SEROLOGICAL AND PARTICIPATORY EPIDEMIOLOGICAL STUDY OF CAMELPOX AND FIELD EVALUATION OF VACCINE IN GABI RASU ZONE, AFAR REGION, ETHIOPIA

MSc Thesis

By

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June, 2015

Bishoftu, Ethiopia

SEROLOGICAL AND PARTICIPATORY EPIDEMIOLOGICAL STUDY OF CAMELPOX AND FIELD EVALUATION OF VACCINE IN GABI RASU ZONE, AFAR REGION, ETHIOPIA

A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Tropical Veterinary Epidemiology

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June, 2015

Bishoftu, Ethiopia

Addis Ababa University

College of Veterinary Medicine and Agriculture

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by **Weldegebrial Gebrezgabher Aregawi** entitled: **SEROLOGICAL AND PARTICIPATORY EPIDEMIOLOGICAL STUDY OF CAMELPOX AND FIELD EVALUATION OF VACCINE IN GABI RASU ZONE, AFAR REGION, ETHIOPIA** and recommended that it be accepted as fulfilling the thesis requirement for the Degree of Master of Science in Tropical Veterinary Epidemiology

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DEDICATION

This thesis manuscript is dedicated to my best friend, Mr. Elias Kebede Hailu.

SIGNED DECLARATION SHEET

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

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LIST OF ABBREVIATIONS

ATIP	A-type inclusion protein
CAM	Chorioallantoic membrane
CI	Confidence Interval
CMLV	Camelpox Virus
CPE	Cytopathic Effect
CSA	Central Statistic Agency of Ethiopia
DNA	Deoxyribo Nucleic Acid
ELISA	Enzyme Linked Immunosorbant Assay
HA	Haemagglutinin
LAMP	Loop Mediated Isothermal Amplification
NAHDIC	National Animal Health Diagnostic and Investigation Center
SSI	Semi-structured Interview
NVI	National Veterinary Institute
OIE	Office Internationale des Epizooties
OPV	Orthopoxvirus
PAs	Pastoral Associations
PCR	Polymerase Chain Reaction
TEM	Transmission Electron Microscopy
VNT	Virus Neutralization Test

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ABSTRACT

Camelpox virus (CMLV) causes a smallpox-like illness in a unique host, the camel. Although the disease is enzootic in almost all regions where camel husbandry is practiced, and is responsible for severe economic losses, its epidemiology has not extensively investigated. Thus, sero- and participatory- epidemiological investigation of the disease was carried out in Gabi Rasu zone using cross sectional study design to determine its seroprevalence and associated risk factors as well as using seasonal calendar to identify its seasonal occurrences. In addition camelpox vaccine, the only available vaccine for the species of animal in Ethiopia, was evaluated under field condition by seroconversion using the before and after with control experimental study design. The study was conducted in two selected districts of Gabi Rasu zone, from November 2014 to May 2015.

Out of 384 camel sera samples tested by virus neutralization test, 74 were seropositive for camelpox, giving an overall seroprevalence of 19.3% (95% CI= 15.3-23.2%) in the study area. A seroprevalence of 21.6 (95% CI= 16.0-27.2) and 16.7 (95% CI=11.2-22.1) were recorded in Amibara and Awash Fentale districts, respectively. At least one seropositive camel was detected from 25 herds among 30 examined giving 83.3% (95% CI = 70.0-96.6) herd level prevalence. Univariable and multivariable logistic regression analysis of the assumed risk factors showed that age of the camel was the only risk factor associated with the occurrence of serpositivity to camelpox. The results indicated that camelpox seroprevalence was significantly higher in adult (24.4%, 95% CI= 18.4-30.4) than young age group (13.9%, 95% CI= 8.9-18.8) with a 2 fold greater odds of occurrence. The small-scale vaccine evaluation trial indicated that, vaccination of camels with the live attenuated camelpox vaccine resulted in a significantly higher seroconvesrion rate compared to unvaccinated camels. However, among 58 vaccinated camels with pre-vaccination seronegative results, 32 found seropositive at post-vaccination serum analysis using VNT, indicating low (55.2%) seroconversion rate. Various explanations have suggested for the failure of post vaccination seroconversion on 44.8% vaccinated camels. Interview of pastoralists has revealed that camelpox is one

of the most common camel diseases in the area. The disease was reported commonly to occur during the minor (Sugum) and major (Kerma) rainy seasons by informant groups.

In conclusion camelpox seems to be endemic in Gabi Rasu zone with seasonal occurrence during rainy season. Therefore, considering the economical significance of the disease, vaccination of young camels along side training of pastoralists for improved management strategies could have a significant importance in diminishing the virus from circulation. However, further extensive studies that lead to conclusive results on the efficacy of the vaccine under evaluation are recommended.

Key words: *Afar, Camelpox, Participatory Epidemiology, Risk Factors, Seroprevalence, VNT.*

1. INTRODUCTION

According to the United Nations Food and Agriculture Organization, the total world camel population is approximately 25.9 million animals (http://faostat.fao.org). Camel (*Camelus dromedaries*) is highly adapted domestic animal to arid and semi-arid environment, particularly in the lowlands of the horn of Africa, including Somalia, Sudan, Ethiopia, Kenya, Eritrea and Djibouti. With in Ethiopia the camel is found in arid and semi-arid areas of Somali, Afar and Southern Oromiya regions where the majority of the people are nomadic pastoralist. According to the report of FAOSTAT (2011), the overall population of camels in Ethiopia is estimated to be 2.4 million which ranks it third in the world after Somalia (7 million) and Sudan (4.5 million).

In Ethiopia, as in most dry lands of Africa and Asia, camels are the principal source of income and food for millions of pastoralists. The commonest uses of camels by the pastoralists are for milk and meat production, transportation (grain, water, salt and other goods), income generation from sale, providing draught power and determining the wealth and social status of pastoralists (Tefera and Gebreab, 2004; Megersa, 2010). They are very reliable milk producers and reduce vulnerability to food insecurity even during the dry season and drought periods, when milk from cattle and goat becomes scarce (Gebre and Kayaa, 2008). Camels as part of livestock diversification have also economic and ecological advantages in arid and semiarid areas and represent a minimal competition with other ruminants (Megersa, 2010). In addition, camels play a central role on the national economy by generating foreign currencies from export markets.

Inspite of the valuable economical and ecological contribution to the pastoral communities, as well as to the National Gross Domestic Product (NGDP), until recently little effort has been made to address the constraints of camel production by researchers and development planners in Ethiopia (Megersa, 2010).This has been probably due to the fact that camel production is in remote, migratory and poor infrastructure condition.

However, currently as result of frequent droughts and extending arid and semi-arid zones, there has been increased attention paid to identify camel production constraints. A few

studies have been conducted and these indicated that among other constraints, camel diseases are the major problems faced by camel producing communities in Ethiopia (Tekle and Abebe, 2001; Tefera and Gebreab, 2004; Gebre and Kaaya, 2008; Megersa, 2010) and in the horn of Africa (Dirie and Abdurahman, 2003). Infectious and parasitic diseases including trypanosomiasis, camelpox, contagious skin necrosis, pneumonia, mange mite infections and internal parasites are among the major reported health problems hampering the potential performances of the animals in the country (Demeke, 1998; Megerssa 2010).

One of the most common important infectious and contagious viral diseases diagnosed in the old-world camelids is camelpox, caused by *Orthopoxvirus cameli* (Azwai *et al.*, 1996). The disease is known in almost all camel raising countries except Australia and it is the most important cause of severe economic losses (Fenner *et al.*, 1989; Azwai *et al.*, 1996). To herdsmen, camelpox is a well known and feared disease (Azwai *et al.*, 1996). Its considerable economic importance is due to the high morbidity, a relatively high mortality in younger animals, loss of condition in all ages, reduced milk production in lactating ones (Azwai *et al.*, 1996) and abortion in pregnant camels (Al Zi'abi *et al.*, 2007; Mahmoud *et al.*, 2012). In addition, the appearance of camelpox in herds may favor secondary infections from other circulating diseases from which camels might die (Duraffour *et al.*, 2011a). The mortality rate in adult animals is between 5% and 28% and in young animals between 25% and 100% (Mayer and Czerny, 1990). Nationally, existence of the disease leads to trade restriction of camels and their by products (Bhanuprakash *et al.*, 2010).

Camelpox is also important because of its potential zoonotic problem (Azwai*et al.*, 1996). Several cases in humans have been described (Kriz, 1982); the most recent from India (Bera *et al.*, 2011) with clinical manifestations such as papules, vesicles, ulceration and finally scabs on fingers and hands. The Office Internationale des Epizooties (OIE), World Organization for Animal Health, lists camelpox as a reportable disease (OIE, 2014b). The disease is also classed in Risk Group 2 for human infection and recommended to be handled with appropriate measures (OIE, 2014a).

Camelpox Virus (CMLV) is transmitted via the contaminated environment through skin abrasions and by aerosols, although a mechanical transmission might be considered (Wernery and Kaaden, 2002). Clinical manifestations of camelpox range from inapparent and mild local infections, confined to the skin, to moderate and severe systemic infections, possibly reflecting differences between the strains of camelpox or differences in the immune status of the animals (Wernery and Kaaden, 2002). In general, young calves and pregnant females are more susceptible (Kriz, 1982; Al Zi'abi *et al.,* 2007).

Camelpox is routinely diagnosed based on clinical signs, pathological findings and cellular and molecular assays. Tentative diagnosis can be made based on clinical signs and pox lesions, but will confuse with other viral diseases, such as contagious ecthyma (parapoxvirus) and papillomatosis (papillomavirus) (Wernery and Kaaden, 2002); therefore differential diagnosis is needed either by identification of the agent or antibody detection from serum samples.

Camelpox virus is host-specific and dependent on a single host, which could potentially target for control and eradication. Control of the disease contributes to increased camel productivity and international trade. Unfortunately, effective control programmes such as quarantine measures and restriction of camel movement appear to be of limited success owing to the migratory pattern of camels and the difficulty of reaching the animals. Therefore, vaccination seems to be the only option to control this viral disease in enzootic countries (Khalafalla and El Dirdiri, 2003). As a result, camelpox vaccine production reports have come from different countries including Saudi Arabia (Hafez *et al.,* 1992), United Arab Emirates (Wernery and Zachariah, 1999), Morocco (El Harrak and Loutfi, 2000) and Mauritania (Nguyen *et al.,* 1996). Recently, the vaccine was developed in Ethiopia by the National Veterinary Institute (NVI), which is the first type of vaccine for dromedary in the country.

To carry out an effective control or even eradication programmes of camelpox through strategic vaccination, a thorough understanding of the epidemiology of the disease in a given geographical area as well as a reliable supply of pure, safe, potent, and effective camelpox vaccine have a paramount importance. However, eventhough the disease was

confirmed to occur in Ethiopia (Ayelet *et al.,* 2013) and known for its economic importance; review of literatures revealed that, few works has been done on the epidemiological features of the disease in Ethiopia. Furthermore, despite the development and production of the vaccine by NVI, no work has been published on the field efficacy of the vaccine. This study was therefore designed with the following general and specific objectives:

General objective:

- To partly contribute towards the understanding of the epidemiology of camelpox in Ethiopia and to reduce the adverse health impact of camelpox on the livelihood of pastoralists through field assessment of camelpox vaccine.

Specific objectives:

- To determine the seroprevalence of camelpox and its associated risk factors,
- To evaluate the efficacy of newly developed camelpox vaccine at field level
- To identify the seasonal occurrence of camelpox outbreak and suggest appropriate vaccination schedules to prevent and control the disease.

2. LITERATURE REVIEW

2.1. Etiology

Camelpox virus (CMLV), the causative agent of camelpox, belongs to the genus *Orthopoxvirus* (OPV), of the subfamily *Chordopoxvirinae* of the family Poxviridae (Moss, 2007). Poxviridae is a large family of dsDNA containing viruses that infect mammals, birds, and insects. Eleven genera are recognized, eight of which infect vertebrates and three of which infect invertebrates. Viruses that infect vertebrates are classified in the subfamily *Chordopoxvirinae* and insect poxviruses are classified under *Entomopoxvirinae*. Genus *orthopoxvirus* are among the best and longest known viruses to mankind, and they are among the most feared viruses of livestock animals and humans (Yousif *et al*., 2010). The genus composes four human pathogenic species, i.e., variola virus (VARV), vaccinia virus (VACV, including the isolate buffalopox virus), monkeypox virus (MPXV) and cowpox virus (CPXV), and species of veterinary importance, i.e., CMLV, CPXV, buffalopox virus (BPXV, a variant of VACV), and ectromelia virus, as well as taterapox virus, the North American OPVs (volepox virus, raccoonpox virus and skunkpox virus) and an unclassified OPV species, uasin gishu disease virus (Duraffour *et al.*, 2011a ; Venkatesan *et al.,* 2012a).

The identification of CMLV agent was an alarm when it was described as smallpox-like disease during smallpox eradication campaign (Baxby, 1972), which led to the discovery of the CMLV. Based on sequence analysis, it has been determined that the camelpox virus is the most closely related to variola virus, the aetiological agent for smallpox, a dreadful disease eradicated in 1980 (Duraffour *et al.*, 2011a).

Orthopoxviruses are large (250 nm350 nm), brick-shaped enveloped viruses (Damon, 2007; Moss, 2007)**,** and the outer membrane is covered with irregularly arranged tubular proteins (OIE, 2014a). The average size of virion (CMLV) is 265–295 nm, which consists of an envelope, outer membrane, two lateral bodies and a core (OIE, 2014a).

CMLV, like other OPVs, shows variable responses to physical/chemical agents (Balamurugan *et al.,* 2013). Poxviruses are generally susceptible to various disinfectants including 1% sodium hypochlorite, 1% sodium hydroxide, 1% peracetic acid, formaldehyde, 0.5–1% formalin and 0.5% quaternary ammonium compounds (OIE, 2014a). Camelpox virus is ether resistant and chloroform sensitive (Tantawi *et al.*, 1974; Davies 1975). The virus is sensitive to pH 3–5 and pH 8.5–10 (Davies *et al.,* 1975). The virus can be destroyed by autoclaving or boiling for 10 minutes and is killed by ultraviolet rays (245 nm wave length) in a few minutes (Coetzer, 2004).

2.2. Genome and Phylogeny of the Virus

The Orthopoxvirus genome is a double-stranded DNA molecule that replicates entirely in the cytoplasm of the host cell (Moss, 2007). The genome size is approximately 200 kbp, with size variations related to the virus strain (Yousif and Al-Naeem, 2012). The central genomic region (approximately 100 kbp) is highly conserved and carries genes primarily involved in RNA transcription, DNA replication, and virion assembly (Duraffour *et al.,* 2011a). In contrast, the terminal genomic regions of OPVs vary both in length and patterns of restriction enzyme cleavage sites, and encode genes that are primarily responsible for host range, virulence, and immunomodulation (Bhanuprakash *et al.,* 2010; Yousif and Al-Naeem, 2012). For these reasons, the sequences of the terminal regions and the organization of the open reading frames (ORFs) are more variable between OPVs (Duraffour *et al.,* 2011a).

The CMLV genome encodes more than 211 putative proteins (Bhanuprakash *et al.,* 2010) and consists a single linear double-stranded DNA molecule terminated by a hairpin loop that replicates in the cytoplasm (Moss, 2007). The genome is AT-rich (66.9 %) having cross-links that join the two DNA strands at both ends. Each end of DNA strand has long inverted tandem repeats that form single-stranded loops. The central region of the genome contains genes that are highly conserved amongst all sequenced OPVs (Fenner, 1989). Like other poxviruses, the genes are tightly packed with little non-coding sequences.

The sequencing of full-genome of CMLV strains revealed that CMLV is closest to VARV, sharing genes involved in basic replication and host related functions and probably, they may share a common ancestor (Afonso *et al.*, 2002). Genomic differences between CMLV and other OPVs are located in terminal regions. In this region, open reading frame (ORF) co-linearity and average amino acid identity decreases (82 % to VACV) due to small and large nucleotide insertions, deletions, and translocations (Balamurugan *et al.*, 2013). However, the arrangement of ORFs close to and within the inverted terminal repeat (ITR) of CMLV and VARV showed a higher degree of similarity in comparison with other OPVs (Afonso *et al.*, 2002; Gubser and Smith, 2002). Phylogenetic analyses have thus revealed that CMLV is distinct but closely related to VARV (Fig. 1) (Duraffour *et al.*, 2011a).

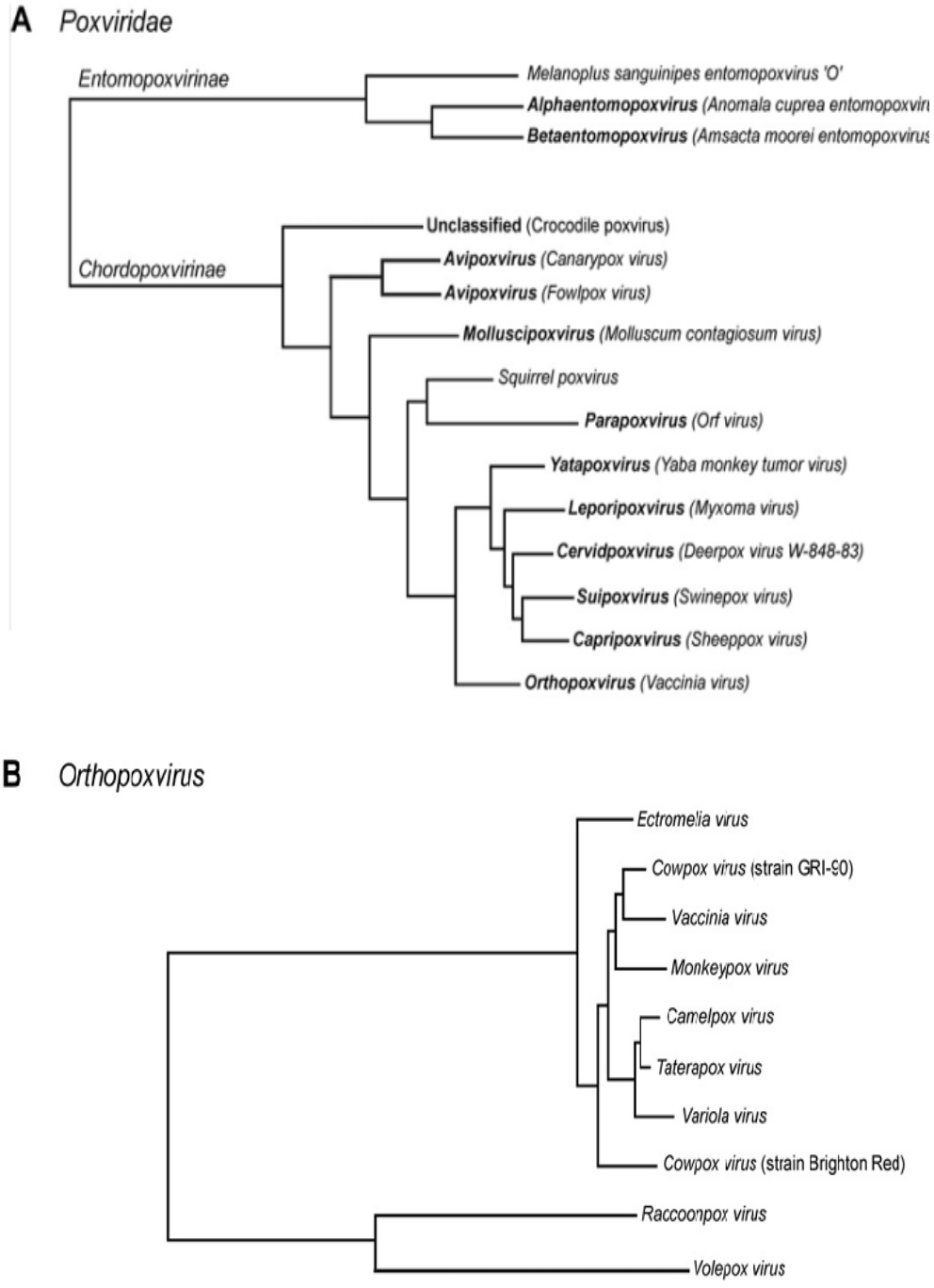

Figure 1: Phylogenetic trees of (A) the poxviruses and (B) the orthopoxviruses**.**
Source: Duraffour *et al.*, 2011a.

2.3. Epidemiology

Camelpox occurs in almost every country in which camel husbandry is practised apart from the introduced dromedary camel in Australia and tylopods (llama and related species) in South America. The disease was initially described in Punjab, India, in 1909 (Wernery and Kaaden, 2002). Subsequently, outbreaks have been reported in the Middle East (Bahrain, Iran, Iraq, Oman, Saudi Arabia, Syria, United Arab Emirates and Yemen), in Asia (Afghanistan and Pakistan), in Africa (Algeria, Egypt, Ethiopia, Kenya, Mauritania, Morocco, Niger, Somalia and Sudan) and in the southern parts of Russia and India (Mayer and Czerny, 1990; Duraffour *et al.*, 2011a; OIE, 2014a). The disease is endemic in these countries and a pattern of sporadic outbreaks occurs with a rise in the seasonal incidence usually during the rainy season (Wernery and Kaaden, 2002). The circulation of CMLV infections in herds has also been confirmed by sero-epidemiologic studies which showed a 9.8% prevalence in Libya (Azwai *et al.,* 1996) and 9.14% in Saudi Arabia (Housawi, 2007). In contrast, a prevalence of neutralizing antibodies of 72.5% was measured in Sudan in an unvaccinated population following the outbreak of 1992–1994 (Khalafalla *et al.,* 1998). Table 1 shows an overview of reports on the occurrences of camelpox disease from various countries.

Currently, CMLV is considered to solely naturally infect old world camelids, including *Camelus dromedaries* (dromedary camel) and *Camelus bactrianus* (Bactrian camel) (Feffer *et al.,* 1996; Wernery and Kaaden, 2002). It appears to be enzootic in camels but natural infections in other animal species, including cattle, sheep and goats are not known (Al Zi'abi *et al.,* 2007; OIE, 2014a). There have been several attempts to infect animals other than camels with CMLV in order to define its host range and develop animal models of camelpox. However, the virus was found to be non-pathogenic to cattle, sheep, goats, rabbits, guinea pigs, rats, hamsters and mice when inoculated by intra-dermal route (Pfeffer, 1998a).

Various studies have demonstrated that the incidence of camelpox outbreaks increases during rainy seasons with the appearance of more severe forms of the disease, while milder form occurs during the dry season (Wernery *et al.,* 1997a,b; Khalafalla and Ali,

2007; Megerssa, 2010). This may be due to the fact that moisture may enhance virus stability in the environment and increase subsequent transmission to susceptible animal (Megerssa, 2010). The increased density of the tick population during the rainy season may also be responsible for the spread of the disease (Wernery *et al*., 1997a).

As a general pattern, young camels and pregnant females appear more susceptible to camelpox (Duraffour *et al.,* 2011a). The disease mostly affects young calves aged 2–3 years in a herd with fatal severe form (generalized form) causing high mortality occasionally due to waning of acquired immunity after 5–8 months (Nothelfer *et al.*, 1995). Abortion rates can reach 87%, as observed in Syria (Al Zi'abi *et al.,* 2007), albeit this high percentage might be explained by the absence of immunity as CMLV circulation had never been reported in this country before. Considering the sex, the morbidity and mortality rates are higher in females than in males (Mahmoud, 2012). In contrast other study reported a higher proportion of cases in males than females (Kriz, 1982; Khalafalla and Mohamed1996).

The morbidity and mortality rates of CMLV varies depending on the geographic location, season, whether the virus is circulating in the herd, the presence of intercurrent diseases, stress, the age structure and the general nutritional status of the herd at the time of the outbreak as well as the virulence of the virus strain involved (Davies *et al.,* 1975; Jezek *et al.,* 1983; Al Hendi *et al.,* 1994; Wernery *et al*., 1997a; Mahmoud, 2012). Generally, mean morbidity rates can be as high as 92%, while the mean mortality rates may vary from 0% to 15% and the case fatality rates may range from 0% to 25% (Table 1) (Duraffour *et al.,* 2011a). Because oral lesions severely impair the ability of young calves to suckle or feed, the case fatality rate may reach 25% (Fenner *et al.,* 1993).

Generally, the major risk factors associated with higher incidence of camelpox have been defined and include age of the animals (less than four years old), the rainy season of the year, the introduction of new camels in a herd and the common watering (Khalafalla and Ali, 2007)**.**

Table 1: Overview of reports of camelpox clinical cases from different countries

Country	**District (number)**	**Year**	**Period (months)**	**No. of animals (no.of herds)**	**No. of cases (No. of deaths)**	**Mean Morbidity rate (%)**	**Mean Mortality rate (%)**	**References**
Somalia	(4)	1978	May to Sep	1052 (30)	295 (16)	28	1.52	Kriz (1982); Jezek *et al.*, (1983)
	Kismayo	1976	April to Jun	-	20 (0)	-	-	
Iraq	Al-Etha	1977	December	-	450	-	-	Falluji *et al.*, (1979)
Saudi Arabia	Eastern region	1989	October	100	10 (0)	10	0	Al Hendi *et al.*, (1994)
Kenya	Turkana	1992	September	600 (25)	36 (0)	6	0	Gitao, 1997
	Samburu	1992	October	500 (20)	135 (2)	27	0.4	
Sudan	Butana	1992–1994	Jan- Dece	2560 (35)	230 (32)	9	1.2	Khalafalla and Mohamed (1996); Khalafalla and Ali (2007)
UAE		1993–1994	Oct to March	- (16)	15 (1)	-	-	Wernery *et al.*, 1997a, b
		1995-1996	Nov to Apri	- (22)	22 (3)	-	-	
Ethiopia	Dire Dawa, Harar Zuria, Jijiga, Gewane	-	May to June	350 (100)	11	3	-	Tefera and Gebrea, 2001
Syria	Duma and Hama	2005	Summer and Spring	875 (7)	489	28–92	1–15	Al Zi'abi *et al.*, 2007
Saudi Arabia	Eastern rovince	2010	-	15 (1)	3 (0)	20	0	Yousif *et al.*, 2010
Ethiopia	Gabra and Borana	2008	April to May	752 (70)	2 (0)	0.3	0	Megersa, 2010
		2008	Oct to Nov	459(70)	65 (4)	14.2	0.9	
Egypt	Alexandria and Matrouh	2009	Winter	141	74 (8)	52.4	5.7	Mahmoud *et al.*, 2012
Ethiopia	Chifra Jigjiga	2012/13	Novemeber to April	953	36	3.8	-	Ayelet *et al.*, 203

Source: Moidified from Duraffour *et al.*, 2011a

2.4. Mode of Transmission

The CMLV is transmitted by either direct or indirect contact via a contaminated environment (Al Zi'abi *et al.,* 2007; Salem *et al.,* 2008). The direct transmission occurs in between infected and susceptible animals either by inhalation or through skin abrasion (Wernery and Kaaden 2002).The affected camels may shed the virus through scab materials and secretions like milk, saliva and ocular and nasal discharges in the environment such as in water which becomes the source of infection to susceptible animals (Khalafalla and Ali, 2007). The dried scabs shed from the pox lesions may contain live virus particles for 4 months and contaminate the environment (Elliot and Tuppurainen, 2010).

The virus can also be transferred mechanically by ticks and other biting arthropods. This idea is supported by the isolation of CMLV from *Hyalomma dromedarii* ticks during the occurrence of camelpox outbreak in United Arab Emirates (Wernery *et al.,* 1997a, b). However, the question remains whether ticks might transmit CMLV mechanically or whether they might be a true reservoir of the virus. Further studies are needed to ensure the involvement of arthropods in the transmission of CMLV, but if confirmed, CMLV would be the first OPV transmitted via arthropods (Duraffour *et al.,* 2011a). Other potential vectors may also be involved, such as biting flies and mosquitoes (Azwai *et al.,* 1996; OIE, 2014a). Transmission of poxviruses through arthropods is not surprising. This has been reported with yatapoxviruses (biting insects), capripoxviruses (mosquitoes and stable flies), leporipoxviruses (mosquitoes, fleas, black flies, ticks and mites), suipoxvirus (louse) and avipoxvirus (mosquitoes) (Duraffour *et al.,* 2011a).

2.5. Pathogenesis

The CMLV enters commonly through skin. However, the oro-nasal infection is also reported. After local replication and development of a primary skin lesion, the virus spreads to local lymph nodes leads to a leukocyte-associated viremia, which may be associated with pyrexia. During this period, virus can be isolated from various tissues, including the skin, turbinates, lungs and also lymphoid organs. Widespread secondary

skin lesions appear a few days after the onset of viremia, and new lesions continue to appear for 2–3 days, at that time the viremia subsides (Balamurugan *et al.,* 2013).

Camelpox can produce severe disease, suggesting CMLV may interfere with the host response to infection. Like other OPVs, CMLV encode multiple genes that antagonize or affect the antiviral host immune response by interfering with the interferon (IFN) response, key pro-inflammatory cytokines [(Interleukin-IL-1b, IL-18 and tumor necrosis factors (TNFs)], chemokines and the complements (Duraffour *et al.,* 2011a).

2.6. Clinical Signs

The disease is characterized by an incubation period of 9–13 days (varying between 3 and 15 days) with an initial rise in temperature, followed by enlarged lymph nodes, skin lesions and prostration (Duraffour *et al.,* 2011a; OIE, 2014a). The clinical manifestations of camelpox range from inapparent and mild local infections, confined to the skin, to moderate and severe systemic infections, possibly reflecting differences between the strains of camelpox or differences in the immune status of the animals (Wernery and Kaaden, 2002). Skin lesions appear 1–3 days after the onset of fever, starting as erythematous macules, developing into papules and vesicles, and later turning into pustules. Crusts develop on the ruptured pustules. These lesions first appear on the head, eyelids, nostrils and the margins of the ears. In severe cases the whole head may be swollen. Later, skin lesions may extend to the neck, limbs, genitalia, mammary glands and perineum. In general, the lesion takes 4–6 weeks to heal (OIE, 2014a). The lesion is usually localized in skin but occasionally, it leads to generalized form. The later form is frequently seen in young animals aged 2–3 years in a herd associated with weaning and poor nutrition (Balamurugan *et al.,* 2013). In the generalised form, pox lesions may cover the entire body. CamelPox infection can also cause blindness due to the presence of pox lesion in eyes (Khalafalla and Mohamed, 1996; Megersa, 2010).

In the systemic form of the disease, pox lesions can be found in the mucous membranes of the mouth, respiratory tract and digestive tracts (Kriz, 1982; Kinne *et al.,* 1998; Wernery and Kaaden, 2002). The affected animals may show anorexia, salivation,

lacrimation and mucopurulent nasal discharge and diarrhoea. Pregnant females may abort (Khalafalla and Mohamed, 1996; Al Zi'abi *et al.,* 2007; Mahmoud *et al.,* 2012) and mortality in affected animals is due to septicaemia caused by secondary bacterial infections like *Staphylococcus aureus* (Nothelfer *et al.,* 1995; Wernery and Kaaden, 2002). Animals that survive the disease are immune for life, and there is no chronic carrier state (Mayer and Czerny, 1990).

2.7. Pathology

Camelpox can either occur as a localized benign infection or as a generalized malignant disease especially in younger animals (Hafez *et al.*, 1992). There are only a few detailed pathological descriptions of internal camelpox lesions. The lesions observed on post-mortem examination of camels that die following severe infection with camelpox are multiple pox-like lesions on the mucous membranes of the mouth and respiratory tract (Kinne *et al.,* 1998; Pfeffer, 1998a). The size of the lesions in the lungs may vary in diameter between 0.5 and 1.3 cm, occasionally up to 4–5 cm. Smaller lesions may have a haemorrhagic centre. Pox lesions are also observed in the mucosa of the trachea and retina of the eye causing blindness (OIE, 2014a).

The histopathology of skin lesions reveals characteristic cytoplasmic swelling, vacuolation and ballooning of the keratinocytes of the outer stratum spinosum of the epidermis (Duraffour *et al.,* 2009; Duraffour *et al.,* 2011b). The rupture of these cells produces vesicles and localized oedema associated with perivascular cuffing of mononuclear cells, neutrophils and eosinophils. Marked epithelial hyperplasia may also occur at the borders of the skin lesions (Yager *et al.,* 1991). The lung lesions are usually characterized by hydropic degeneration, proliferation of bronchial epithelial cells associated with proliferative alveolitis and bronchiolitis infiltrated by macrophages, necrosis and fibrosis, which leads to obliteration of normal architecture (Pfeffer *et al.*, 1998a,b).

2.8. Diagnosis

Camelpox is routinely diagnosed based on clinical signs, pathological findings and cellular and molecular assays. Tentative diagnosis can be made based on clinical signs and pox lesions, but will confuse with other diseases, such as contagious ecthyma, papillomatosis and reaction to insect bites (Yousif *et al.,* 2010); therefore differential diagnosis is needed using laboratory-based diagnostic methods (Wernery and Kaaden, 2002). Identification of the agent and antibody detection from serum samples can be used for the differential diagnosis of the disease. Few complementary techniques might be advised for camelpox diagnosis namely transmission electron microscopy (TEM), cell culture isolation, immunohistochemistry, demonstration of neutralizing antibodies, PCR and LAMP assays (Bhanuprakash *et al.,* 2010; Duraffour *et al.,* 2011a). The choice of the test method depends on the purpose of the investigation and the availability of the test methods at a time. Generally isolation and identification of the virus using TEM, Immunohistochemistry, cell cultures and molecular techniques are advised for the confirmation of clinical cases and serological tests are valuable for secondary confirmatory testing (OIE, 2014a).

2.8.1. Transmission electron microscopy (TEM)

TEM is a reliable and rapid method to demonstrate camelpox virus in scabs or tissue samples (Salem *et al.,* 2008; Duraffour *et al.,* 2011a; OIE, 2014a). This technique enables the differentiation between OPVs, which are brick-shaped, and parapoxviruses, which are ovoid-shaped (Damon, 2007). Currently, it is the best method for distinguishing clinical cases of camelpox and orf caused by camelpox and parapox viruses, respectively (OIE, 2014a), although the viruses can be differentiated by serological techniques and by PCR (Mayer and Czerny, 1990). However, a relatively high concentration of virus in the sample is required for positive diagnosis and camelpox virus cannot be differentiated from other *Orthopoxvirus* species (Duraffour *et al.,* 2011a; OIE, 2014a). The technique is also complex, required high skills to operate electron microscopy and not usually available for veterinarians in the field services (Nagarajan *et al.,* 2011).

2.8.2. Immunohistochemistry

Immunohistochemistry for the detection of the infectious agent of camelpox is a relatively fast method and can be used instead of electron microscopy (Nothelfer *et al*., 1995). The camelpox antigen in the infected scabs and tissues can be identified by using this technique (Wernery and Kaaden, 2002; Yousif *et al.,* 2010). It is based on the use of monoclonal antibody directed against the epitope A 1 of the orthopoxvirus fusion protein (Czerny and Mahnel, 1990; Czerny *et al.,* 1994). However, immunohistochemistry couldn't differentiate OPV members unless specific monoclonal antibodies are used (Guarner and Zaki, 2006). Almost any polyclonal antibody against vaccinia virus is likely to produce reasonable results in this test because of the wide homology between vaccinia and camelpox viruses (Nothelfer *et al*., 1995).

2.8.3. Virus isolation in cell cultures

Camelpox virus can be propagated in a large variety cell cultures including the following cell lines: Vero (transformed green monkey kidney), MA-104 (African green monkey kidney), BHK (baby hamster kidney), and Dubca (transformed camel skin fibroblasts) as well as primary cell cultures including lamb testis, lamb kidney, camel embryonic kidney, calf kidney, and chicken embryo fibroblast (Duraffour *et al.,* 2011a; OIE, 2014a). However, Vero, MA-104 or Dubca cells, in which the virus replicates easily are generally preferred (Pfeffer *et al.,* 1998b). Blood, serum and homogenized tissue samples can be used to infect cell cultures. Cultures should be monitored for cytopathic effects (CPE) for 10–12 days. However, depending on the virus concentration, plaque-type cytopathic effects can be appeared at one day post-infection (Duraffour *et al.,* 2011a; OIE, 2014a). CPE includes the formation of multinucleated syncytia, rounding, ballooning and syncytia with degenerative changes (Balamurugan *et al.,* 2013).

Camelpox virus can also be isolated on the chorioallantoic membrane (CAM) of 11 to 13 day old embryonating chicken eggs (Sheikh Ali *et al.,* 2009). The eggs should be incubated at 37°C and after 5 days, the eggs containing living embryos are opened and the CAM examined for the presence of characteristic pock lesions: dense, greyish-white

pocks, flat in shape, with sizes varying between 0.5 and 1.5 mm diameters (Duraffour *et al.,* 2011a; OIE, 2014a). Camelpox virus does not cause death in inoculated embryonated chicken eggs. The maximum temperature for the formation of pock lesions is 38.5°C degrees (OIE, 2014a). If the eggs are incubated at 34.5°C, the pocks are flatter and a haemorrhagic centre may develop (Tantawi *et al*., 1974).

2.8.4. Serological tests

A battery of serological tests including haemagglutination, haemagglutination inhibition, neutralization, Indirect ELISA, complement fixation, and fluorescent antibody tests/assays are available for the detection of antibody to CMLV (Balamurugan *et al.*, 2013). All the viruses in the genus *Orthopoxvirus* cross-react serologically; However, within the genus only camelpox virus can cause pox-like lesions in camels. Parapox and camelpox viruses do not cross-react and so infections of camelpox and camel orf can be distinguished serologically (Azwai *et al.*, 1996; Duraffour *et al.,* 2011a; OIE, 2014a). Most of the conventional serological tests are very time- and labour-consuming, which makes them not suitable for primary diagnosis. However, serological tests are a valuable tool for the purposes of screening population freedom from infection, determination of prevalence of infection in those areas where vaccination against camelpox is not practised, surveillance and immune status in individual animals or populations post-vaccination (OIE, 2014a).

2.8.5 Polymerase chain reaction (PCR)

PCR is a highly sensitive technique for quickly amplifying a desired DNA fragment (target DNA) to a detectable level. The advent of PCR is therefore meant that insufficiencies in the quantity of DNA were no longer a limitation in molecular biology research or diagnostic procedures (Viljoen *et al.,* 2005). PCR method has since been adopted for the detection of camelpox virus DNA (Meyer *et al.,* 1994) and it is rapid and sensitive method which can detect even a few copies of viral DNA from the clinical samples (Nagarajan *et al.,* 2011). DNA can be extracted from cell culture samples and clinical material using numerous commercial kits. Recently, a reliable and low-cost two-

step extraction procedure has been developed for isolating CMLV DNA from skin samples (Yousif *et al.,* 2010).

The PCR assays available to identify CMLV are based on the detection of different target genes using different specific oligonucleotides/primers. The PCR techniques targeting A-type inclusion protein (ATIP gene) (Meyer *et al.,* 1994) and Haemagglutinin (HA) gene (Ropp *et al.,* 1995) are generic PCR assay enables the differentiation of OPV species by producing amplicons of different sizes (Duraffour *et al.,* 2011a; OIE, 2014a; Balamurugan *et al.,* 2013). The ATIP gene-based PCR for example produces a PCR product size of 881 bp, which is specific for the camelpox virus (Meyer *et al.,* 1994). An extra step consisting of a *Bgl*II or *Xba*I restriction digestion allows the unequivocal identification of the virus species (Meyer *et al.,* 1994; Meyer *et al.,* 1997). The HA-PCR amplicon *Taq*I restriction fragment length polymorphism (RFLP) permits to differentiate between OPV species, but species-specific primers within the HA open reading frame (ORF) of OPVs have also been described (Ropp *et al*., 1995). Differentitation of camelpox from other orthopoxvirus (OPXV) and parapoxvirus infections by the use of restriction enzyme analysis (REA) needs virus isolation involving tedious and laborious cell culture methods (Venkatesan *et al.,* 2012a). Recently, a PCR assay targeting the encoding ankyrin repeat protein (C18L gene) has been developed, to specifically identify CMLV and to differentiate it from other OPVs, capripoxviruses and parapoxviruses (Balamurugan *et al.,* 2009). Further, a duplex PCR based on the C18L and DNA *polymerase* (DNA *pol*) genes for specific and rapid detection and differentiation of CMLV from BPXV has also been developed (Singh *et al.,* 2008; Balamurugan *et al.,* 2009). These assays have the advantage of avoiding an extra step of restriction analysis (Duraffour *et al.,* 2011a).

As an improvement over conventional PCR approaches, the real-time PCR techniques targeting A36R gene using fluorescence resonance energy transfer (FRET) method (Pfeffer *et al.,* 1996) have been developed. Similarly, the real-time PCR targeting A13L, rpo18 and viral early transcription factor (VETF) genes (Nitsche *et al.,* 2004) using melting curve analysis have been in use for rapid, highly sensitive and specific detection and quantitation of CMLV and other related OPVs. Recently, C18L gene based real-time

PCR based on either SYBR green chemistry (Balamurugan *et al.,* 2009) or *Taq*Man hydrolysis probe (Venkatesan *et al.,* 2012b) have also been optimized for specific detection of CMLV in clinical samples (Balamurugan *et al.,* 2013)

2.5.6. Loop mediated isothermal amplification (LAMP) assay

LAMP is a rapid, accurate, simple and cost-effective novel nucleic acid amplification method under isothermal conditions (60–65^{o}C), with great potential application in developing countries for diagnosis without requiring sophisticated equipments and skilled personnel (Notomi *et al.,* 2000). The LAMP since its first report by Notomi and his coworkers, it has been used widely for the diagnosis of various diseases (for review see Dhama *et al.,* 2014).

LAMP assay based on the highly conserved region of ankyrin repeat protein gene (*C18L*), which is specific only for CMLV (Balamurugan *et al.,* 2009), has been developed for the diagnosis of CMLV and evaluated using field clinical samples (Venkatesan *et al.,* 2012a). The amplicon size of the LAMP product is 198 bp. The amplified LAMP product can identified by agarose gel electrophoresis and subsequent direct visualization under UV light or observation by naked eye for the presence of turbidity and color change following the addition of SYBR Green I dye and hydroxy naphthol blue (HNB) (Venkatesan *et al.,* 2012a). This assay appears to be potential as rapid and sensitive diagnostic tool for its application in less equipped rural diagnostics laboratory settings in developing countries.

2.9. Differential Diagnosis

Pox and pox-like diseases of camels are a group of exanthematous skin conditions that have become increasingly important economically (Nagarajan *et al.,* 2011). They may be caused by three distinct viruses: *Orthopoxvirus cameli* (camelpox), papillomavirus (camel papillomatosis) and parapoxvirus (camel contagious ecthyma) (Munz, 1992). Clinical signs of camelpox are often indistinguishable from lesions caused by parapox or papilloma viruses (Munz, 1992). Simultaneous infections with both ortho- and parapox

viruses have been described (Renner-Muller *et al.,* 1995; Pfeffer, 1996; Megersa, 2010; Azwai *et al.,* 1996). Infection with papillomavirus can also lead to localized pox-like lesions and may be misdiagnosed as a pox disease (Munz *et al.,* 1990; Nagarajan *et al.,* 2011). Definitive diagnosis of camelpox must, therefore, be based on laboratory-based diagnostic tests (Azwal *et al.,* 1996).

2.10. Treatment

The use of antiviral drugs may be of choice particularly in young camels less than 6 years old as an alternative treatment (Duraffour *et al.,* 2011a). There are potent antiviral molecules active *in vitro* and *in vivo* against poxviruses, including OPVs and could be envisaged for the treatment of camelpox (Snoeck *et al.,* 2007; Smee, 2008). They include molecules belonging to the acyclic nucleoside phosphonate (ANP) family, i.e., cidofovir and its lipid derivative CMX001 (De Clercq *et al.,* 1987, Kern *et al.,* 2002) and the compound ST-246 (Yang *et al.,* 2005). Cidofovir and CMX001 are active against a broad range of DNA viruses including poxviruses. Both compounds target the viral DNA polymerase of OPVs and inhibit its functions (Andrei *et al.,* 2006). ST-246 is a potent inhibitor only of orthopoxviruses. It targets the protein F13L of VACV, which is required for the wrapping of intracellular mature viruses and the production of extracellular enveloped viruses (Yang *et al.,* 2005; Duraffour *et al.,* 2008). Numerous studies have also shown that ST-246 administered for 10–14 days at a dose of 100 mg/kg once per day protects OPV-infected animals from disease development (Duraffour *et al.,* 2011a). In the case of CMLV, the activities of the molecules (Cidofovir, CMX001 and ST-246) have only been evaluated *in vitro* and are potent inhibitors of CMLV replication. In mouse models of camelpox infection, cidofovir either formulated as cream or for systemic use protected animals from disease development and/or death. Nevertheless, CMX001 and ST-246 offer the advantage of being orally available which may render them more attractive for veterinary use (Duraffour *et al.,* 2011a).

Application of a combination of long acting Oxytetracyclin (10mg/kg. b. wt.) twice a week at 3 day's interval and antihistaminic (1 ml/30 kg. b. wt.) at 24 hours of interval,

may protect diseased animals against secondary bacterial infection. A local antiseptic solution (Betadine) can also be used as wound dressing (Mahmoud *et al.*, 2012)**.**

2.11. Prevention and Control

The prevention and control of sporadic cases of camelpox infection in camel husbandry is of prime importance in developing countries (Balamurugan *et al.,* 2013). In this context, camelpox vaccines have been generated from different countries. To date, protection against camelpox can be achieved using CMLV-based vaccines, but camels under the age of 6 months showed poor immune responses, possibly due to the immaturity of the immune system (Khalafalla and El Dirdiri, 2003). Access to antivirals might also be beneficial for treating affected young animals**.**

Currently two types of vaccines, live attenuated and inactivated camelpox vaccines, are commercially available (OIE, 2014a). Live attenuated vaccines provide protection for at least 6 years, probably longer (Wernery and Zachariah, 1999). However, a booster vaccination is recommended for young animals at the age of 8–12 months, 2–3 months after the initial vaccination to avoid interference by maternal antibodies (OIE, 2014a). Inactivated vaccine reported to provide 1 year of protection (Elliot and Tuppurainen, 2010). The inactivated vaccine gives good protection against camelpox after a double injection administered at a 3 to 6 month interval followed by the annual booster. The vaccine is recommended from the age of 8–12 months to avoid interference by maternal antibodies (OIE, 2014a). Limiting herd contacts, care for sick animal and treatment of other parasitic diseases that may reduce the defence mechanism of animals might also help in the prevention of the disease (Megersa, 2010).

2.12. Public Health Importance

Camelpox is important because of its potential zoonotic problems. Camelpox virus is difficult to distinguish from smallpox virus and it occurs in areas where smallpox vaccination has now ceased (Azwai *et al.,* 1996). Although rare, pox-like lesions

associated with camelpox have been described in man (Jezek *et al.,* 1983); and confirmed serologically (Kriz, 1982); it has also been suggested that camelpox virus could be as pathogenic for man as cow pox and monkey pox (Marrenikova *et al.,* 1974). Among the human cases, people drinking milk from camelpox-affected animals have been reported to develop ulcers on the lips and in the mouth, but these observations could not be visualised or laboratory confirmed (Davies *et al.,* 1975).

From the 1970s until recently, it has been well accepted that CMLV rarely infects humans. The immunological status of the individuals (i.e., previous smallpox vaccination or history of smallpox) might have been a bias for estimating the possible human cases of camelpox (Duraffour *et al.,* 2011a); because cross-immunity between VACV, VARV and CMLV has already reported (Baxby *et al.,* 1975; Falluji *et al.,* 1979). Baxby *et al.,* 1975 and Azwai *et al.,* 1996, predicted the occurrence of camelpox as the smallpox immunity of the human population wanes. Consequently, recently the first conclusive evidence of zoonotic CMLV infection in humans (unvaccinated smallpox individuals) associated with outbreaks in dromedarian camels in India has been reported and it is considered as emerging zoonotic infection (Bera *et al*., 2011). The lesions were confined to the hands and fingers of camel handlers, and passed through all the stages of pock lesions until the formation of scabs. Serum samples of the three suspected cases showed neutralizing antibodies against CMLV and, of importance, none of these patients had ever been vaccinated against smallpox. In addition, in one of the three human cases, viral DNA could be detected by conventional PCR for CMLV-specific genes (Bera *et al*., 2011).

In the future it is possible that human infections with camelpox could be increased as the smallpox immunity of the human population wanes; this would only be detected by long-term serological monitoring (Azwai *et al.,* 1996). If camelpox were to infect man, it would probably, as with other pox virus infections, be most important in immunosuppressed individuals. Further epidemiological and immunological studies of camelpox on these endemic regions are necessary to assess the circulation of CMLV, both in camels and humans in order to know its public health significance (Duraffour *et al.,* 2011a) as well as to determine the possible transmissibility of camelpox to man (Azwai *et al.,* 1996).

3. MATERIAL AND METHODS

3.1. Description of the Study Area

The present study was conducted in two selected districts of Gabi Rasu zone of Afar National Regional State, which is situated in the North Eastern part of Ethiopia. Afar region is located in the Great Rift Valley, comprising a total geographical area of 270,000 km^2 (CSA, 2008). It is geographically located between $39^o34'$ and $42^o28'$ East Longitude and $8^o49'$and$14^o30'$North Latitude. The region has a great potential of livestock resources comprising of 2.3 million cattle, 4.3 million goats, 2.5 million sheep, 0.8 million camels and 0.19 million equines that support the region and contributes to the national economy (CSA, 2010). Administratively, Afar region consists of 5 administrative zones, 32 woredas (districts) and 401 kebelles or pastoral associations.

Gabi Rasu (formerly known as zone three) is one among the 5 administrative zones, located in the southern part of the region (Fig. 2). The zone consists of six districts, predominantly occupied by pastoral and agro-pastoral communities. The two selected districts of the zone, namely Amibara and Awash Fentale, are located in the dry lowlands of the rift valley, at about 230 and 280 km northeast, respectively from the capital Addis Ababa. Gabi Rasu zone is characterized by arid and semi arid agro-climatic condition with ranging annual rainfall of 200 to 700 mm. Specifically, a long term average annual rainfall of 550 mm was reported for Awash Fentale by Abule *et al.* (2007), while 560 and 578 mm were reported for Amibara by Kidane (2005) and Kidanie (2010), respectively. The mean annual minimum and maximum temperature at Awash Fentale is 17.4 and 32.7°C (Abule *et al.,* 2007), respectively, while the temperature is 19.5 and 34.4°C, respectively at Amibara (Kidanie, 2010). The area has two (a bimodal) rainy seasons with the main rainy season occurring from July to September and a short rainy season occurring from February to April (Abule *et al.*, 2007). Land is generally flat and fertile with altitude ranges from 500 to 1500 metres above sea level. The predominant vegetation includes Acacia species, Mesquite (*Prosopis juliflora),* different bushes and other thorny shrubs (Kidane, 2005; Kidanie, 2010). Some of the common important tree

species in the area are *Acacia senegal, Acacia nilotica, Acacia melifera, Acacia nubica and Balenitus spp.* Nowadays, *Prosopis juliflora* an exotic invasive tree species, is dominating the grazing and irrigated land of the zone.

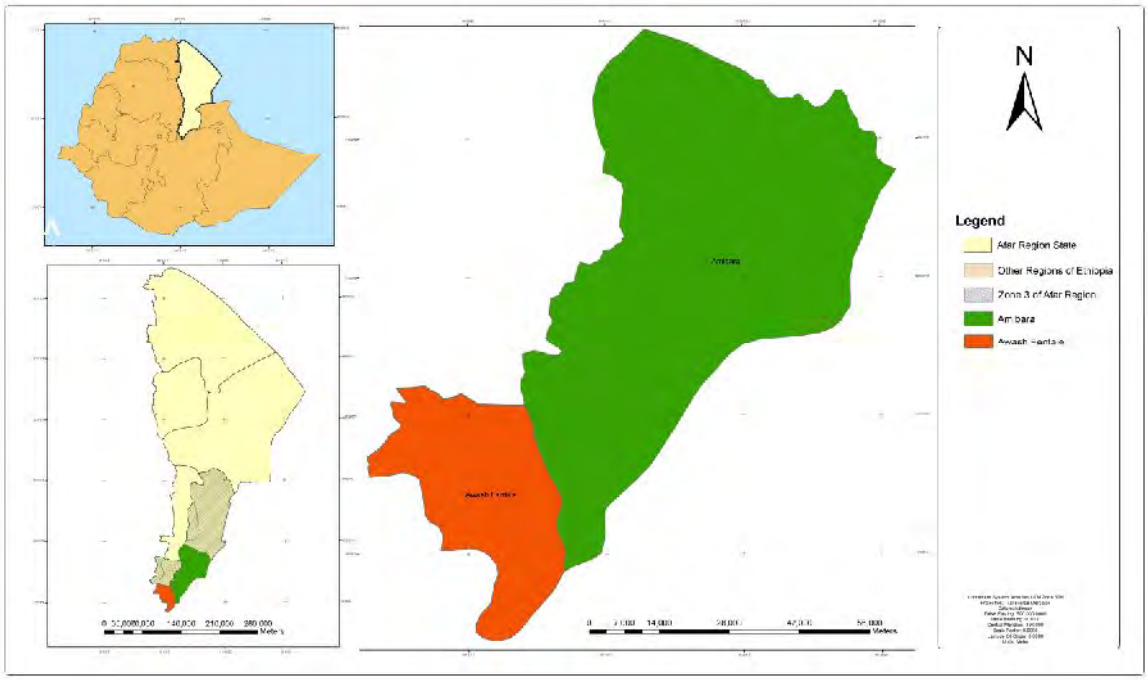

Figure 2: Geographical location of Gabi Rasu zone and selected study districts

3.2. Study Design

The study was carried out from November 2014 to May 2015 using a cross sectional observational study design (Dohoo *et al.*, 2003; Thrusfield, 2005) and the before-and-after with control experimental study design (Kothari, 2004) for the sero-epidemiological study of camelpox disease and field evaluation of camelpox vaccine, respectively. In addition, a participatory epidemiological study method was used to identify the seasonal occurrence of camelpox in the area (Catley *et al.*, 2012).

3.3. Sampling Strategies and Study Animals

3.3.1. Seroprevalence study

Both probabilistic and non probabilistic sampling approaches were used at different stages of the sampling units, because strict random sampling procedure is difficult, if not impossible in pastoral production system where the study population are scattered over a wide and remote areas. Hence, among the six districts of Gabi Rasu zone, two representative districts, namely Amibara and Awash Fentale, were selected purposively (Pfeiffer, 2002; Thrusfield, 2005) based on their camel production potential and accessibility to vehicles. However, simple random sampling method (Pfeiffer, 2002; Dohoo *et al.,* 2003; Thrusfield, 2005) was used to select pastoral associations (PAs), commonly called Kebelle from the list provided by the Districts Pastoral Agriculture and Rural Development Office. PAs with low camel production potential were excluded from the list during random sampling and a total of three PAs from each district were selected. Village selection from the randomly selected PAs was purposive, according to the camel production potential, willingness of camel owners to cooperate and physical accessibility of the villages during the study period.

A total of 30 herd (15 herd from each district) were selected by systematic random sampling method (Pfeiffer, 2002; Dohoo, 2003; Thrusfield, 2005) by starting from one herd that show cooperativeness and proceeded for the rest by jumping one to three herds based on the number of available herds in that particular village until the number of camels required from the village was met. When owners of target herd refused their camels to be bled, the adjacent herd was replaced. A proportion of camels in the selected herd older than 8 months of age were selected using simple random sampling depending on the population of camels within the herd. Commonly, 30 percent of the herd population was sampled for the primary cross sectional seroprevalence study of camelpox.

The total study animals were determined according to the formula set by Thrusfield, 2005 for simple and systematic random sampling, using 95% confidence level and 5% absolute precision. Hence the relevant formula for a 95% confidence interval is:

$$N = 1.96^2 \times (P_{exp})\ (1 - P_{exp})/D^2$$

Where N is required sample size, P_{exp} is expected prevalence and D is level of absolute precision (5%).

Due to the absence of reports on estimates of the prevalence of antibodies against camelpox in the country, an arbitrary estimate of 50% was chosen to give the maximum sample size. As a result, a total of 384 camels (*C. dromedarius*) were included for the determination of antibody prevalence against camelpox. The study animals included camels of different ages and of both sexes reared under extensive husbandry management system. Information of each sampled camel including sex, age, herd size, and health status was recorded using structured data sheet format. The age of selected camels were determined based on the information obtained from the owners and were categorised in to young (8 month to 4 years) and adult (> 4 years old) considering the maturity age of camels (Wilson, 1998). Camel herd sizes were categorized as ≤ 25 camels (small herd size), > 25 and ≤ 50 camels (medium) and > 50 camels (large) considering the herd structure of the study area.

3.3.2. Field evaluation of vaccine by seroconversion

Of 30 herds studied in both districts for seroprevalence study (described under subsection 3.3.1), 15 herds and 204 camels selected from Amibara district were included for this study. Amibara district was chosen because of its proximity for close follow up and supervision of experimental animals from vaccination to postvaccination serum collection. In addition, in order to minimize the risk of losing some animals due to migration, death, selling and owners uncooperativeness for the second post vaccination serum collection, additional 100 camels were included from the same district. Thus, a total of 304 camels were selected and their serum was collected. Of these 304 camels, 200 were exposed at the spot to attenuated camelpoxvirus vaccine purchased from NVI

after the serum sample collection. The remaining 104 camels were left unvaccinated (negative controls). Individual sampled camel was identified by a code (number) written on the neck using permanent marker. In addition, identification methods including name of the camel, type and location of brand (if any), number of birth and physiological status of each sampled camel were recorded. Post-vaccination serum sample were collected 21–30 days after vaccination (OIE, 2014a) from both vaccinated and negative control groups. A total of 190 post-vaccination serum samples were recovered from vaccinated (110) and negative control (80) groups.

Initially, the intention was to analyse 150 pre and post vaccination serum sample (100 from vaccination and 50 from control groups); However due to logistic constraints to analyse all of the collected sera samples, only 70 from vaccination and 30 from control postvacciation serum samples were analysed**.**

3.3.2.1. Camelpox vaccine and vaccination protocol

A live attenuated vaccine commercially produced by NVI was purchased for the experiment. The vaccine was presented as bulk pack of 100 doses per freeze dried vial, supplied with saline water for reconstitution. The vial content was dissolved in the sterile physiological saline water and the study experimental animals were vaccinated once after initial serum sampling. The dose and route of vaccination were 1ml per animal and subcutaneously around the neck, respectively, according to the recommendations of the manufacturer. Dromedaries were observed every three days for any signs of the disease until post vaccination serum sample were collected. The efficacy of the live attenuated vaccine was later evaluated by measuring the antibody levels against camelpox virus 21–30 days after the last vaccination using virus neutralization test.

3.4. Data Collection

3.4.1. Serum sample collection

After physical restraining of each selected camel, blood was withdrawn from jugular vein in to plain vacutainer tubes. Each sample was labeled using codes specific to the individual animal and information about sampled animal were recorded on the prepared data sheet. The blood tubes were allowed to stand overnight at room temperature, after which the blood is centrifuged at 3000 rpm for 15 min to collect serum samples. Separated serum samples were harvested and held at 4°C until submitted to virology laboratories and preserved at -20°C until they used for detection of camelpox antibodies.

3.4.2. Laboratory examination procedures

Serum samples collected from camels were analysed by virus neutralization test, a recommended method by the Office Internationale des Epizootics (OIE, 2014a), for detecting camelpox virus specific antibodies by *in vitro* neutralisation of the viral cytopathic effect on cell culture.

This method is based on a reaction between the virus and specific antibody in the test serum. The serum samples (in duplicate rows) were diluted in serum-free cell culture medium (25 µl volumes) in a two-fold dilution series from 1:2 to 1:32 in a flat-bottomed 96-well tissue culture plate. The camelpox virus suspension with a titer of 100TCID50 in 25 µl was added to each serum well and the mixture incubated for 1 h at 37^{O}C in a humidified CO_2 incubator. A 100 µl aliquot of Vero cell suspension was added to each well and the plates incubated. Serum (positive and negative control serum), virus and cell controls were included in the test. The plates were observed daily for CPE in the infected cells for 7 days. The presence of unneutralized virus was detected by plaque formation/cytopathic effect, indicating a negative result. On the other, positive sera resulted in loss of infectivity of cells caused by interference by the bound antibody with any of the steps leading to the release of the viral genome from the host cells including attachment, infection, or viral release. The test method was conducted at NVI and National Animal Health Diagnostic and Investigation Center (NAHDIC) and details of

the test principle, materials and reagents used, procedure and interpretation of the results are described at Appendix I.

3.4.3. Questionnaire and participatory epidemiological (PE) survey

This part of the study was conducted in two phases in both districts. The first phase was questionnaire to get baseline information about the common camel disease in the study area. It was conducted on 30 respondents from both districts. In this phase the respondents listed major camel diseases by their local names, their clinical symptoms and ranked them based on the frequent occurrence of each listed disease using open-ended question (SSI). The top 5 ranked diseases were selected to be studied in detail using participatory epidemiology (phase 2 below). Camelpox was one of the 5 top ranked priority diseases to be scored during the subsequent participatory epidemiological studies.

The second phase was the actual participatory epidemiology of camelpox study using 12 independent groups whereby each group composed of 5-10 respondents. Explicitly, 6 groups were interviewed per district and informants included those people whose camels were being sampled. Other people present nearby with good camel herding experience and rich indigenous knowledge related to camel diseases and health care were also invited to join the discussion by the research team. The investigation was carried using selected tools including seasonal calendars and semi-structured interviews (SSI) according to the objectives and context of the study (Catley *et al.*, 2012; FAO, 2000). All PAs and villages selected for serum sample collection were included in this study. The method was practiced (pre-tested) on some animal health workers and camel owners before using it for the actual field work in order to make sure that the method was understood and the questions were clear. The survey team composed of three interviewers comprising of the team leader (researcher), a community mobiliser and a translator. The community mobiliser made prior arrangements and preparations with the pastoralists in each village and ensured time and place for the interview.

3.4.3.1. Seasonal calendar

Seasonal calendars, a time-related data source, were used to describe the seasonal occurrence of the five important camel diseases selected using simple ranking (ILRI, 2009). To construct a seasonal calendar, the four Afar seasons by their local names (Appendix II) were represented by objects: a stick with green leaves represented *'Kerma'* (July-August), followed by green stick without leaf *'Gilal'* (September-February), stick with few green leaves *'Sugum'* (March-Apr) and a dry stick with out leaves *'Hagay'* (May-June) on the X-axis. Pieces of papers with pictures and local names of the diseases printed on them placed along the Y-axis. These were placed on the flip chart and explained to the informant group after they were arranged to sit in convenient places. The informants were then requested to explain the meaning of each symbol to know whether they have understood what it represented. The informants were then given 30 stones and asked to show the relative occurrence of each disease in each season. When placing of the stones for one disease against the season was complete, the group was requested to thoroughly check the scores and if they wanted, rearrange the scores until they were contented with the result. The seasons, diseases and number of stones were kept constant across all informant groups to make the technique more reproducible.

3.4.3.2. Semi-structured interviews (SSI)

Following scoring of the seasonal calendar, the results were discussed with the participants using open and probing questions through the use of SSIs. The informant groups were specifically probed more on the disease of interest (camelpox) with regard to the seasonal occurrence, impact, age group affected and predisposing factors.

3.5. Data Analysis

Field and laboratory data were coded, entered and stored in Microsoft Excel spreadsheet MS office (2007) programme to create a database and simple descriptive statistics such as percentage and proportions were applied to compute some of the data. The data were then imported to STATA 11 (StataCorp., 2009).

The seroprevalence for animal level was calculated by dividing the number of camelpox antibody positive individuals to the total number of tested animals multiplied by 100. Similarly, herd level prevalence was computed as the number of herds with at least one positive animal divided by the total number of herds tested. The 95% CI for proportions were calculated using the formula given by Petrie and Watson (2006).

Univariable logistic regression analysis was employed to determine the associations of hypothetical risk factors with the seropositive camels. Odd ratio (OR) was used to point out the degree of risk factors association with the disease occurrence indicated by 95% confidence intervals. Risk factors having $p < 0.2$ value on univariable analysis was subjected to multivariable analysis using logistic regression. *Goodness-of-fit* of the model was also tested using backward elimination, by taking away the variables sequentially; starting from the variable which contributes the least until deletion of a variable significantly reduces the amount of the explained variable on dependent variable (Petrie and Watson, 2006). The full and the other reduced (simpler) models were compared by the computer estimates of their likelihood ratio statistic (LRS) or -2log likelihood using chi-square test for significance.

Post vaccination seroconversion rate between vaccinated and unvaccinated control groups were analysed by Chi-square test for statistical differences.

A significance level ($P < 0.05$) and confidence level (95%) was set to determine the presence or absence of statistically significant difference between the given parameters. Differences were considered significant and highly significant when P-values were less than 0.05 and 0.01, respectively, using logistic regression and Chi-square test.

Data collected by seasonal calendar was analysed using statistical package for social sciences software version 20.0 (SPSS, v. 20.0). Scores were summarised using median scores, minimum and maximum scores and 95% confidence intervals. The Kendall's coefficient of concordance (W) was used to assess agreement between informant groups.

4. RESULTS

4.1. Seroprevalence and Distribution of Camelpox

Camelpox antibodies were detected in 74 camels among 384 examined gving an overall seroprevalence of 19.3% (95% CI= 15.4-23.3). According to study districts, the prevalence rate of the disease was 21.6 and 16.7% at Amibara and Awash Fentale, respectively (Table 2).

Table 2: Serological prevalence of camelpox in selected districts of Gabi Rasu zone

District	No. Examined	No. of positive	Prevalence (95% CI)
Amibara	204	44	21.6 (16.0-27.2)
Awash Fentale	180	30	16.7 (11.2-22.1)
Over all	384	74	19.3 (15.3-23.2)

All the six villages included in the study were found to have at least one herd that was seropositive to camelpox and had a range between 14 and 24% seroprevalence rate. Camelpox antibodies detected in 25 herds among 30 examined and the overall herd level seroprevalence rate was 83.3% (95% CI = 70.0-96.6). Animal and herd level camelpox antibodies distributions in the 6 villages are shown in (Appendix III).

4.2. Camelpox Seroprevalence and Assumed Risk Factors

The associations of the hypothetical risk factors such as age groups, sex, herd size and study districts with the seropositive camels are shown in Table 3. The results indicated that camelpox seroprevalence was significantly higher in adult (24.4 %) than young age group (13.9 %) with a 2 fold greater odds of occurrence. With regard to sex, although the serological prevalence was higher in female camels (20.6%) than males (10.2%), the difference was not statistically significant ($p>0.05$).

Comparison of three herd size categories showed similarity of seropositivity ($p > 0.05$). The results highlighted highest prevalence in large herds possessing more than 50 camels

(21.7%), followed by 19.4 and 14.1% in small and medium herds possessing less than 25 and 26 to 50 camels, respectively. District wise analysis revealed higher infection rate in Amibara compared to the other district. However, the difference was not statistically significant (P>0.05) (Table 3).

Table 3: Risk factors for camelpox positivity by univariable logistic regression

Risk Factors	**Group Category**	**No. examined**	**No. positive**	**% Positives (95% CI)**	**P-value**	**OR (95% CI)**
[a]Age group	Young	187	26	13.9 (8.9-18.8)	0.01*	2.0 (1.2-3.4)
	Adult	197	48	24.4 (18.4-30.4)		
Sex	Male	49	5	10.2 (1.7-18.7)	0.08	2.3 (0.9-5.9)
	Female	335	69	20.6 (16.3-24.9)		
[b]Herd size	Small	103	20	19.4 (11.8-27.0)	0.52	1.1 (0.8-1.5)
	Medium	106	15	14.1 (7.5-20.7)		
	Large	175	38	21.7 (15.6-27.8)		
Study district	Amibara	204	44	21.6 (16.0-27.2)	0.2	0.7 (0.4-1.2)
	Awash F.	180	30	16.7 (11.2-22.1)		

* Significant at 95% level of significance

[a]Age group : Young = 8months to 4 years; Adult= above 4 years old

[b]Herd size: Small= ≤ 25 camels; Medium= >25 and ≤ 50; Large= > 50 camels

Risk factors such as age group, sex and study districts were selected from the univariable analysis for multivariable logistic regression analysis based on their p-value (≤ 0.2) in order to identify the major risk factors.

Multivariable logistic regression analysis of selected risk factors determined age group was the only marginally explanatory variable that significantly associated with the occurrence of seropositivity to camelpox. The odds ratio of camelpox seroprevalence,

comparing adult to young age group was 1.8, after the effect of sex and study district were removed (Table 4).

Table 4: Risk factors for camelpox positivity by multivariable logistic regression analysis

VNT	Odds Ratio	Std. Err.	z	P-value	[95% Conf. Interval]
Age	1.8	0.5	2.00	0.045	1.0-3.1
Sex	1.7	0.9	1.04	0.299	0.6- 4.6
District	0.9	0.2	-0.56	0.579	0.5-1.5

Log likelihood = -183.99155; LR chi2 (3) = 8.43; Prob > chi2 = 0.0378

In order to identify the best fit model which have the major risk factor/s, backward elimination was conducted by taking away the variables sequentially, starting from study district (variable which contributes the least) and then sex because of its insignificant contribution after adjusting the effect of age (Data not shown). Consequently, no significant differences ($P > 0.05$) were recorded between the saturated and reduced models at different levels. Thus, the model having only age as explanatory variable was selected as the best fit, which implies age was the only risk factor for the occurrence of camelpox seropositivty among the hypothetical risk factors screened in this study. From the final best model (Table 5), there was a two fold increase in seropositivty of camelpox if the camel was adult than being young.

Table 5: The final best fit model for camelpox positivity using age as a risk factor

VNT	Odds Ratio	Std. Err.	z	P-value	[95% Conf. Interval]
Age	2.0	0.5	2.6	0.01	1.2-3.4

Log likelihood = -184.78619; LR chi2 (1) = 6.84; Prob > chi2 = 0.0089

The major risk factor, age group, was later stratified in to four groups: calf, (8 month to ≤ 1 years) young (>1 years and ≤4 years), adult (>4 years and ≤10) and old (>10 years), considering the age structure of the study herds, and analysed for the degree of significance among groups. Hence, a significantly highest ($P < 0.05$) seroprevalence of camelpox was recorded in old age group (31.6%), followed by 17.2 , 14.7 and 11.8% in adult, young and calf age groups, respectively (Table 6). Age was considered as linear

trend in the model and progression in age from one category to the next resulted in increasing seropositivity on average by odds of 1.6 (95% CI= 1.2-2.0).

Table 6: Seroprevalence of camelpox according to four categorised age groups

Risk Factor	Category	No. examined	No. positive	% Positives (95% CI)	P-value	OR(95% CI)
Age* group	Calf	51	6	11.8 (2.9-20.6)	0.001*	1.6 (1.2-2.0)
	Young	136	20	14.7 (8.7-20.6)		
	Adult	99	17	17.2 (9.6-24.4)		
	Old	98	31	31.6 (22.4-40.8)		

[a]Age groups: Calf= 8 month to 1 year, Young= above 1 year to 4 years, Adult= above 4 years to 10 years and old= above 10 years old

To classify which specific age group was significantly associated with seropositivty of camelpox, separate comparsion of each age groups were carried out using univariable logistic regression by assuming age as a categorical variable as shown on table 7. This was to identify the specific age group/s significantly associated with seroconversion of camelpox which might be helpful to target specific age group/s for vaccination.

Table 7: Separate comparsion of four age groups for significanct association with camelpox seropositivity

Age groups[a]	Odds Ratio	95% C.I.	Z-Statistic	P-Value
2 vs 1	1.3	0.5 - 3.4	0.5	0.6
3 vs 1	1.5	0.6 - 4.2	0.7	0.4
4 vs 1	3.5	1.3 - 9.0	2.6	0.01*
3 vs 2	1.2	0.6 - 2.4	0.5	0.6
4 vs 2	2.7	1.4 - 5.1	3.0	0.002*
4 vs 3	2.2	1.1 - 4.4	2.3	0.02*

[a]Age groups: 1= Calf; 2 = Young; 3 = Adult and 4 = Old

The result indicated that camels under old age category were significantly ($p < 0.05$) associated with camelpox seroprevalence comparing to calf, young and adult age groups.

The odds of occurrence of the disease in old camels were 3.5, 2.7 and 2.2 comparing to calf, young and adult camels, respectively. However, there was no significant difference among calf, young and adult age groups on the occurrence of seropositivity.

4.3. Field Evaluation of Vaccine

4.3.1. Post-vaccinal reaction

Field based experimental camels were monitored for any post-vaccination reaction. Neither the camels vaccinated nor the contact negative control camels showed any local lesion at the site of inoculation. Clinically, throughout the experiment, experimental animals appeared healthy and behaved normally, without any clinical signs.

4.3.2. Pre- and post-vaccination antibody prevalence

Antibody prevalence of experimental camels prior to inoculation with camelpox vaccine was determined by VNT and the result indicated that twelve camels from vaccinated and five from control groups contained neutralising antibodies against camelpox (Table 8).

Production of neutralising antibodies against CMLV was also detected in the vaccinated and unvaccinated camels. Out of 70 vaccinated camels bled for post-vaccination seroconversion assessment starting from the 21st post vaccination day, 42 (60%) camels were seropositive to camelpox by VNT (Table 9 and Fig. 3). Of these, as mentioned above, 12 (17.1%) were previously seroconverted camels and later on postvaccination serum analysis, two of them found seronegative albeit a positive result was anticipated (Table 9). Generally, 32 (55.2%) among the 58 pre-vaccination seronegative camels were found to be seropositive at post-vaccination serum analysis while the remaining 26 (44.8%) camels fails to give a seroconversion result (Table 8). On the other hand, no post-vaccination neutralising antibodies were detected from 30 unvaccinated (control) camels except on three among the five camels which had a pre-vaccination antibody against camelpox (Table 8).

Table 8: Cross tabulation of pre- and post-vaccination seroconverted camels from vaccinated and control group

		Post-vaccination		
	Results	Positive	Negative	Total
Vaccinated camels				
Pre-vaccination	Positive	10	2	12
	Negative	32	26	58
	Total	42	28	70
Control camels				
	Results	Positive	Negative	Total
Pre-vaccination	Positive	3	2	5
	Negative	0	25	25
	Total	3	27	30

The post-vaccination antibody prevalence of the vaccination group was compared with the unvaccinated control. A statistically highly significant ($P < 0.01$) seroconversion rate was observed on vaccinated camels. However, the proportion of pre-vaccination seroconverted camels between the two groups (17.1% and 16.6%) was not significant as expected (Table 9).

Table 9: Comparative evaluation of antibody prevalence of camelpox before and after vaccination in vaccination and control groups

Group Category	**N**	**Pre-vaccination**				**Post-vaccination**			
		No. positive	Prevalence (%)	Chi2	P-value	No. positive	Prevalence (%)	Chi2	P-value
Vaccination	70	12	17.1	0.003	0.95	42	60	21.2	0.000*
Control	30	5	16.7			3	10		

*Highly Significant at 95% level of significance

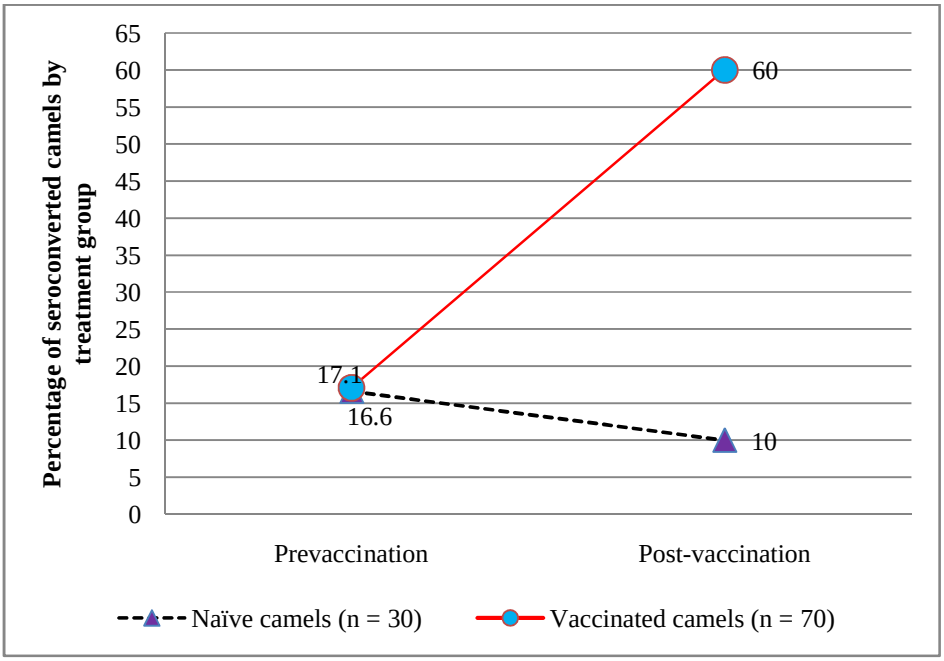

Figure 3: Seroconversion rate of camelpox before and after vaccination in vaccination and control groups

4.4. Questionnaire and Participatory Epidemiological (PE) Survey

During the initial questionnaire survey, various camel diseases with their symptoms were mentioned by the pastoralists based on their common occurrences (Appendix IV). Among which five, including camelpox (Fig. 4) were selected by simple ranking method (ILRI, 2009) for the seasonal calendar study. In addition to these, occurrence of camel tick infestation was included intentionally for the seasonal calendar as reference control.

Results of the 12 seasonal calendars are summarized in Figure 4. Agreement between informant groups was quantified using the Kendal coefficient of concordance and were categorised according to critical values for W as weak, moderate and good if W-values were less than 0.26 ($p>0.05$), between 0.26 and 0.38 ($p<0.05$) and greater than 0.38 ($p<0.01$ to <0.001), respectively. This was by assuming ranking of four objects (seasons) by 12 judges (groups of informants) (Seigel and Castellan, 1994, cited from Catley *et al*, 2002).

Good agreement was evident among the 12 informant groups concerning seasonal patterns of the selected camel diseases except for *galideli* (abscessation) which scores weak agreement (W= 0.171). Camelpox (*galiguduf/ambraruk*) incidence peaked during minor *(Sugum)* and major (*Kerma)* rainy seasons with a median score of 16(8-28) and 13.5 (2-22), respectively (Fig. 4).

According to the explanation of informant groups, the high incidence of camelpox during the rainy seasons was due to the returning back of camels from where they had migrated and congregation in small areas that favour transmission of the disease. Increasing humidity (hot temperature and rain) during *sugum* (minor rain) season was also explained as a predisposing factor and the reason for the highest occurrence of the disease in the season. The disease was reported mainly to affect young camels that allowed grazing during the rainy seasons. Increasing frequency of contact with other camels and grazing on thorny plant species and bushes which are abundant during rainy seasons were mentioned as predisposing factors of the disease in young camels.

On the hand, occurrences of the other selected camel diseases including respiratory diseases, mange mites, trypanosomosis and ticks were associated with the long and short dry season of the year, *Gilal* (September-February) and *Hagay* (May-June), respectively (Fig. 4). This was mainly due to shortage of feed and starvation during these seasons.

Diseases	**Afar Seasons**			
	***Kerma* (July-August)**	***Gilal* (Septem-February)**	***Sugum* (March-April)**	***Hagay* (May-June)**
Galideli Abcession (W=0.171 ^{ns})	10.5(2-19)	10(5-24)	4(2-9)	2(0-15)
Galiguduf /Ambraruk Camelpox (W=0.671***)	13.5(2-22)	0(0-2)	16(8-28)	0(0-3)
Kahu / Bahu Respiratory disease (W=0.492**)	0.5(0-6)	25(18-30)	0.5(0-6)	2(0-15)
Agara Mange (W=0.588***)	0(0-4)	26(15-30)	0(0-4)	2.5(0-15)
Dahan Trypanosomosis (W=0.512**)	0(0-8)	5(0-22)	0(0-4)	24(8-30)
Kilim Ticks (W=0.594***)	1(0-4)	12.5(5-20)	1.5(0-4)	15(6-23)

Figure 4: Summarized seasonal calendar on the occurrence of selected camel diseases in Gabi Rasu zone, Afar.

Key: N= 12; W= Kendall's coefficient of concordance ($^{ns}p > 0.05$ (non significant); $^{**}p < 0.01$; $^{***}p < 0.001$). W values vary from 0 to 1.0; the higher the value, the higher the level of agreement between the informant groups. The black dots represent the median scores (number of stones) that were used during construction of the seasonal calendars. The minimum and maximum limits are shown in parentheses.

5. DISCUSSION

5.1. Seroprevalence of Camelpox Virus

The epidemiology of most camel diseases including camelpox have not extensively investigated. Most of the reports on camelpox are paid attention on the isolation and characterization of the causative agent (Renner-Muller *et al.,* 1995; Pfeffer, 1998; Salem *et al.,* 2008; Yousif and Al-Naeem, 2012; Ayelet *et al.,* 2013). In the present study, sero-epidemiological investigation of the disease has been carried out to determine the extent of poxvirus infection in camels and provides data for prevalence of infection.

In this investigation, out of 384 camels, 30 herds and 6 villages sampled from two districts of Gabi Rasu zone, camelpox antibodies were detected in 74 camels(19.3%), 25 herds (83.3%). and all villages. These findings show that there is a high prevalence of antibody to camelpox in herds examined regardless of their geographical location. Serological evidence of the present study, together with the confirmatory report of the disease by Ayelet and his coworkers, 2013 from Afar region suggests that the disease is enzootic in the area. According to the information collected from the animal health sector of Pastoral Agriculture and Rural Development Office of the districts and herd owners, the camel population in the study area has never been vaccinated against any diseases including camelpox.

The seroprevalence result of this study was relatively higher than other sero-epidemiologic studies of camelpox that reported the presence of neutralizing antibodies in 9.8% of the animals in Libya (Azwai *et al.*, 1996) and 9.14% in Saudi Arabia (Housawi, 2007). In these two studies, it was stressed that camelpox vaccinations were not practiced. In contrast, a prevalence of neutralizing antibodies of 72.5% in Sudan and 100 % in Egypt was measured in an unvaccinated population by Khalafalla *et al.,* 1998 and Mahmoud *et al.,* 2012, respectively. However, the former study was conducted following outbreak occurrence of the diseases and the other study by Mahmoud *et al.,* 2012, was from camels with clinical cases of the disease and from contact apparently healthy camels.

Camelpox prevalence based on clinical cases have also reported from various countries (Table 1) and different parts of Ethiopia by Tefera and Gebreab, 2001; Megersa, 2010 and Ayelet *et al.,* 2013, who reported a 3%, 14.2% and 3.8% prevalence, respectively. It was not unexpected to find higher serological prevalence than clinical prevalence as poxvirus antibodies can be detected in animal sera much more frequently than poxviruses can be isolated from them (Marennikova, 1975**).** Similar findings were also reported for other camel diseases such as trypanosomosis that parasite antibody prevalence was observed by far higher than the prevalence of live parasites (Hagos *et al.,* 2009; Weldegebrial *et al.,* 2015).

Among the risk factors screened for an association with seropositivity of camelpox antibody, age group was found the only significantly associated factor. The study revealed seroprevalence was significantly higher in adults than young dromedaries. This finding could be explained by adult animals consistently get exposure with cumulative increase in neutralizing antibody titer than the young animals. However, camelpox has fatal outcome in young camels, thus antibody detection is less likely. Young aged below 4 years old in a herd experiences severe (generalized) form causing high mortality occasionally due to waning of acquired immunity after 5–8 months (Nothelfer *et al.,* 1995).

Sex wise analysis revealed higher prevalence of camelpox antibody in female (20.6%) than male dromedaries (10.2%). However, the difference was not statistically significant. The role of sex for susceptibility to camelpox is unsettled in the body of science as the findings of others also contradict each other on this aspect (Kriz, 1982; Khalafalla and Mohamed, 1996; Mahmoud, 2012). Moreover, seroconversion was not significantly affected by herd size as well as study districts. These might be attributed to the contagious nature camelpox virus which is not considered as a disease of herd importance. In addition, the pastoral production system familiar with migratory nature of camel herders together with sharing the same communal grazing and watering points as well as close proximity of the two districts (Fig. 2) might be the possible reasons for these findings.

5.2. Field Evaluation of Vaccine

CMLV is considered to solely naturally infect old world camelids. Therefore, camels have been used as animal models of camelpox infection, mainly for evaluating the efficacy of camelpox vaccines **(**Duraffour *et al.,* 2011a). For that reason, evaluation of camelpox vaccine produced by NVI was conducted on dromedaries under field condition through seroconversion. Camels inoculated with the vaccine remained apparently healthy without any adverse local reactions and signs of illness, indicating that the vaccine is safe and that there is an absence of residual virulence.

The pre-vaccination seroprevalence result from vaccinated group was 17.1% prior to vaccination, but an overall rise to 60% seroconversion had been observed after vaccination. Post-vaccination seroconversion rate from vaccinated camels was significantly higher compared to control animals. Potentially, the vaccine tested may be useful as 83.3% of the herds included in this study were positive for camelpox exposure of infection that might develop to clinical disease to cause adverse economic consequences to camel herders and the country.

Nevertheless, the post-vaccination seroconversion rate from vaccinated group with no pre-vaccination antibody history was 55.2%. That means post-vaccination antibody production was still not detected in 44.8% vaccinated camels. Various explanations could be suggested for the failure to detect post-vaccination antibodies from these animals. These can be categorized in to three such as inherent to the vaccine, the animal or the diagnostic method.

Successful vaccination depends on several factors inherent to the vaccine, the immunogenic virus quality employed in its manufacturing, antigen stability, the amount of virus used per dose and the virus profile matters. Thermostability is the other factor to be considered for this failure because thermoliable vaccines have a serious drawback for their efficient use in hot climatic environments where the camel was reared (Abdellatif *et al.,* 2002).

Since the disease seems to be endemic in the present study area as well as in other camel breeding areas, the presence of pre-vaccination undetectable circulating antibodies, which have neutralizing effect on the virus, might also contribute on lowering post-vaccination serconversion rate. Abdellatif and his coworkers, 2014 has observed camels with low levels of neutralising antibodies can be protected when challenged with wild-type virus. Alternatively, the vaccine may have been initiated other components of the immune system, cell-mediated immunity seeing that this type of immunity was detected in camels after vaccination using delayed type of hypersensitivity test by Khalafalla and El-Dirdiri, 2003.

The accuracy of the test method (VNT) should also be considered as an important factor for lowering post-vaccination seroconversion detection of antibody. Eventhough, it is one of the two tests recommended by OIE for such studies and the most commonly used test for diagnosis of orthopoxvirus infections (Khalafalla and El-Dirdiri, 2003; Mahmoud *et al.,* 2012; Abdellatif *et al.,* 2014; OIE, 2014a), undoubtedly questions remain concerning the reliability of the neutralization test for the detection of antibody (Azwai *et al.,* 1996). Surprisingly, two sera samples had inconsistent results in vaccinated group in that they were positive before but negative after vaccination in the present study. This could be due to neutralization of the vaccine by the pre-vaccination antibody leading to vaccine unrecognition by memory cell (Khalafalla and El-Dirdiri, 2003), lower performance of the test (Azwai *et al.,* 1996; Prabhu *et al.,* 2012), standardization of test reagents, the expertise and time also factors for lower repeatability and inconsistencies of serological tests (Prabhu *et al.,* 2012). On the other hand, neutralisation methods require highly specific neutralizing antibody against the vaccine, which however is not full proof method as one hundred percent neutralization is highly unlikely. This raises the possibility of the presence of antibodies against these organisms in experimental animals used in vaccine trials that may complicate the interpretation of the results (Prabhu *et al.,* 2012).

Generally, it is important to note that evaluating whether the level of antibody is protective or not at laboratory and field level by measuring the antibody titer and

conducting post-vaccination challenge trial, respectively can help to overcome some complications. However, details regarding antibody titration and challenge trial are beyond the scope of this thesis work.

In the present study the absence of seroconversion among non-vaccinated control animals that were in contact with vaccinated camels may show that the viruses had not been excreted by vaccinated animals and/or transmitted to contact susceptible animals which indicate the safety of the vaccine under evaluation. Consistent findings were also reported by Khalafalla and El-Dirdiri, 2003 and Abdellatif *et al.,* 2014 on their field evaluation study of camelpox vaccine in Sudan. However, further field testing of the vaccine under evaluation and more detailed testing of residual virulence are needed; because the duration from vaccination to post-vaccination serum collection was short (21 to 30 days) to conclude about the safety of the vaccine on control experimental camels.

5.3. Participatory Epidemiology

Seasonal calendar study was conducted to complement the diagnostic investigation of camelpox and field evaluation of vaccine. The information could be useful for improving camelpox mitigation strategies such as timing of prophylactic (vaccination) or therapeutic interventions.

Camelpox was reported to occur during the short and rainy seasons by informant groups. This is in general agreement with the reports of Wernery and Kaaden 2002; Khalafalla and Ali, 2007 and Wernery *et al.,* 1997a,b, who demonstrated higher occurrence of camelpox outbreaks during rainy seasons with the appearance of more severe forms. Specifically, the relatively higher incidence report in *sugum* (short rainy season) than *kerma* (major rainy season) of this study was consistent with finding of Megerssa, 2010, who reported the highest occurrence of the diseases during the minor wet followed by the major wet seasons. The author suggested moisture as enhancing mechanism of virus stability in the environment and increase subsequent transmission to susceptible animal in rainy seasons. On the other hand, Wernery *et al*., 1997a associated occurrence of the disease with the increased density of the tick population during the rainy season. This

contradicts with the present finding where higher density of the tick population was reported to occur in the long and short dry seasons (Fig. 4). Of importance, further studies are recommended to ensure the involvement of arthropods in the transmission of CMLV (Duraffour *et al.*, 2011a).

Pastoralists have clear knowledge of the seasonality of camelpox which is in agreement with modern scientific laboratory based reports. However, camel diseases like trypanosomosis were reported to occur in the dry seasons by informant groups although the diseases was known to occur commonly during the wet season when the biting fly population is abundant (Luckins *et al.*,1988). This was associated with feed and water shortage that results in immuno-deficiency of camels. The situation in camelpox was however different which still reported to occur in the rainy season that shows synergic effect with the scientific reports.

6. CONCLUSION AND RECOMMENDATIONS

The ability of the camel to perform efficiently in harsh environmental conditions and its productive potentialities are convincing reasons for the improvement of its resource. Diseases are considered one of the most important constraints in improving camel health and production. The economical significance of camelpox is evident as losses are not only due to mortalities but also due to loss of weight, poor growth rate and decreased milk production in addition to the cost of medicines and treatment. According to the present seroepidemiological study, it seems that camelpox is endemic in Gabi Rasu zone with 19.3% animal level and 83.3% herd level seroprevalence. Antibody of the disease was detected from all villages included in the study that implies the wide distribution of the disease regardless of geographical location in the zone. Furthermore, camelpox was listed to be one of the most common five camel diseases in the area by pastoralists. The major risk factor associated with seropositivity of the disease was age of the camel both at univariable and multivariable logistic analysis, where seroprevalence was increased positively with age. Vaccination of camels resulted in a significant seroconversion rate with no adverse effect. The seasonal calendar of camelpox was outlined by informant groups and it was claimed to occur during the minor and major rainy seasons of a year. Taking in to consideration the results of this study, the following recommendations are suggested:

- Vaccination program, especially young camels, along with improved management strategies could have a paramount importance in the improvement of herd immunity and in reducing the circulation of camelpox. However, further large scale studies on the evaluation of the vaccine should be conducted to conclude about the efficacy of the vaccine.

- According to the baseline information collected from informant groups, camelpox vaccination programmes should be carried out before the start of rainy season. However, this should be validated by longitudinal observational studies.

- Protection of camels with the immunity induced by the live attenuated vaccine used in the present study should be confirmed by challenging the vaccinated animals with the wild strain of the virus.

- Considering the fact that camels are the single reservoir host of camelpox, in the long term there should be national strategic vaccination and surveillance policy targeting eradication of the disease. In this context, the development of heat stable camelpox vaccine would facilitate its use in hot, dry and inaccessible regions where the camel reared by involving community based vaccination programmes.

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8. APPENDICES

Appendix I: Test Method for Neutralization of Camelpox Virus

A. Principle

To detect the presence of serum specific antibodies against camelpox virus by *in vitro* neutralisation of the viral cytopathic effect on cell culture. This method is based on a reaction between the virus and specific antibody in the test serum. Virus and products containing a neutralizing antibody are mixed under appropriate conditions and then inoculated into cell culture. The presence of unneutralized virus is detected by plaque formation/cytopathic effect/. A loss of infectivity is caused by interference by the bound antibody with any of the steps leading to the release of the viral genome from the host cells including attachment, infection, or viral release.

B. Abbreviations

- VN - Virus Neutralization
- VNT- Virus Neutralization Test
- CMLV – Camelpox Virus
- $TCID_{50}$: 50% Tissue Culture Infective Dose
- FCS: Foetal Calf Serum
- CPE: Cytopathic Effect
- CO_2: carbon dioxide
- °C:degree centigrade
- µl: micro litre
- MEM: minimum essential medium
- V: volume

C. Materials

- graduated pipettes of 1ml, 2ml,5ml and 10ml

- Pipetter
- tips of 100µl, 200µl and 1000µl
- multichannel 50-300 µl and single channel 5-50
- Sterile tubes
- 96 flat bottom wells sterile plate (microplates tissue culture grade)
- Troughs for reagent distribution
- Water bath set at 56°C
- Incubator 37 °C with 5% CO_2 atmosphere
- Bio Safety cabinets
- Inverted microscope
- Refrigerator and freezer
- Vortex mixer
- Centrifuge
- Pipette cylinder containing water and disinfectant

D. Reagents

- VERO Cells
- Titrated CMLV suspension in media with out FCS
- Cell culture medium (MEM)
- L Glutamine
- Foetal Calf Serum
- Antibiotic-antimycotics
- Test sera
- Ethanol 70%
- Other disinfectant (for example Bleach or virkon's)

E. Procedure

i. Working sheet

Take the record sheet and fill in the layout of the 96-plate according to the samples to be tested. Each sample must be recorded individually/, camelpox virus stock used, the sera dilution (inverse log) and all information required.

ii. Preparation of the medium used in the plate

Prepare a sufficient volume of medium: MEM + 2% antibiotic/antimycotic + 1% /v/v/ L Glutamine of 200 mM with and without serum.

iii. Preparation of the serum to be tested

Thaw and de complement the test sera, stored at -20, by heating at 56 °C for 30 minutes in a water bath.

iv. Dilution of the virus

- Remove the viral suspension from -80°C just before use.

- The virus stock, with a known titre, should be diluted to give a 100 TCID50 /ml. Use MEM without serum but with 2% antibiotics antimycotics and 1% glutamine. 25µl (100 TCID50) of this viral dilution will be added to each well, therefore prepare enough volume according to the number of plate you want to do (you need 10 ml for one plate)

- <u>For the control plate</u>, take four sterile tubes labelled (1, 2, 3 and 4) and put 1.5 ml of the 100TCID50/ml virus dilution in the tube (1) and add 1.350 ml of MEM without serum in the other three tubes (2,3 and 4) and prepare serial dilutions (1:10) of the virus at 10^{3} TCID50/ml (doing a serial dilution of the 100 $TCID_{50}$ virus suspension (150 µl in 1350 µl of medium, thus giving tubes containing:

- 1.5 ml of virus 1000 $TCID_{50}$/ml → (100 µl) = 100 $TCID_{50}$/100µl
- 1.350 ml of virus 100 $TCID_{50}$/ml → (100 µl) = 10 $TCID_{50}$/100µl
- 1.350 ml of virus 10 $TCID_{50}$/ml → (100 µl) = 1 $TCID_{50}$/100µl
- 1.350 ml of virus 1 $TCID_{50}$/ml = 0.1 $TCID_{50}$/100µl

v. Preparation of the Vero cells suspension

- Vero cells are trypsinized. After counting the viable cells, they are diluted to make a suspension of $4x10^5$ cell/ml in MEM + 10% CSF + 2% antibiotic/antimycotic + 1% L Glutamine (You need 5 ml for one plate)

vi. Addition in the test plate

Test plate:

Take a 96 well tissue culture plate. One plate allows to test 8 sera in duplicate in serial dilutions (1:2).

Addition of medium: 25 µl of medium without serum is distributed in all wells.

Addition of samples: Add in wells A1and B1 25 µl of a serum 1 to be tested.

Add in wells C1and D1 25 µl of a serum 1 to be tested.

Add in wells E1and F1 25 µl of a serum 1 to be tested.

Add in wells G1and H1 25 µl of a serum 1 to be tested.

Repeat the same operation with sera in wells of column 7.

With the multichannel pipette set at 25 µl, perform 2 fold serial dilutions (from colomn1-6, and Colomn7-12) with initial dilution 1/2 and discard 25 µl of suspension from the end point dilution 1/64 i.e from column 6 and 12.

- Addition of virus: Add 25µl of 1000 TCID50/ml viral suspension to each wells.

Control plate: The plate should contain positive and negative controls and prepare separately as shown in lay out 2 (Section-F).

-Negative control: 6 wells in the last row are without virus.

-Positive control: 6 wells in each 4 rows are filled with different dilutions of camelpox virus (100TCID50, 10TCID50, 1TCID50 and 0.1TCID50 in 100 µl suspension) then incubate in 37°c for 1 hour.

- Addition of cells: After 1 hour add in each well of the plate 50 µl of Vero cells suspension of ($4x10^5$ cell/ml**).** Then the plates are incubated at 37°c with 5% CO2.
- Microscope reading: Plate reading to monitor for CPE formation

- Interpretation of the result and Calculation of the serum sample titre: CPE is observed when the serum is negative (the virus is not neutralized), in the contrary, no CPE will be observed if there is an antibody in the serum against CMLV.

· The test result is valid only if CPE does not occur in the Negative control wells and

· For the positive controls the CPE formation should be according to the virus titre in each four rows and expected standard protocol would be (++++, +++, +- +-, ----).

$100TCID_{50}/100\mu l$: + + + + +

$10TCID_{50}/100\mu l$: + + + + +

$1TCID_{50}/100\mu l$: + - + - +

$0.1TCID_{50}/100\mu l$: - - - - -

Calculation is done automatically in the work sheet. On the two dilutions series made for a serum sample, the titre can only be calculated if the difference between the two negative results (no CPE) and the two positive results(CPE) is not more than two dilutions: the difference acceptable possibilities are shown in the table

Dilutions	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1/10	-	-	-	-	-	-	-	-	-	-	-	-
1/20	-	-	-	-	-	-	+	-	-	+	-	-
1/40	+	+	-	+	+	-	-	-	+	-	+	+
1/80	+	+	+	+	+	-	+	+	+	+	-	-
1/160	+	+	+	+	+	+	+	+	+	+	+	+

The titre of the sample is equal to the inverse of the first dilution where the two wells are positives plus inverse of the last dilution where the two wells are negative, all being divided by two.

$$T = \frac{\left(\frac{1}{\text{first dilution with two wells positives}} + \frac{1}{\text{last dilution with two wells negatives}}\right)}{2}$$

Therefore, in the above example, the titre of sample 1 and 2 are respectively 30 and 50.

Example of moving positive wells:

Dilutions	Sample 3		Sample 4		Sample 5		Sample 6	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1/10	-	-	-	-	-	-	-	-
1/20	-	-	+	-	-	+	-	-
1/40	+	-	-	-	+	-	+	+
1/80	+	+	+	+	+	+	-	-
1/160	+	+	+	+	+	+	+	+

If the progression of CPE is not regular in the two series of dilution, (jump of well as in the case of samples 3 to 6 where CPE's do not match with dilutions), the calculation of the titre is done as before, after having moved the positivity of the well to the dilution just above (higher dilution) Therefore, the titre of sera 3, 4, 5 and 6 are respectively 60, 50, 30 and 60.

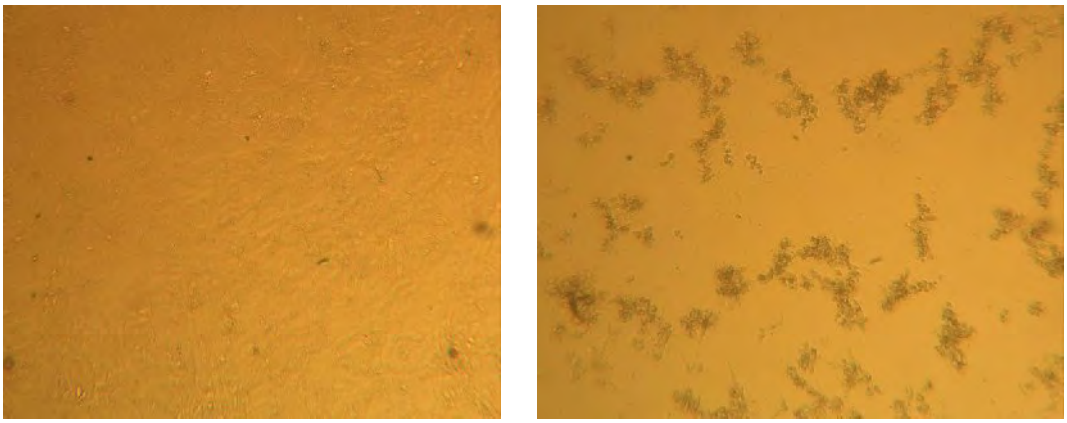
F: Plate lay out of VNT

Layout 1: Test plate layout in duplicate for camelpox test sera. Plate No.----------

	1/2	1/2	1/4	1/8	1/16	1/32	1/2	1/2	1/4	1/8	1/16	1/32
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A												
B												
C												
D												
E												
F												
G												
H												

Layout 2: Control plate layout for the positive and negative controls.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	
A													
B													100 TCID50 / 50 µl
C													10 TCID50 / 50 µl
D													1 TCID50 / 50 µl
E													0.1 TCID50 / 50 µl
F													
G													No virus, only cells
H													



A B

Figure 5: Positive (A) and negative (B) result of serum samples from this study

Appendix II: Seasons of the year in Afar Region

Afar Seasons	**Afar Months**	**Ethiopian Calendar Months**	**Rainfall Pattern**
'Gilal'	Hagayile	September	Cold and dry
	Leine	October	
	1st Gumazidi	November	
	2nd Gumadi	December	2-3 days rain (Dadaa)
	3rd Gumadi	January	Cold and Dry period
	4th Gumadi	February	
'Sugum'	Hara Asila	March	Short rains
	Tsom Bahe	April	
'Hagay'	Tsom	May	Hot and Dry Period
	Arfabahe	June	
'Kerma'	Erfa	July	Main Rains
	Waybuy	August	

Appendix III: Seroprevalence of camelpox by study village in Gabi Rasu zone, Afar Region, Ethiopia

District	**Village**	**Number examined**		**Number positive**		**Prevalence (%)**	
		Herd	Animals	Herd	Animals	Herd	Animals
Amibara	Halidegi	6	125	5	30	83.3	24
	Uduleisi	7	10	6	56	85.7	17.9
	Bedhamo	2	23	2	4	100	17.4
Awash	Arab Hara	8	100	7	14	87.5	14
	Ali Bete	6	65	4	13	66.7	20
	Gini Daho	1	15	1	3	100	20
Overall	6	30	384	25	74	83.3	19.3

Appendix IV: Camel Diseases Listed by Pastoralists in Gabi Rasu Zone Based on their Frequent Occurrence

Local Name (Afar)	***Scientific Name**
Galidelli	Abcessation
Kahu/Bahu	Respiratory Diseases (Coughing)
Galiguduf /Ambraruk	Camelpox/ orf
Agara	Mange
Dahan	Trypanosomosis
Firra	Anthrax/Sudden death
Boda/Derahot	Chronic and Acute Mastitis
Gini	Neck twisting
Uruga	Diarhea
Rebreba/Andela	Toxic plants (Plant poisoning)
Gindo	Lameness
Entigilli	Eye disease
Kilim	Ticks
BerEi	Abortion
Beghira	Constipation in new born calves
Hobia	Tick infestation of camel calf

*Scientific Name= Interpretation of local names to vernacular name is based on clinical symptoms mentioned by pastoralists and observation of some clinical cases

Appendix V: Seasonal calendar data recording sheet

Camel diseases	**Afar Seasons**				**Reason (Probing)**	**Remark**
	Hagay	*Sugum*	*Gilal*	*Kerma*		
Galidelli						
Galiguduf						
Kahu/Bahu						
Agara						
Dahan						
Kilim						

Appendix VI: Selected pictures taken during seasonal calendar data collection