MOLECULAR DIAGNOSIS AND CHARACTERISATION OF ORF VIRUS IN SYMPTOMATIC GOATS IN COAST AND DAR ES SALAAM REGIONS, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ONE HEALTH MOLECULAR BIOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA

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ABSTRACT

Orf virus (ORFV) is a member of the parapoxvirus genus that causes orf, a zoonotic and epitheliotropic highly contagious disease mainly affecting sheep, goats, wild ruminants and humans. In the present study, an outbreak of a disease in goats with clinical signs suggestive of orf in 11 flocks with a total of 259 goats was investigated between May and June 2015. Eight villages in districts of Bagamoyo (2), Ilala (1), Kinondoni (1) and Kisarawe (4) in Coast and Dar es Salaam regions of Tanzania were involved. The aim of the present study was to confirm ORFV involvement in diseased goats and to provide the genetic characteristics of ORFV. Upon visiting of goat flocks, a total of 72 goats presented orf-like clinical signs and 24 were reported to have died with similar clinical presentation. The presence of ORFV in oral swabs, scabs and skin scrapings was investigated by partial amplification of the ORFV *RNA polymerase* gene using Orf1 and Orf2 primers by polymerase chain reaction (PCR). A total of 16 out of 22 goats tested positive for ORFV upon PCR. Afterwards, molecular characterisation of ORFV was performed by amplification and nucleotide sequencing of the *B2L* gene encoding the major envelope protein. The results of nucleotide sequencing showed that orf was caused by closely related ORFV belonging to cluster I. ORFV were found to be genetically closely related to OV-SA00 (Accession number AY386264) strain of ORFV isolated in 2003 from scab material of a kid in United States of America and ORFV collected from a goat in Kyela, Tanzania in 2013. To our knowledge, this is the first report of the phylogenetic analysis of *B2L* gene of ORFV from goats in Tanzania. More studies are required to determine the extent of spread and genetic diversity of ORFV in livestock, wildlife and humans.

DECLARATION

I, Charles Mayenga, do hereby declare to the senate of Sokoine University of Agriculture that this dissertation is my original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

Charles Mayenga Date

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The declaration is hereby confirmed;

_____ _____

Prof. Gerald Misinzo Date

(Supervisor)

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DEDICATION

This work is dedicated to my late father who demanded excellent performance that can bring the best in life through manifesting hard work and decent character.

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LIST OF ABBREVIATIONS AND SYMBOLS

APC	antigen presenting cells
ATI	A-type inclusion bodies
BLAST	Basic Local Alignment Search Tool
bp	base pair
BPSV	bovine popular stomatitis virus
CDC	Center for Disease Control and Prevention
CFT	complement fixation test
COSTECH	Tanzania Commission for Science and Technology
CPE	cytopathic effect
DNA	deoxyribonucleic acid
EEV	extracellular enveloped virion
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organisation
FMD	foot-and-mouth disease
FMDV	foot-and-mouth disease virus
GM-CSF	granulocyte-macrophage colony stimulating factor
HIV	human immunodeficiency virus
IAEA	International Atomic Energy Agency
IMV	intracellular mature virion
Kbp	kilobase pair
MDBK	Madin-Darby bovine kidney cells
MDOK	Madin-Darby ovine kidney cells
MEGA	Molecular Evolution Genetic Analysis
mRNA	messenger ribonucleic acid

MV	mature virion
NCBI	National Center for Biotechnology Information
nm	nanometer
ORFV	orf virus
PBS	phosphate-buffered saline
PCPV	pseudo cow poxvirus
PCR	polymerase chain reaction
PPR	*peste des petits ruminants*
PVNZ	parapoxvirus of red deer in New Zealand
qPCR	quantitative polymerase chain reaction
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
SNT	serum neutralization test
SPPV	squirrel parapoxvirus
SPV	seal poxvirus
SUA	Sokoine University of Agriculture
TVLA	Tanzania Veterinary Laboratory Agency
USA	United States of America
V	Volts
VEGF	vascular endothelial growth factor
VIR	virus interferon resistance factor
VLTF	vaccinia virus late transcription factor

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Orf virus (ORFV) is a member of the genus Parapoxvirus, subfamily Chordopoxvirinae of the *poxviridae* family (Li *et al*., 2012). The family *Poxviridae* is characterized by viruses with linear double-stranded deoxyribonucleic acid (DNA) molecule of 130 to 300 kilobase pair (kbp) with a hairpin loop at each end (Moss, 2001). Viruses belonging to this family replicate entirely in the cytoplasm because their virions contain enzymes that synthesize messenger ribonucleic acid (mRNA). Parapoxviruses are distinguished from other members of the family by having an ovoid shape, with a crisscross patterned tubule-like structure on the particle surface and relatively small size and high guanine and cytocine (GC) content of the genome (Delhon *et al*., 2004; Mercer *et al*., 2006). Other members of the genus parapoxvirus include bovine papular stomatitis virus (BPSV), pseudocowpox virus (PCPV), parapoxvirus of red deer in New Zealand (PVNZ), seal poxvirus (SPV), squirrel parapoxvirus (SPPV), Auzdyk disease virus and Chamois contagious ecthyma virus (Mercer *et al.*, 1997; Moss, 2001; Delhon *et al*. 2004).

Parapoxviruses cause generally mild papular dermatitis around the mouth, teats and/or skin of infected animals and also occasionally infect humans (Robinson and Lyttle (1992), cited by Inoshima *et al.* (2000). Zoonotic parapoxviruses include BPSV and PCPV, maintained in cattle and ORFV which is maintained in sheep and goats (Mercer *et al*., 1997).

Orf virus is the causative agent of orf, one of the most widespread viral diseases, affecting mostly small ruminants and, sometimes other species, including wild animals and humans

(Hosamani *et al*., 2009). The disease is highly contagious primarily affecting sheep and goats, characterized by proliferative lesions in the skin of the lips, around the nostrils, and in the oral mucosa (Glover (1928), cited by Delhon *et al*. (2004). Mortality rate of up to 10% and 93% have been reported in lambs and kids respectively and, this occur when lesions in lips and udders prevent infected animals from suckling and grazing and also from associated secondary infections of bacterial and fungal origin and, maggot infestation (Mazur and Machado, 1989). Affected young animals experience reduced growth and slaughter of the affected animals is curtailed when the lesions become extensive and severe. This disease obviously impacts on the economic well being of poor farmers in developing countries who rely on small ruminants for their livelihood and as source of income.

1.2. Problem statement and justification

Orf is a highly contagious disease primarily affecting sheep and goats that cause significant losses to livestock keepers. It is the most common viral disease with a global implication (Hosamani *et al*., 2009). The disease has high morbidity rate and mortality of up to 10% and 93% in lambs and kids respectively (Mazur and Machado, 1989). High mortality in lambs and kids is attributed to inability of the animals to feed due to oral lesions leading to anorexia and associated secondary infections of bacterial and fungal origin (Hosamani *et al*., 2009). Orf outbreaks therefore threaten the contribution of goats and sheep to food security and economy.

Although among animal poxviruses, ORFV is one of the most extensively studied virus owing to its worldwide distribution and zoonotic significance (Hosamani *et al*., 2009). Little is known about ORFV affecting goats and sheep in Tanzania. A less detailed report by Mwanandota *et al*. (2014) reported on diagnosis and molecular characterisation of a

case of ORFV infection that occurred in 2013 in Kyela district located in Southern Highlands Zone. The disease was confirmed by partial amplification by Polymerase Chain Reaction (PCR) of the ORFV granulocyte-macrophage colony-stimulating factor (GM-CSF)/Il-2 gene.

Beginning in May 2015, goat keepers in the villages of Mapinga and Kwambwera in Bagamoyo district, Kakubiro, Dondwe, Mwanzomgumu and Msongora in Kisarawe district, Mbezi in Kinondoni district and Kimanga in Ilala district reported a disease in goats which manifested itself by affecting the skin of the mouth, causing formation of thick scab and nodular lesions which are typical manifestation of orf disease. This study was conducted to thoroughly investigate and confirm ORFV involvement by PCR and provide the genetic characteristics of ORFV circulating in these villages. The findings would give a confirmation of an outbreak of orf disease and create awareness about the presence of the disease to stakeholders. Moreover, the findings would be used by authorities involved in formulation of policies and strategies for control of animal diseases in the country. The study also would benefit the worldwide scientific community by providing the genetic characteristics of ORFV circulating in Tanzania.

1.3. Objectives

1.3.1. Overall objective

To perform molecular diagnosis and charactisation of ORFV in suspected outbreaks in goats of Coast and Dar es Salaam Regions in Tanzania.

1.3.2. Specific objectives

1. To detect ORFV in samples collected from symptomatic goats of Coast and Dar es Salaam Regions in Tanzania by polymerase chain reaction (PCR)

2. To determine nucleotide sequence of the *B2L* gene of detected ORFV, and
3. To establish the phylogenetic relationship between ORFV circulating in Coast and Dar es Salaam Regions in Tanzania and previous ORFV isolates across the world.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Overview of orf virus

ORFV, is the causative agent of orf, an epitheliotropic disease that affects sheep, goats, wild ruminants, and humans which is worldwide distributed (Li *et al*., 2012). It is the prototype of the genus Parapoxvirus belonging to the sub-family Chordopoxvirinae of the family Poxviridae. Other members of this genus include bovine popular stomatitis virus (BPSV) and pseudocowpox virus (PCPV) in cattle, squirrel parapoxvirus (SPPV), and parapoxvirus of red deer in New Zealand (PVNZ) (Hosamani *et al*., 2009; Zhang *et al*., 2010).

Orf, also known as contagious ecthyma, contagious pustular dermatitis, infectious labial dermatitis, scabby mouth, or sore mouth is the disease caused by ORFV and affects sheep, goats, wild ruminants, and humans (Hosamani *et al*., 2009; Lojkic *et al*., 2010). It can be found in any part of the world where sheep and goats are raised. The disease has a high morbidity rate and, mortality rates of up to 10% and 93% have been reported in lambs and kids, respectively (Mazur and Machado, 1989). The disease is characterized by proliferative lesions in the skin of the lips, around the nostrils, and in the oral mucosa (Glover (1928) as quoted by Delhon *et al*. (2004). Orf is one of trade sensitive diseases, the impact of disease is beyond local economies (livestock keepers). Economic impact is broadly considerable if one considers village, district, region and cross border small stock trade. The disease is zoonotic and it causes painful pustules on hands and fingers which start as erythematic lesions then progress to be vesicles, pustules, and scabs (Fleming *et al*., 2007).

Orf in sheep was recognized as early as 1787 and the earliest known report of human case was made in 1879 (Robinson and Lyttle (1992), cited by Inger (2001) and has been reported worldwide where sheep and goats are reared including those in Europe, the Middle East, the United States, Africa, Asia, Alaska, South Africa, Canada, New Zealand and Australia.

2.2 Orf virion structure

The orf virion particle (Fig. 1) like those of poxviruses is ovoid in shape and relatively smaller than other members of the poxviridae family. It contains a single copy of the viral DNA within its core, which also contains enzymes necessary for transcription of the early viral genes. The particle can appear in three forms: the first is the one which is surrounded by a single lipid bilayer membrane referred to as mature virion (MV). It is present in the cytoplasm and may be released when the infected cell is disrupted. The second type is that which is released through the plasma membrane of the cell where it acquires a second lipid bilayer membrane and is referred to as extracellular enveloped virion (EEV). The third type is a mature virion (MV) which may become embedded within large protein inclusion bodies, the A-type inclusion (ATI) bodies, which form in the cytoplasm of cells. All of the three virion particle types are infectious and this may account for the diversity in alternative surface structure, which affects the properties and functions of each type of particle (Pickup, 2015).

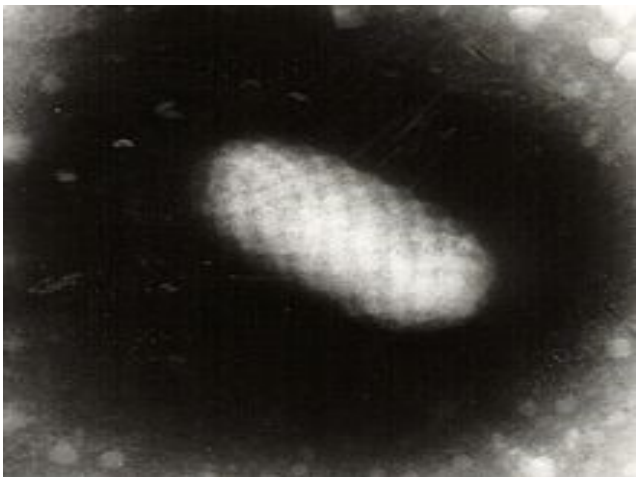

Figure 1: Electron micrograpgh of the ORFV particle. The particle reveals ovoid virion shape with external tubules showing a criss-cross pattern on the surface. Source: Said *et al*. (2013).

2.3 Orf virus genome

ORFV genome is a linear double stranded DNA composed of approximately 138-140 kbp with covalently-closed terminal hairpins on both ends (Kottaridi *et al*., 2006). The virus has 132 putative genes that include 89 highly conserved genes and some variable genes (Delhon *et al*., 2004; Mercer *et al*., 2006; Mahmud *et al*., 2014) located on both strands with a bidirectional orientation. ORFV is usually rich in GC content, about 64% and low in AT content (Moss, 2001; Delhon *et al*., 2004). Conserved genes are found at the central region of the genome while variability is observed in the terminal ends (Kottaridi *et al*., 2006).

2.4 Replication cycle of orf virus

Orf virus like all other poxviruses contains a double stranded DNA genome with enzymes and transcription factors. Being a large virus it replicates in the cytoplasm of host cells (Fig. 2). When a MV comes in contact with the cell membrane of host cell, a signaling cascade is triggered that results in membrane rearrangements and the formation of actin

and ezrin-containing protrusions that envelop the virus. At this stage the core of the virus is released into the cytoplasm. There are no specific proteins receptors identified to date that are required for penetration (Moss, 2001).

Once in the cytoplasm the core synthesizes early messenger RNA (mRNA) that is translated into several proteins including growth factors, immune defense molecules, enzymes and factors for DNA replication and intermediate transcription factors. This is followed by the core uncoating to allow DNA replication forming concatemeric molecules. Then transcription of intermediate genes occurs in the progeny DNA and the mRNA produced is translated to form late transcription factors. Virion structural proteins come from translation of mRNA transcribed from late genes. All of the products then start being assembled with the formation of distinct membrane structures. The concatemeric DNA intermediates are resolved into units genome and packaged in immature virions. Then maturation of the virion progresses to form an infectious intracellular mature virion. Then modified trans-golgi and endosomal cisternae wrap up the MVs to form wrapped virions (WV) which are transported to the periphery of the cell along microtubules. Upon reaching the plasma membrane, the WVs fuses with the cell membrane resulting in release of extracellular enveloped virion (EEV), which then go about to infect other cells (Moss, 2001).

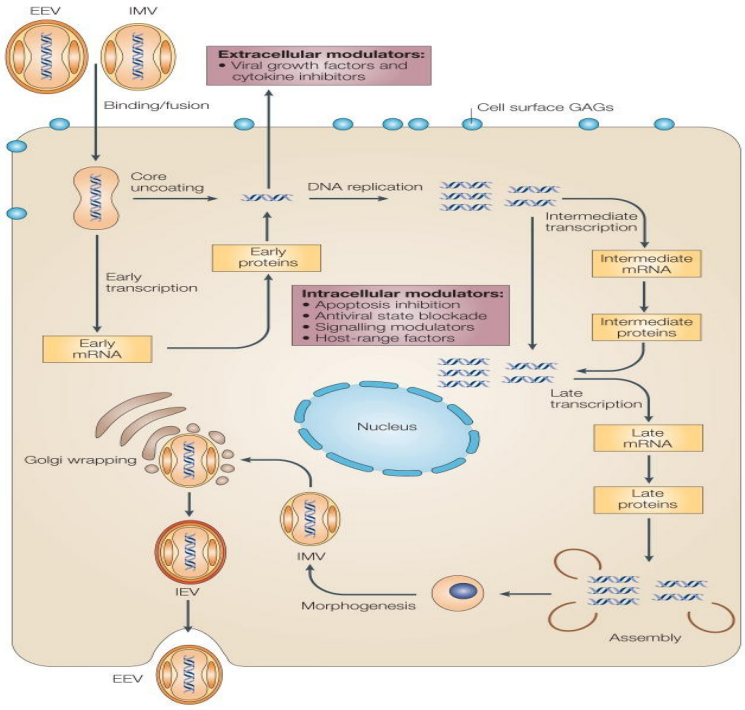

Figure 2: Replication cycle of parapoxviruses. The viral genome, structural and non-structural proteins are synthesized and afterwards mature virions are released by exocytosis and budding. Source: McFadden (2005).

2.5 Molecular epidemiology of orf virus

Initial molecular characterization of ORFV was done by restriction fragment length polymorphism (RFLP). This is a method based on the differences in homologous DNA sequences that can be detected by the presence of fragments of different lengths after digestion of the DNA samples in question with specific restriction enzymes then separated by gel electrophoresis and identified using labeled RFLP probe that hybridize with one or more fragments of the digested DNA sample. Restriction enzymes which have been used for this purpose include *Eco* RI, Bam HI and *Hind* III (Nandi *et al*., 2011). This method has been used to differentiate strains of ORFV (Robinson *et al*., 1982; Rafii and Burger, 1985; Mazur *et al*., 1991; Gilray *et al*., 1998; Mazur *et al*., 2000; Guo *et al*., 2003; Guo *et al*., 2004). Later, molecular characterization was worked out by direct sequencing of partial nucleotide sequence of major envelope gene (*ORFV011* or *B2L*) (Guo *et al*., 2003;

Guo, *et al*., 2004; Klein and Tryland, 2005; Lojkic *et al*., 2010; Zhang *et al*., 2010) and *virus interferon resistance factor* gene (*VIR*) (Guo *et al*., 2004; Kottaridi *et al*., 2006; Oem *et al*., 2009). Currently, full genome sequences of ORFV have been reported (Delhon *et al*., 2004; Mercer *et al*., 2006; Li *et al*., 2012; McGuire *et al.*, 2012).

Full genomic sequence of orf virus from Tanzania is not available. Only one partial sequence based on granulocyte-macrophage colony-stimulating factor (GM-CSF) inhibitor (*GIF*) gene is listed in GeneBank database with accession number KJ767194. (Mwanandota *et al*., 2014).

2.6 Economic importance of orf virus

ORFV causes disease in both animals and humans. The disease is known by several names, commonly orf in animal and humans but also contagious ecthyma, sore mouth, contagious pustular dermatitis or scabby mouth (Thomas *et al*., 2003) in animals. It is an acute, contagious, debilitating and economically important zoonotic viral skin disease that primarily affects sheep, goats and some other domesticated and wild ruminants (Nandi *et al*., 2011). The disease is characterized by proliferative lesions on the mouth and muzzle which begin as erythematous macule then progress to papule, vesicles, pustules and scab formation that usually resolve in 4-8 weeks (McKeever *et al*., 1988). Young animals are severely affected with facial, oral lesions and lesions on the udder interfering with suckling resulting to death.

ORFV infections pose an economic loss to farmers especially in the rural poor population of developing countries who rely on small ruminants for food security and as source of income and other social benefits. Losses do not only result from death but also from reduced growth rate, premature culling of ewes and does due to orf-induced mastitis,

reduction in reproductive performance of a flock when rams are infected with the venereal form of orf making them unwilling to mate, extra cost for veterinarian involvement and delays in trading and slaughtering (Hosamani *et al*., 2009).

Orf disease is highly zoonotic and cases of human infection have been reported worldwide (Robinson and Lyttle (1992), quoted by Inger, (2001) and Leavell *et al*. (1968); Dupre *et al*. (1978); Huerter *et al*. (1991); Bassioukas *et al*. (1993) and Georgiades *et al*. (2005), quoted by Nandi *et al*. (2011). Most people who are at risk of acquiring the disease are those who are likely to be in contact with infected animals, carcasses or contaminated nonliving material. These include shepflocks, veterinarians, farmers, butchers and meet porters.

Apart from causing disease in animals and humans, ORFV has been used as a vector in recombinant vaccine preparations owing to its ability to survive in the presence of pre-existing immunity. The vascular endothelial growth factor (VEGF), a virulence determinant, which is essential for growth, is a candidate locus for expression of foreign genes for protection against heterologous organisms (Hosamani *et al*., 2009). Example of ORFV recombinant vaccine developed include D1701 for delivery of pseudo-rabies antigen (Dory *et al*., 2006) and ORFV recombinant that expresses an antigenic peptide (EG95) of *Echinococcus granulosus* (Marsland *et al*., 2003).

2.7 Pathogenesis and clinical signs of orf disease

A broken or abraded skin is the entry point of ORFV and this is where the virus replicates and produces the characteristic lesions. Replication starts in the epidermal cell layer derived from the wall of hair follicle. It takes 4 to 8 days for the first signs of infection to be noticed. There is initial rise in body temperature followed by development of papules

and pustules at oral commissure, skin of lips and nose which then become thick, tenacious scabs covering a raised area of ulceration, granulation and inflammation (Fig. 3). Kids below two months of age also develop similar lesions on the lips then spread to the skin of face, ears, feet, flanks, and scrotum (de la Concha-Bermejillo *et al*., 2003). In lactating animals lesions may appear on the udder or teats. Lesions can also appear on skin of vulva of ewes and scrotum of ram causing the venereal form of orf. Hosamani *et al*. (2009) described the lesions as being proliferative, cauliflower-like in appearance that commence on the lips, muzzle, mucocutaneous junctions, nostrils, gums and sometimes spread to internal organs including the tongue even in the absence of systemic involvement. Systemic involvement have been reported where the infection extends to alimentary tract and trachea leading to severe gastroenteritis and bronchopneumonia (de la Concha-Bermejillo *et al*., 2003; Nandi *et al*., 2011).

Figure 3: Clinical signs of orf in sheep. Proliferative lesions on the skin of the lips around the mouth, nostrils, and the eyelids. Source: Li *et al*. (2012).

Clinical signs of orf infection in humans occurs 3-7 days post entry of the virus. The disease manifests itself by a single skin lesion or a few lesions of small firm, red to blue papule on a finger, hand or other bare parts of the body (Fig. 4). Other symptoms reported include pain, pruritus, lymphangitis and axillary adenitis or sometimes fever or malaise. Unless secondary infection gets involved, the lesions heal in 3-6 weeks (Nandi *et al*., 2011). Like in animals re-infection with orf virus is possible though the lesions are less severe and resolve quickly. Persons with weakened immune system due to infections with diseases like HIV or cancer therapy can develop serious symptoms following orf virus infection, including large tumor-like lesions, progressive disease, development of multiple lesions, or erythema multiforme reactions involving rashes on the mucous membranes and skin (CDC, 2012).

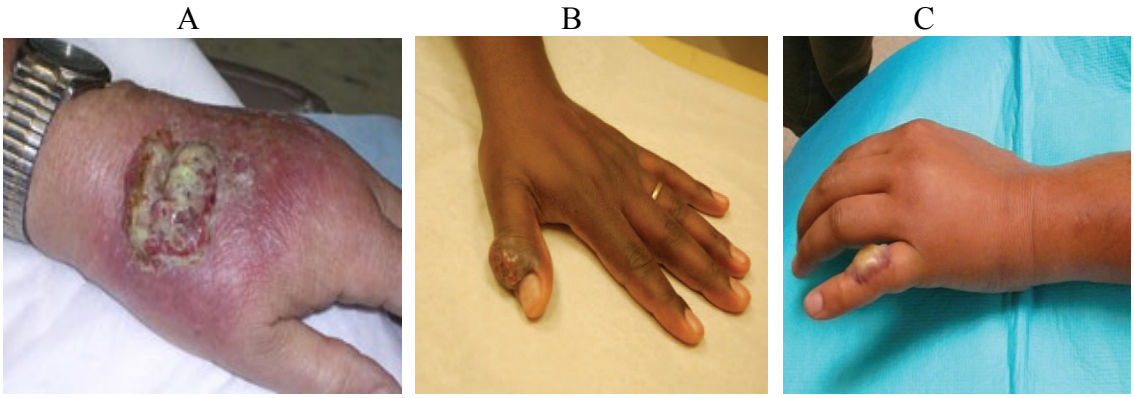

Figure 4: Clinical signs of orf in humans. The lesions include a single papule on a finger (B and C) and a large tumor-like lesion (A) on a hand of a person with a weak immune system. Source: CDC (2012)

2.8 Epidemiology of orf disease

Orf disease is found worldwide in sheep and goat rearing countries. People who are in direct contact with infected animals can get the human form of orf disease. The disease is considered to be one of the most distressing skin disease and more serious in lambs and

kids than adults, being characterized by scabby erythematous lesions, around the nares and mouths of animals (Hosamani *et al*., 2009).

ORFV is said to be robust in a dry environment and can survive for months or even years but its life span may be shortened in cold and wet conditions. The virus has high affinity to epithelial cells, normally entering via broken or damaged skin and start replicating in regenerating epidermal keratinocytes. Lesions are localized, progressing from erythema to pustule and scab (Nandi *et al*., 2011); the virus is not known to spread systemically from the site of infection, rather it is shed with scab material and remain in the environment only to infect susceptible animals once conditions are conducive.

Transmission of orf is through contact from infected animals to susceptible animals. Clinically normal animals have been reported to transmit the disease to orf naïve animals as well as dipping can save as a means of transmission (Nettleton *et al*. (1996); Sargison *et al*. (2007), quoted by Nandi *et al.* (2011). Minor or major surgical intervention, hand contact, drenching and ear tagging have been reported to be source of transmission of orf (Allworth *et al*., 1987). Individual or certain breeds of animals and immune status are suspected to be contributing factors in ORFV persistence and progression (Yeruham *et al*., 2000; de la Concha-Bermejillo *et al*., 2003). It has been reported that orf outbreak occur secondary to primary infection such as peste des petits ruminants (PPR) hence exacerbating disease conditions and associated losses. For example Saravanan *et al*. (2007) reported a mixed outbreak of peste des petites ruminants (PPR) and orf. Other predisposing factors reported for ORFV infection in both animals and humans include immunosuppression induced by corticosteroid therapy, pregnancy, autoimmune disorders and prolonged chemotherapy (Hosamani *et al*., 2009).

The actual prevalence of orf disease among livestock is said to be greatly underestimated (Hosamani *et al*., 2009). Whereas morbidity can go as high as 100%, mortality rate is usually less than 1% but secondary infections of bacteria and fungus can increase the number to between 20 and 50% but depending on the age of the animal it can go as high as 93% (Hosamani *et al*., 2009). Following clinical disease or vaccination, animals are partially protected with recurrent infection being possible to occur in 1-3 months but with less severe lesions and healing is rapid (Nandi *et al*., 2011).

Currently there is no published data on the prevalence of orf disease in Tanzania. However, farmers visited in Kisarawe district said that the disease almost occurs every year following the onset of rainy season and they call it a '*dew disease*' thinking that animals get infected by eating grasses covered with dew (Kisarawe goat keepers, personal communication, 2015).

2.9 Host range

Sheep, goats and humans are the common species affected by ORFV. Other animal species that have been reported to succumb to orf infection include:- cattle, alpacas, camels, reindeer, sichuan takin, deer, prong horn antelope, wapiti and seal squirrels (Robinson and Mercer, 1995; Azwai *et al*., 1995; Thomas *et al*., 2003; Guo *et al*., 2004 and Tryland *et al*., 2005), quoted by Nandi *et al*. (2011). Hagis and Ginn (2001) reported that dogs can contract infection from eating orf-contaminated carcasses.

2.10 Immunity against orf disease

Infection and vaccination does not confer long lasting immunity against ORFV and therefore re-infection with orf in not uncommon although the clinical severity and the course of secondary exposure are reduced. This is the same for animals and humans

(Hosamani *et al*., 2009). Both humans and animals have demonstrated humoral immune response and cell-mediated immune response following ORFV infection (McKeever *et al*., 1987; Lloyd *et al.*, 2000). The ability of ORFV to evade immune response through induction of apoptosis of antigen presenting cells (APCs) and ability to inhibit apoptosis of ORFV infected cells has been described (Westphal *et al*., 2007).

2.11 Diagnosis of orf disease

2.11.1 Clinical signs

Orf can easily be diagnosed based on the characteristic lesions on the predilection sites, however, it should be differentiated from other viral diseases that share similar clinical signs such as foot-and-mouth disease (FMD), capripoxvirus infection, rarely bluetongue disease, bacterial diseases such as staphylococcal dermatitis or dermatophilosis and ulcerative dermatosis (Wilson *et al*., 2002; Watson, 2004). When describing differences in lesions caused by these diseases, Nandi *et al*. (2011) said that oral lesions due to FMDV are not proliferative as those caused by ORFV. Ulcerative dermatosis is characterized by ulcers and crusts on skin of the face, feet and genitalia but the lesions are not elevated because epithelial hyperplasia is absent. Dermatophilosis results in multiple small superficial pustules and rarely causes prominent skin lesion whereas staphylococcal dermatitis (periobital eczema) is characterized by hair loss, edema and deep necrosis of eyelids and adjacent areas. Capripoxvirus infection is characterized by elevated papules over the entire body also involving profound systemic reaction that is often fatal.

Orf disease symptoms in humans are said to provide sufficient evidence for the diagnosis, especially when the person had a history of coming in contact with infected animals or materials. However, laboratory diagnosis of human parapoxvirus infections is important

since clinical manifestations in immune compromised individuals can be shared with potentially life-threatening zoonoses like cutaneous anthrax (Lederman *et al*., 2007).

2.11.2 Laboratory tests

Several laboratory tests have been developed which can be used to confirm orf infection including both those targeting the virus and those analyzing antibody reaction after infection or vaccination. The following is a brief explanation of each of the commonly employed laboratory tests.

2.11.2.1 Electron microscopy

This is a direct method for quick diagnosis and differentiation of poxvirus infections in humans and animals. Lesion extract, negatively stained and viewed under electron microscopy reveal ovoid virion particles with external tubules showing a criss-cross pattern and approximately 200 nm x 160 nm (Guo *et al*., 2004; Hosamani *et al*., 2009). This method is also used to differentiate parapoxvirus infection from capripoxvirusses in animals owing to their differences in shape.

2.11.2.2 Histopathology

Histopathological sections made from affected skin tissue reveal epidermal hyperplasia with hyperkeratosis, ballooning and degeneration of keratinocytes. Hyperkeratosis, parakeratosis and acanthosis of the epidermis, degenerative changes in stratum spinosum and infiltration of mononuclear cells including macrophages, lymphocytes and neutrophils are seen. The cytoplasm of infected cells may contain eosinophilic inclusion bodies (Barraviera (2005), quoted by Nandi *et al*. (2011).

2.11.2.3 Cell culture isolation

ORFV has been isolated on both primary and cell line cultures. Primary cells include lamb testis, lamb kidney, fetal lamb dermis cells, fetal lamb muscle cells, ovine fetal turbinate cells, fetal bovine muscle cells and fetal bovine lung cells whereas cell lines include Madin-darby bovine kidney (MDBK), Madin-darby ovine kidney (MDOK) and Vero cells (Inoshima *et al*., 2000; Delhon *et al*., 2004). Cytopathic effects (CPE) start by ballooning, then rounding and degeneration of cells with detachment from the surface usually on initial passage or after 2-3 blind passages (Hosamani *et al*., 2009).

2.11.2.4 Virus neutralization and complement fixation test

These are serological tests developed for detection of antibodies. A sample is considered positive if it has a titre of 8 for serum neutralization test and 20 for complement fixation test (Zarnke *et al*., 1983).

2.11.2.5 Enzyme-linked immunosorbent assays (ELISAs)

This test detects antibodies against ORFV in serum samples of different species of animals. Inoshima *et al*. (1999) described an indirect ELISA that employs purified antigens and peroxidase conjugated protein A or G or chimeric A/G. This test has been used for detection of antibodies to ORFV in humans (Yirrell *et al*., 1994), sheep (McKeever *et al*., 1988) and camel (Azwai *et al*., 1995).

2.11.2.6 Western blotting

Western blotting is a test used to detect specific proteins in a sample of tissue homogenate or extract. The proteins are separated by gel electrophoresis depending on their sizes and then stained with specific antibodies. A number of proteins have been noted to react in sera from ORFV infected animals including 40, 22, 20, 56, 66, 39, 24, and 25 kDa. Three

intensely stained bands were visualized at 66, 39 and 22 kDa. Two enveloped proteins of 39 and 22 were the prominent antigens recognized by the immune system (Czerny *et al*. (1997) as quoted by Hosamani *et al*. (2009).

2.11.2.7 Real time polymerase chain reaction (qPCR)

Real time Polymerase chain reaction, also known as quantitative polymerase chain reaction (qPCR) amplifies and simultaneously detects or quantifies a targeted DNA molecule. The major envelope gene (*B2L*) has been used to develop real time PCRs. This test has been optimized to distinguish four members of the parapoxvirus genus in the same sample-ORFV, PCPV, BPSV and seal parapoxvirus (Gallina *et al*., 2006; Nitsche *et al*., 2006).

2.11.2.8 Conventional polymerase chain reaction (PCR)

This test amplifies copies of ORFV DNA found in the sample and then separated on an agar gel by electrophoresis and visualized under UV transiluminator. Several genes have been targeted and therefore different PCR formats for quick diagnosis of ORFV infections have been developed. Table 1 summarizes different PCR methods in use.

Table 1: Different primers for diagnosis of orf using polymerase chain reaction**.** These primers are used to diagnose ORFV both in animals and humans.

Gene target	Primer (5'-3' direction	Amplicon (bp)	Reference
Major envelope protein gene (*B2L*)	PPP1: gtcgtccacgatgagcagct	554	(Robinson and Balassu, 1981)
	PPP4: tacgtgggaagcgcctcgct	234*	
	PPP3: gcgagtccgagaagaatacg		
RNA polymerase gene	Orf1: cgcagacgtggctgagtacgt	140	(Torfason and Guonadottir, 2002)
	Orf2: tgagctggttggcgctgtcct		
GM-CSF/IL-2 inhibitor factor	GIF5: gctctaggaaagatggcgtg	408	(Hausawi *et al*., 2004)
	GIF6: gtactcctggctgaagagcg		
Virus interferon resistance gene	VIR1: acaatggcctgcgagtg	552	(Deane *et al*., 2000)
	VIR2:ttagaactgatgccgcag		
Vascular endothelial growth factor	GF1: gcgggatccgccatgaagttgctcgt	399	(Gallina *et al*., 2006a)
	GF2: gcggaattcctagcggcgtcttctgg		
Late transcription factor (VLTF-1)	045F: cctacttctcggagttcagc	392	(Jenkinson *et l*., 2008)
	045R: gcagcacttctcctcgtag		
IMV protein (F1L), a homologue of vaccinia virus H3L gene	OVS: aggcggtggaatggaaaga	708	(Esposito and Fenner, 2001)
	OVA:ccagcaggtatgccaggatg		

*Semi-nested PCR. GM-CSF: Granulocyte-macrophage colony-stimulating factor; IMV: Intracellular mature virus; VLTF: Vaccinia virus late transcription factor

Source: Hosamani *et al*. (2009)

2.12 Treatment and control of orf disease

Orf disease like other viral diseases does not have specific treatment, however, symptomatic treatment with antibiotics, dressings and local antiseptics application are helpful (Nandi *et al*., 2011). Although several vaccines have been developed, they are not recommended in a flock that has not suffered from the disease. This is because the vaccine is likely to cause the disease. Vaccination is recommended in already infected flocks to fasten the recovery process and even though it does not confer resistance against future outbreaks (Hosamani *et al*., 2009).

Several measures have been suggested in order to prevent spread of the disease during an outbreak in flocks or flocks. These include quarantine of new animals before mixing with other animals in the farm. It is very important that, sick animals be separated, fed and treated. People should avoid consumption of milk from infected ewes and does presenting lesions on the teats and udder. Animal handlers should wear gloves and facemasks when handling sick animals to avoid getting infected. Proper disinfection of premises of animal houses and proper handling of all contaminated materials should be carried out. These measures are essential to reduce the risk of new infection. Finally the migration of animals from one place to another particularly the infected ones should be prohibited. Most important is for farmers to supply well balanced diet to their animals which can help build their immunity and resist infection by ORFV which once enters a flock or flock is difficult to eradicate (Nandi *et al*., 2011).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1 Study area

This study was conducted in Bagamoyo and Kisarawe districts of Coast region and Ilala and Kinondoni districts of Dar es Salaam region in Tanzania. These districts are located in the eastern part of Tanzania (Fig. 5). The study was carried out following reports of a disease suspected to be orf based on clinical signs. Sampling of goats was conducted in farms where this disease was reported. The disease was reported in eight villages where 11 family flocks were affected. The names and locations of villages where the outbreak occurred and sampling was done are shown in Fig. 5.

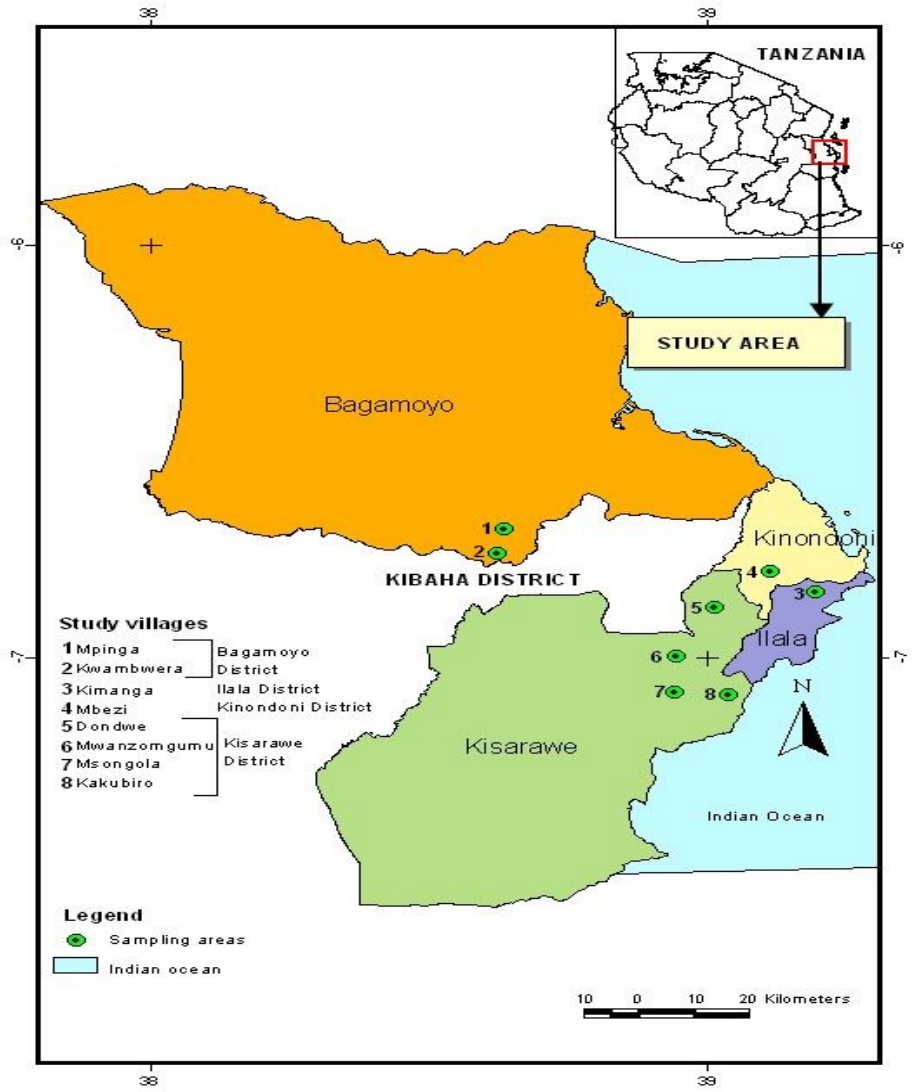

Figure 5: Map of Coast and Dar es Salaam regions showing sampling locations. Eight villages (indicated with cycles) from Ilala and Kinondoni districts of Dar es Salaam region and Kisarawe and Bagamoyo districts of Coast region were involved. The insert is the map of Tanzania showing the location of study area.

3.2 Sampling and sample collection

This study involved flocks of goats suspected of ORFV infection. Ward livestock extension personnel in the areas where farmers reported the disease were contacted and afterwards farmers were visited for the purpose of assessing the health status of their flocks. Clinically sick goats were identified and purposively sampled. During sampling, flock history was also taken for the purpose of seeking to establish as to what could have been the source of the disease (Appendix 1). Collected samples were taken to Center for Infectious Diseases and Biotechnology (CIDB) laboratory of Tanzania Veterinary Laboratory Agency (TVLA) in Dar es Salaam for testing. Two types of samples were collected, dry scabs or skin scrapings and oral swabs. Scabs and/ or skin scrapings were collected into capped sterile tubes using a sterile forceps. Oral swabs were collected using cotton swabs and placed in vials containing 1.5 - 2 ml sterile phosphate-buffered saline (PBS) containing antibiotics and antifungal. These samples were uniquely labelled, stored in a cool box containing ice packs, and transported to the laboratory for testing. Flock history and clinical signs were recorded.

3.3 Laboratory testing

3.3.1 Sample processing in the laboratory

Each tube containing a swab was vortexed to dislodge cells from the swabs into PBS. Then, the tubes were centrifuged at 8000 *g* for five minutes at 4 ^{o}C and supernatant was transferred into a new sterile tube and labeled. Scabs or skin scrapings were mechanically homogenised in PBS at the ratio of 1:10 using a sterile motor and pestle. The homogenate was centrifuged at 8000 *g* for 5 minutes at 4 ^{o}C. The supernatant was collected into a sterile tube and labelled accordingly. Finally the products (swab, scab or skin scrapping supernatants) were stored at -20 ^{o}C until DNA extraction.

3.3.2 DNA extraction

Samples from at least two animals representative of each flock were purposively chosen and DNA was extracted from these samples. One previously collected sample from Kyela was included as positive control during confirmatory diagnostic PCR. Deoxyribonucleic acid extraction was carried out using QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany), according to manufacturer's instructions.

3.3.3 Amplification of ORFV *RNA polymerase* gene for diagnosis of ORFV

Extracted DNA samples were subjected to classical PCR to amplify a 140 bp fragment of DNA polymerase gene of ORFV. ORFV specific forward Orf1 (5'- CGC AGA CGT GGC TGA GTA CGT-3') and reverse Orf2 (5'- TGA GCT GGT TGG CGC TGT CCT-3') primers were used as previously described by Torfason and Guonadottir, (2002). Polymerase chain reaction reagents included IQ supermix (Bio-Rad, Munich, Germany), primers and RNase-free water mixture as shown on Table 2.

The amplification was carried out in a GeneAmp 9700 (Applied Biosystem, Foster City, CA) thermo-cycler with initial denaturation at 95 ^{o}C for 5 minutes followed by 40 cycles of denaturation at 94 ^{o}C for 40 seconds, annealing at 55 ^{o}C for 40 seconds and extension at 72 ^{o}C for 40 seconds, and a single cycle of final extension at 72 ^{o}C for 5 minutes. Then, six µl of PCR product mixed with four µl of loading die were loaded onto a 1.5% agarose gel mixed with five µl of gel red and electrophoresed at 100 v for one hour. Visualization of PCR products was done using a UV Transilluminator (VWR International BVBA, Leuven, Belgium).

Table 2: Master mix for amplification of ORFV *RNA polymerase* gene. Amplification was done using IQ supermix (Bio-Rad) and primers Orf1 and Orf2.

Reagent	**Volume of one reaction composition in µl**
IQ Supermix	10
Orf1 primer (5 pmol/µl)	2
Orf2 primer (5 pmol/µl)	2
RNase-free water	3
Template DNA	3
Final Volume	**20**

3.4 Genotyping of ORFV

3.4.1 Amplification of full length of the *B2L* gene

Positive samples were chosen for amplification of full length (1137 bp) of the *B2L* gene encoding the major envelope protein of ORFV using primers ORFV-B2Lf-For (5'-GAC CTT CCG CGC TTT AAT TT-3') and ORFV-B2Lf-Rev (5'-CCC GCC TGC TAA AAG ACT-3') (Gelaye, E. personal communication, 2015). Master mix composition is as indicated in Table 3. A PCR product of 1210 bp was expected.

The PCR protocol comprised of initial denaturation at 94 ^{o}C for four minutes followed by 40 cycles of denaturation at 95 ^{o}C for 45 seconds, denaturation at 52 ^{o}C for 1 minute and extension at 72 ^{o}C for 1 minute and 30 seconds and a single cycle of final extension at 72 ^{o}C for seven minutes. Confirmation of amplification was done by gel electrophoresis using 6 µl of PCR product mixed with 4 µl of loading dye (Qiagen, Hilden, Germany) loaded onto a 1.5% agarose gel mixed with 5 µl of gel red (Biotium, Phoenix, CA) and electrophoresed at 100 V for 1 hour. Visualization of PCR products was done using a UV

Transilluminator (VWR International BVBA, Leuven, Belgium).

Table 3: Master mix for amplification of ORFV *B2L* gene. The PCR for amplification of *B2L* gene for sequencing was carried out using primers ORFV-B2Lf-For and ORFV-B2Lf-Rev

Reagent	**Volume for one reaction composition in µl**
Buffer (10x)	5
Primer ORVB2LfFow (5 pmole/µl)	5
Primer ORVB2LfRev (5 pmole/µl)	5
dNTPs (2mM)	5
RNase-free water	22.5
Enzyme Tag (Qiagen)	0.5
DNA template	7
Total volume	**50**

3.4.2 Preparation of PCR products for sequencing

PCR products were cleaned up using Wizard®SV Gel and PCR Clean-Up System (Promega, Madison, USA) and sequenced using dideoxynucleoside cycle sequencing. Because the dideoxynucleotide sequencing method produces good quality chromatogram for sequences not exceeding 1000 bp, two internal primers were designed to allow sequencing of the complete *B2L* gene. The two internal primers included the forward internal ORFV-B2Li-For (5'-GTC CGC GTT CTT CCA CTC-3') and reverse internal ORFV-B2Li-Rev (5'-GCG GGC GTC AAC TAC TAC A-3') (Gelaye, E., personal communication, 2015).

3.4.3 Sequence analysis

Sequencing produced four overlapping sequences spanning the whole length of *B2L* gene

for each sample. These were assembled to produce a consensus sequence using Staden software version 2.0.0.b8 (Joint FAO/IAEA Animal Production and Heath Laboratory, Vienna, Austria). Further processing and sequence alignment was done using BioEdit software version 7.0.0 (Ibis Biosciences, Carlsbad, CA). Evolutionary divergence between sequences was analyzed using Maximum Composite Likelihood Model as implemented in MEGA 6.05 software (Tamura *et al*., 2013).

3.5 Phylogenetic analysis of ORFV circulating in eastern Tanzania

Using the BLASTn service on NCBI website, the consensus sequences obtained were used to search for similar sequences at GenBank database. Then the sequences were imported to MEGA 6.05 software in order to construct a phylogenetic tree. Calculation of evolutionary differences among ORFV isolates using the Neighbor-Joining method by Kimura-2-parameter model at 1000 bootstrap replications was also carried out. Clustering patterns in the phylogenetic tree were used to determine the place of ORFV genotypes obtained in the present study in relation to other selected ORFV isolates from around the world available at GenBank.

CHAPTER FOUR

4.0 RESULTS

4.1 Clinical signs

Eleven flocks of goats belonging to 11 smallholder farmers from eight different locations or villages in the districts of Bagamoyo (4), Ilala (1), Kinondoni (2) and Kisarawe (4) were visited for the purpose of clinical examination and sampling. The animals presented typical orf clinical signs (Fig. 3). The clinical signs included slight rise in body temperature for some animal (Apendix 1), pustules around the muzzle and nostrils, proliferative cauliflower-like lesions on the lips, muzzle, mucocutaneous junctions and nostrils. Other animals were severely affected where the muzzle area was swollen with papules forming ulcers (Fig. 6). Lesions were also seen on other parts of animal body including ears, teats and hind limbs.

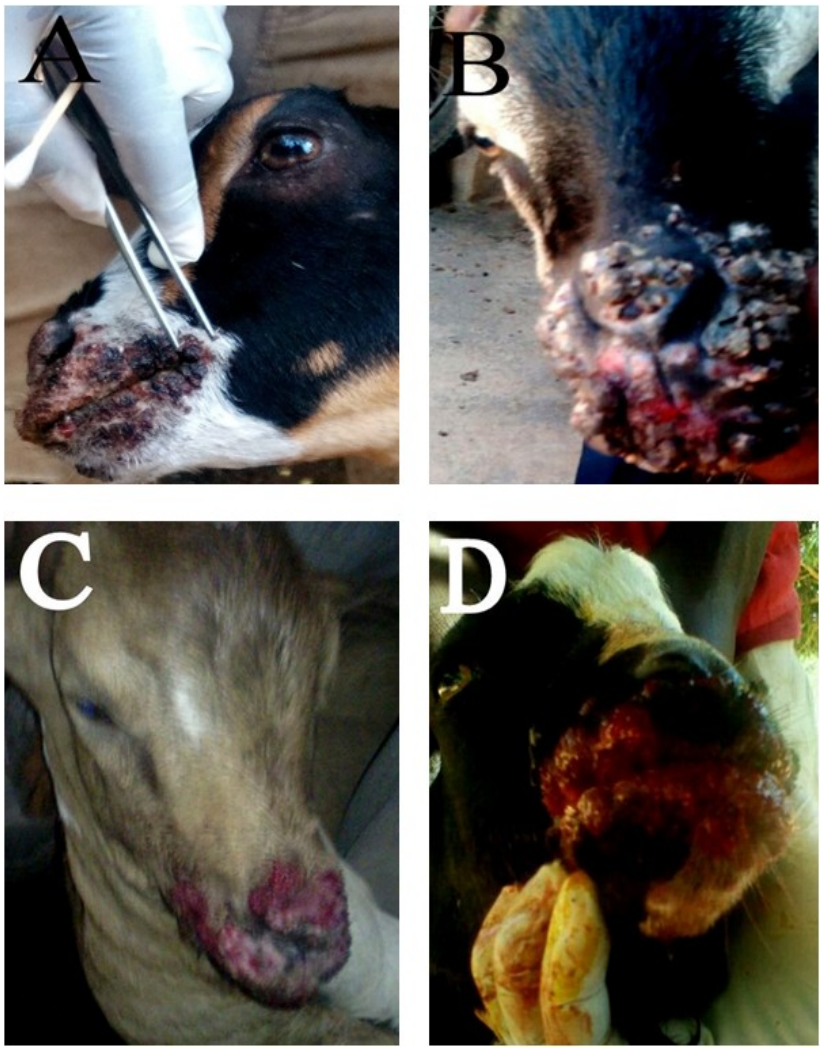

Figure 6: Clinical signs observed in goats with orf. Lesions on the lips, muzzle, nostrils and oral commissures (A and C), proliferative cauliflower-like lesions on the lips, muzzle, mucocutaneous junctions and nostrils (B) and lesions on a severely affected goat where the lips and commissures are swollen and papules forming ulcers (D).

4.2 Confirmatory diagnosis of orf using PCR

Oral swabs and scab or skin scrapings were collected from 22 symptomatic goats. One scab sample collected from a goat in Kyela in 2013 archived at CIDB Laboratory was included as positive control. Confirmatory diagnosis of orf was performed by amplification of *RNA polymerase* gene using primers Orf1 and Orf2. A PCR product of approximately 140 bp was amplified in a positive control sample from Kyela and some of the samples included in the present study (Fig. 4). A total of 16 out of 22 goats were positive for ORFV. The proportion of positive samples was 14 out of 25 swabs, 11 out of 14 scabs and seven out of nine skin scrapings (Table 4).

Table 4: Detection of ORFV in goats. The *RNA polymerase* gene was amplified from swabs, scabs and skin scrapings collected from symptomatic goats in Bagamoyo, Ilala, Kinondoni and Kisarawe districts. The numbers of positive samples are indicated in parenthesis.

Location	District	Swabs	Scabs	Skin scrapings	Total	Goats
Kwambwera	Bagamoyo	7 (0)	3 (0)	0 (0)	10 (0)	4 (0)
Kimanga	Ilala	4 (4)	2 (2)	2 (2)	8 (8)	2 (2)
Mapinga	Bagamoyo	5 (2)	5 (5)	0 (0)	10 (7)	5 (5)
Mbezi	Kinondoni	0	0	2 (0)	2 (0)	2 (0)
Mwanzomgumu	Kisarawe	2 (1)	1 (1)	2 (2)	5 (4)	2 (2)
Dondwe	Kisarawe	4 (4)	1 (1)	2 (2)	7 (7)	4 (4)
Msongola	Kisarawe	1 (1)	1 (1)	0 (0)	2 (2)	1 (1)
Kakubiro	Kisarawe	2 (2)	1 (1)	1 (1)	4 (4)	2 (2)
Total		25 (14)	14 (11)	9 (7)	48 (32)	22 (16)

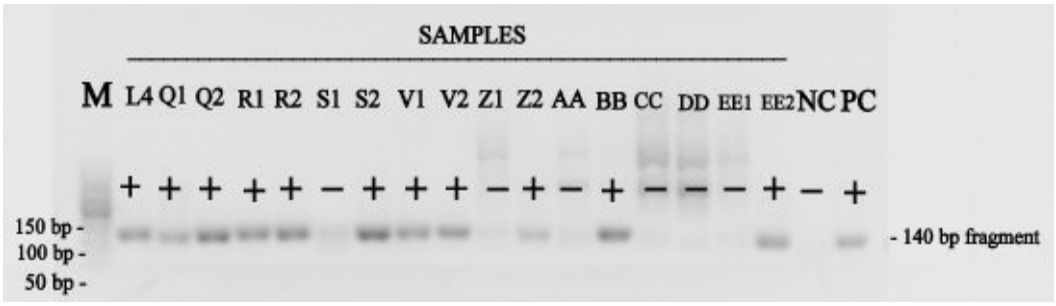

Figure 7: Diagnosis of orf virus in goats. Confirmatory diagnosis was carried out by amplification of *RNA polymerase* gene using Orf1 and Orf2 primers by polymerase chain reaction (PCR). The PCR product after PCR amplification is 140 bp seen in the positive control (PC) and samples in goats with ORFV (+). Samples that are negative are indicated with a (–) sign. M = DNA Maker.

4.3 Genotyping of ORFV

A PCR test was carried out to amplify the full length of *B2L* gene in 11 samples including the positive control using ORVB2LfF and ORVB2LfR primers. Ten out of 11 samples produced a PCR product that was sequenced (Fig. 8 and Table 5).

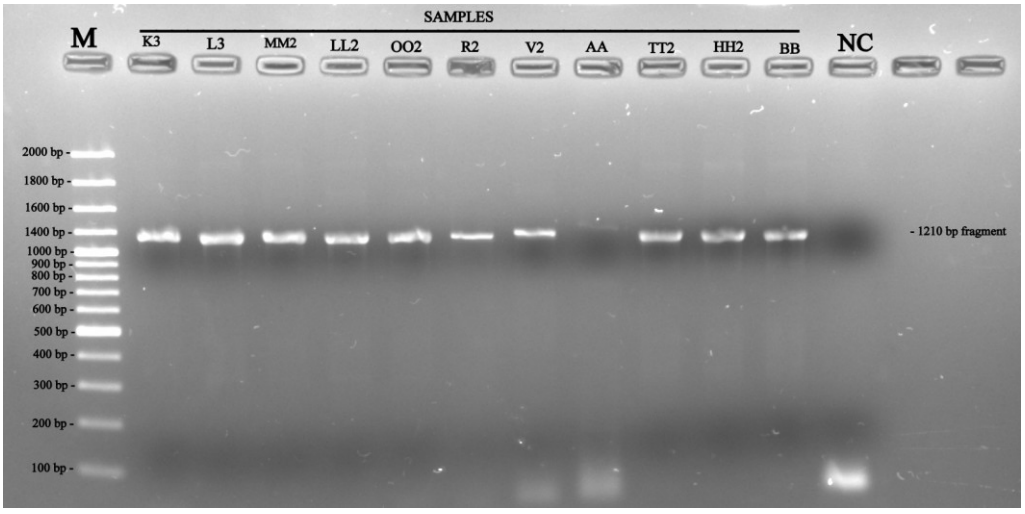

Figure 8: Amplification of ORFV B2L gene for sequencing. The *B2L* was amplified using ORVB2LfF and ORVB2LfR primers. The PCR product after PCR amplification is 1 200 bp seen in positive samples. The negative sample (Z1) does not have a band. M = Marker and NC = Negative Control.

Table 5: Goats with orf whose samples were used for sequencing. The samples which produced a good band worthy of sequencing were collected from Kimanga in Ilala, Mapinga in Bagamoyo and Dondwe, Msongola, Kakubiro and Mwanzomgumu in Kisarawe.

S/n	Sample code	Sample origin	District	Date of sampling
1	K3	Kimanga1	Ilala	26 May, 2015
2	L3	Kimanga2	Ilala	26 May, 2015
3	MM2	Dondwe1	Kisarawe	06 June, 2015
4	LL2	Dondwe2	Kisarawe	06 June, 2015
5	OO2	Msongola	Kisarawe	06 June, 2015
6	R2	Mapinga1	Bagamoyo	17 May, 2015
7	V2	Mapinga2	Bagamoyo	17 May, 2015
8	TT2	Kakubiro	Kisarawe	06 June, 2015
9	HH2	Mwanzomgumu	Kisarawe	06 June, 2015
10	BB	Kyela	Kyela	15 March, 2013

The 10 positive samples for *B2L* gene were sequenced. The sequences were processed and aligned to determine various parameters of interest. There was substitution of nucleotides at some locations (Appendix 2) but most of them did not cause changes in the amino acid sequence. However, three substitutions resulted in change of amino acids. Two sequences (BB ans R2) had alanine at position 126 while all others had Threonine. Six sequences had asperagine at position 267 while the other four had aspartic acid. And at position 328 all sequences had threonine except sequence K3 and L3 that had isoleucine (Apendix 3). Estimation of evolutionary divergence between the sequences revealed an overall average of 0.01% (Table 6).

Table 6: Estimates of Evolutionary Divergence between Tanzania ORFV sequences. The left diagonal shows the number of base substitutions per site from between sequences and the above diagonal shows the standard error estimates.

	1	2	3	4	5	6	7	8	9	10
1		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.01	0.01		0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.01	0.01	0.00		0.00	0.00	0.00	0.00	0.00	0.00
5	0.01	0.01	0.00	0.00		0.00	0.00	0.00	0.00	0.00
6	0.01	0.01	0.01	0.01	0.01		0.00	0.00	0.00	0.00
7	0.01	0.01	0.00	0.00	0.00	0.00		0.00	0.00	0.00
8	0.01	0.01	0.00	0.00	0.00	0.01	0.00		0.00	0.00
9	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00		0.00
10	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	

1 = K3-Kimanga1, 2 = L3-Kimanga2, 3 = MM2-Dondwe1, 4 = LL2-Dondwe2, 5 = OO2-Msongola, 6 = R2-Mapinga1, 7 = V2-Mapinga2, 8 = TT2-Kakubiro, 9 = HH2-Mwanzomgumu, 10 = BB-Kyela

The MEGA software was also used to construct a tree to depict evolutionary relationship of ORFV isolates in this study. ORFV from three villages in Kisarawe district clustered together, whereas ORFV from one village in Kisarawe district clustered together with ORFV from a village in Bagamoyo district. ORFV in Ilala district clustered together and were identical by 100%. ORFV from one location in Bagamoyo did not cluster with any other virus. Likewise, the ORFV collected from Kyela in 2013 was different from all others (Fig. 9).

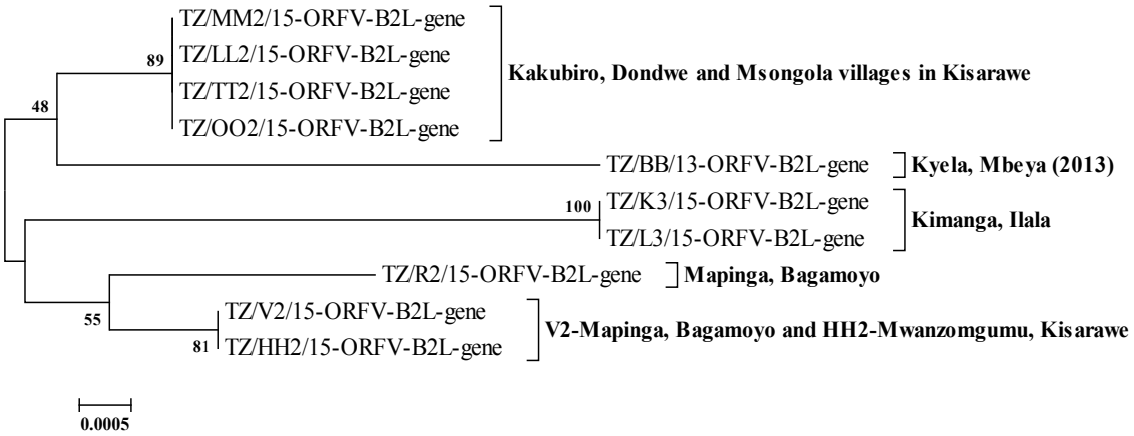

Figure 9: Evolutionary relationship of Tanzania ORFV. The evolutionary history was inferred using the Minimum Evolution (ME) method and the evolutionary distances were computed using the Maximum Composite Likelihood method using MEGA6.05.

4.4 Genetic characteristics of ORFV

The nucleotide sequences obtained in the present study were queried at GenBank using BLASTn in order to search for similar sequences. The results of BLASTn are shown in Table 7. The Tanzanian ORFV were highly similar (99%) to other ORFV of USA previously reported by Delhon *et al.* (2004). However a BLASTn search at GenBank for amino acid sequences of Tanzanian ORFV showed high similarity to ORFV strain for China and South Korea in addition to those of USA (Table 8).

A number of ORFV belonging to cluster I, II, and III were retrieved from GenBank and used for constructing phylogenetic tree along with Tanzanian ORFV. The result show that the Tanzanian isolates cluster to cluster I of ORFV (Fig. 10).

Table 7: Genetically related ORFV isolates based on nucleotide sequence identity. The ORFV isolates resulted from basic local alignment search tool (BLASTn) for each of the nucleotide sequence obtained in this study.

TZ ORFV isolate	GeneBank isolate	Country	Animal isolated from	Year	Max score	Total score	Query cover	Identity	Accession number	Reference
K3	OV-SA00	USA	Goat	2003	1977	1977	100%	99%	AY386264	Delhon *et al*., 2004
L3	OV-SA00	USA	Goat	2003	1977	1977	100%	99%	AY386264	Delhon *et al*., 2004
MM2	OV-SA00	USA	Goat	2003	2010	2010	100%	99%	AY386264	Delhon *et al*., 2004
LL2	OV-SA00	USA	Goat	2003	2010	2010	100%	99%	AY386264	Delhon *et al*., 2004
OO2	OV-SA00	USA	Goat	2003	2010	2010	100%	99%	AY386264	Delhon *et al*., 2004
R2	OV-SA00	USA	Goat	2012	1988	1988	100%	99%	AY386264	Delhon *et al*., 2004
V2	OV-SA00	USA	Goat	2011	1999	1999	100%	99%	AY386264	Delhon *et al*., 2004
TT2	OV-SA00	USA	Goat	2003	2010	2010	100%	99%	AY386264	Delhon *et al*., 2004
HH2	OV-SA00	USA	Goat	2011	1999	1999	100%	99%	AY386264	Delhon *et al*., 2004
BB	OV-SA00	USA	Goat	2012	1971	1971	100%	99%	AY386264	Delhon *et al*., 2004

Table 8: Genetically related ORFV isolates based on amino acid sequence identity. The ORFV isolates resulted from basic local alignment search tool (BLASTn) for each of the corresponding amino acid sequence of ORFV obtained in this study.

TZ ORFV isolate	GeneBank isolate	Country	Animal isolated from	Year	Max score	Total score	Query cover	Identity	Accession number	Reference
K3	ORFD(OV-SA00)	USA	Goat	2003	749	749	100%	99%	NP_957788	Delhon *et al*., 2004
L3	ORFD(OV-SA00)	USA	Goat	2003	749	749	100%	99%	NP_957788	Delhon *et al*., 2004
MM2	ORFD(OV-SA00)	USA	Goat	2003	751	751	100%	99%	NP_957788	Delhon *et al*., 2004
LL2	ORFD(OV-SA00)	USA	Goat	2003	751	751	100%	99%	NP_957788	Delhon *et al*., 2004
OO2	ORFD(OV-SA00)	USA	Goat	2003	751	751	100%	99%	NP_957788	Delhon *et al*., 2004
R2	FJ-SJ1	China	Goat	2012	752	752	100%	99%	AGY41807	Chi *et al*., 2013
V2	ORF/2011/B2L	South Korea	Goat	2011	752	752	100%	99%	AGA96103	Unpublished
TT2	ORFD(OV-SA00)	USA	Goat	2003	751	751	100%	99%	NP_957788	Delhon *et al.*, 2004
HH2	ORF/2011/B2L	South Korea	Goat	2011	752	752	100%	99%	AGA96103	Unpublished
BB	FJ-SJ1	China	Goat	2012	752	752	100%	99%	AGY41807	Chi *et al*., 2013

Table 9: Strains of ORFV (B2L gene) used to construct phylogenetic tree. The strains of ORFV *B2L* gene were retrieved from GeneBank database.

Isolate	Country	Town	Year isolated	Accession Number	Genotype	Reference
K2-SD	Sudan		2013	KR231670	IV	Khalafalla *et al*., 2015
Jodhpur	India	Jodhpur	2008	GQ390365	IV	Lucinda *et al*., 2010
HB1	China	Hubei	2010	KJ610838	I	Unpublished
Mukteswar/09	India	Mukteswar	2009	GU139356	III	Unpublished
OV-IA82	USA	Iowa	1982	AY386263	II	Delhon *et al*., 2004
GX/YB	China		2011	JQ904793	I	Zang *et al*., 2014
NE2	Brazil		1993	JN088051	I	Unpublished
India 67/04	India	Izatnagar	2004	DQ263305	III	Hosamani *et al*., 2006
D1701	Germany		2010	HM133903	I	Unpublished
OV-SA00	USA	Texas	2003	AY386264	I	Delhon *et al*., 2004
ORFV	USA		2003	AY424971	II	Guo *et al*., 2004
SD/DY	China		2010	JQ904794	II	Zang *et al*., 2004
ORFV	USA		2003	AY278208	II	Guo *et al*., 2003
NZ-2	New Zealand		1994	U06671	II	Sulivan *et al*., 1994
Isolate D	Brazil		1992	JN088052	II	Unpublished

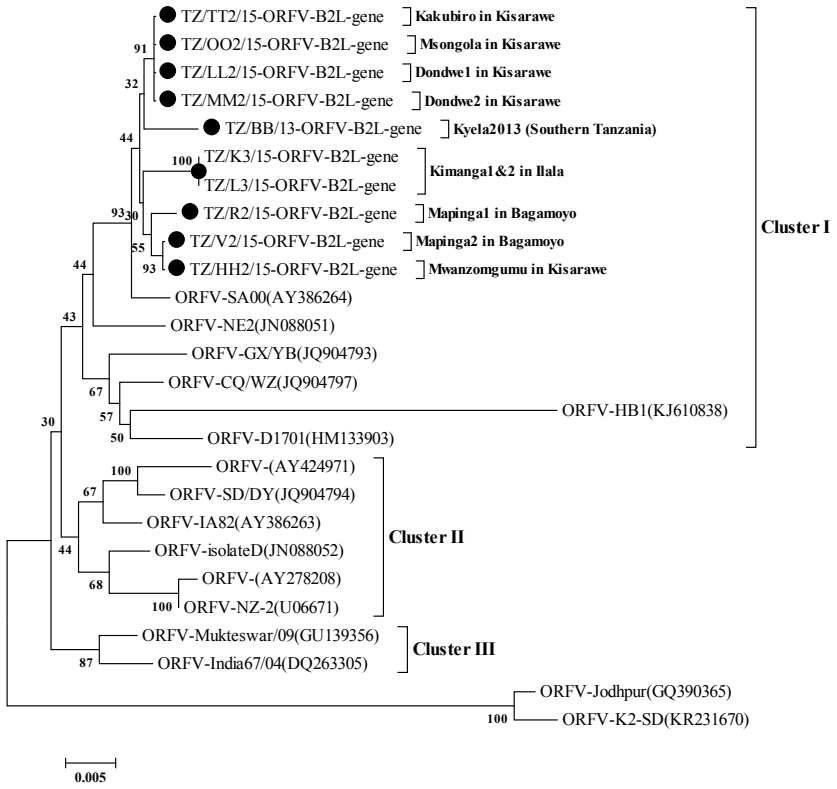

Figure 10: Phylogenetic relationship between Tanzanian and other ORFV. The consensus tree was constructed based on the nucleotide sequences alignments of the *B2L* gene homologue using the Neighbor-Joining method using MEGA6 software. Tanzanian ORFV are indicated by a black dot.

CHAPTER FIVE

5.0 DISCUSSION

Small ruminants form a backbone of livelihood of most rural poor families who rely on them as source of not only milk and meat for family consumption but also of income that is easily mobilized for paying household expenditure. Therefore, any disease affecting these animals has an economic importance more so when that disease also has a potential for infecting human beings. Orf or contagious ecthyma is a highly contagious viral disease of sheep, goats and occasionally humans which is worldwide in distribution (McElroy and Bassett, 2007). It is caused by orf virus a type species of the parapoxvirus genus in the family *Poxviridae* (Robinson and Lyttle 1992). Because of its contagious nature, public health aspects and economic importance this study was carried out to detect and characterize by molecular means the virus causing a disease reported by farmers that presented orf-like clinical signs. The first case was reported by a farmer at Mapinga village in Bagamoyo district who previously bought 10 goats from a village in Kisarawe district of which all died showing similar signs as those were sick at the time of visitation. A nearby farmer also reported similar cases. Upon examination, the affected animals presented typical ORFV lesions including ulcerations at the nasal and oral mucosa accompanied with pustular dermatitis and in some cases severe yellowish to brownish crust formation on the lips commissures which spread to the muzzle and nostrils (Fig. 6) and occasionally trunk skin and teats. Similar clinical signs were reported in other places of districts of Bagamoyo, Kisarawe, Ilala and Kinondoni. These places were visited for the purpose of clinical examination and sampling for laboratory testing and molecular characterization (Appendix 1).

Eleven farmers with a total of 259 goats were visited. 72 goats presented orf-like clinical

signs and 24 goats were reported to have died making crude morbidity and mortality rates of 28% and 9% respectively (Appendix 1). Both crude morbidity and mortality rates observed were lower compared to other outbreaks reported (Lojkic *et al*., 2010; Zhang *et al*., 2010) . Among the sick animals a mild increase in body temperature was observed (39.8 - 40.2^{o}C) in agreement with what was reported by Nandi *et al*. (2011). Oral swabs, scabs and skin scrapings were collected from 22 goats for detection of ORFV as previously described (Yaegashi *et al*., 2013). Sixteen goats (73%) out of the 22 goats tested positive.

A total of 48 samples were tested including 25 swabs, 14 scabs, and 9 skin scrapings (Table 4). Fourteen out of 25 swabs (56%) tested positive, whereas 11 scabs (79%) tested positive and 7 skin scrapings (78%) tested positive. This observation suggests that ORFV virus can easily be detected in scabs or skin scrapings than oral swabs. All samples from Kwambwera village in Bagamoyo district and Mbezi area in Kinondoni district (27%) tested negative suggesting that could be the animals in that village were infected with a different virus like FMD or PPR as previously described by Hosamani *et al*. (2009) and Nandi *et al*. (2011).

Sequencing of the *B2L* gene from 10 isolates produced sequences of 1106 bp that were closely related as revealed by calculations of evolutionary differences (Table 7). The sequences had a GC content of 64. 4% that was in agreement with previously established parameter (Moss, 2001). This suggests that the isolates could have same origin but minor differences might have resulted from geographical differences following separation for a period of time. This observation was similar to that observed by Lojkic *et al*. (2010). Sequence analysis of the nucleotide and deduced amino acids of the ORFV *B2L* gene showed that the Tanzania isolates shared close relationship with other ORFV isolates from

different regions (94% - 99% and 83% - 99% respectively) and all shared the highest homology (99% at nucleotide level) with OV-SAOO isolated in 2003 from USA. At amino acid level, isolates K3, L3, MM2, LL2, OO2 and TT2 shared the highest homology (99%) with isolate ORFD from the same strain of ORFV (OV-SA00), whereas isolates R2 and BB shared the highest homology (99%) with isolate FJ-SJ1 isolated in 2012 from China and isolates V2 and HH2 shared the highest homology (99%) with isolate ORF/2011/B2L isolated in 2011 from South Korea.

Phylogenetic analysis of ORFV obtained from this study produced a distinct branch on genotype/cluster I of the phylogenetic tree (Figure 5). The results presented here suggest that the OV-SA00 strain of ORFV was introduced in Tanzania but it is difficult to determine the route through which the strain entered into Tanzania because there are no sequences in the GeneBank from neighbouring countries which would be of help in explaining the genetic relatedness.

Evolutionary relationship of ORFV isolates in this study (Figure 6) reveal that isolate HH2 (from Mwanzomgumu village in Kisarawe district) clustered together with isolate V2 (from Mapinga village in Bagamoyo district). V2 was collected from a flock whose owner had prior introduced 10 goats from Kisarawe (Appendix 1) before the onset of the disease. It suggests that those animals were infected (all died) and therefore could have introduced the disease in the flock which from where it spread to other flocks which shared grazing area (isolate R2).

Virus spread to other villages might have been contributed by free movement of animals in this region owing to the big livestock market at Pugu in Dar es Salaam which saves as a source of livestock for many farmers. The results indicated that the disease was endemic

in Kisarawe because the virus which was detected in Kyela in 2013 (isolate BB) originated from there. Isotates MM2, LL2, TT2 and OO2 clustered together and all were from neighbouring villages of Kakubiro, Dondwe and Msongola in Kisarawe district whose flocks share grazing and watering area. Isolates K3 and L3 were identical because they were collected from the same flock at Kimanga in Ilala district. This observation agrees with what Li *et al*. (2012) hypothesized that the genetic diversity among ORFV strains mainly relate to geographic location, viral strain differences, flock immunity, genetic susceptibility or method of exposure.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The present study described a severe outbreak of orf or contagious ecthyma in small family flocks of goats in different locations of Bagamoyo, Ilala, Kinondoni and Kisarawe districts in eastern Tanzania and identified the causative agent as an ORFV. However, ORFV could not be confirmed as a causative agent of a disease that presented orf-like clinical signs at Kwambwera village in Bagamoyo district and Mbezi area in Kinondoni district.

The study revealed that the circulating strains of ORFV were closely related to an ORFV detected from a goat in Kyela, Mbeya in 2013. Molecular characterization and phylogenetic analysis revealed that ORFV circulating in eastern Tanzania is genetically closely related with other ORFV isolates from distant geographical locations. This makes difficult in explaining the evolutionary or geographical origin of the ORFV in this study due to lack of *B2L* gene sequences in the GeneBank from neighbouring counties.

Free movement of animals, insufficient veterinary services (testing of animals before purchase) and lack of basic knowledge on disease control and prevention on part of livestock keepers are suggestive of contributing factors of spread of the outbreak from the point of origin. Moreover, the persistent nature of the virus in the environment and the fact that some animals can carry the virus without showing any clinical signs (Hosamani *et al*., 2009) could be the reason for the outbreak of the disease when animals are stressed.

6.2 Recommendations

Although there are limited reports about orf infections in Tanzania, this study suggests that orf disease could be endemic in Tanzania just as many researchers have reported that orf disease is found wherever goats and sheep are reared. Sheep and goats are commonly kept under free range system in Tanzania sharing pasture and watering points with wild animals which also are susceptible to orf infections. The disease is well known to livestock keepers in Kisarawe district and they associate it with dew, thinking that animals get infected when they graze on dew covered grasses (personal communication). This thinking suggests that the onset of the disease follows the beginning of the rain season; however, more studies are required to determine the extent and associated risk factors of ORFV infections in Tanzania not only in livestock but also in wildlife. Complete genome sequencing studies are required to be able to understand fully the genetic diversity and molecular epidemiology of this virus in the country.

ORFV and other Parapoxvirus infections have been identified in other parts of the world as cause of zoonotic diseases in people who are normally in contact with animals including farmers, veterinarians and butcher men (Georgiades *et al*., 2005; CDC, 2012). Such cases however, have never been reported in Tanzania and the reason could be that orf is a neglected zoonosis and they are likely to be under-recognized by medical practitioners. As pointed out in the literature review section, ORFV and other parapoxvirus infections in humans can cause painful lesions on the hands, fingers and face, and in some cases lymphadenopathy (CDC, 2012). It also shares risk factors with life-threatening diseases like anthrax (Leavell *et al*., 1968). Missed or mistaken diagnosis poses huge consequences on certain categories of patients, such as children and immunocompromised individuals (Lederman *et al*., 2014). The results in the current study therefore calls for thorough investigations that will involve human population to determine

the zoonotic capability of the circulating ORFV in Tanzania.

Veterinary laboratories in the country should be well equipped to be able to provide diagnostic services in time. Farmers should be encouraged to report and seek advice from veterinarians once they observe onset of abnormal conditions among their animals. Blanket treatment that is normally provided whenever animals are perceived to be sick should be avoided as it encourages wide spread of viral diseases while inflicting considerable losses.

Farmers, veterinarians and all other stakeholders involved in the livestock industry should be made aware of the risk they face. Farmers should be given basic knowledge of disease control and prevention. For instance, to be sure that newly introduced animals are free from diseases, they should be kept separately for a time period before are mixed with the existing animals. Animals showing signs of ill-health should be separated from the rest of animals to avoid spread of the disease to other animals. Most importantly, animals should be provided with well balanced diet, enough water and clean environment to ensure strong body immunity that will successfully neutralize any invading pathogen.

REFERENCES

Allworth, M.B.; Hughes, K.L. and Studdert, M.J. (1987). Contagious pustular dermatitis (orf) of sheep affecting ear following ear tagging. *Australia Veterinary Journey*, 64(2): 61–62.

Azwai, S.M.; Carter, S.D. and Woldehiwet, Z. (1995). Immune responses of the camel (*Camelus dromedarius*) to contagious ecthyma (Orf) virus infection. *Veterinary Microbiology*, 47(1-2): 119–131.

Barraviera, S.R.C.S. (2005). Diseases caused by poxvirus-orf and milker's nodule-a review. *Journal of Venomous Animals and Toxins Including Tropical Diseases*, 11(2): 102–108.

Bassioukas, K.; Orfanidou, A.; Stergiopoulou, C.H. and Hatzis, J. (1993). Orf: Clinical and epidemiological study. *Australasian Journal of Dermatology*, 34(3): 119–123.

CDC. (April13, 2012). Human orf virus infection from household exposures-United States, 2009-2011. *Morbidity and Mortality Weekly Report*, 61(14): 245–248.

Chi, X.; Zeng, X.; Hao, W.; Li, M.; Li, W.; Huang, X.; Wang, S. and Luo, S. (2013). Heterogeneity among orf virus isolates from goats in Fujian province, southern China. *Journal Plos One*, 8(10): E66958.

Czerny, C.P.; Waldmann, R. and Scheubeck, T. (1997). Identification of three distinct antigenic sites in parapoxviruses. *Archives of Virology*, 142: 807–821.

de la Concha-Bermejillo, A.; Guo, J.; Zhang, Z. and Waldron, D. (2003). Severe persistent orf in young goats. *Journal of Veterinary Diagnostic Investigation*, 15(5): 423–431.

Deane, D.; McInnes, C. J.; Percival, A.; Wood, A.; Thomson, J.; Lear, A.; Gilray, J.; Fleming, S.; Mercer, A. and Haig, D. (2000). Orf virus encodes a novel secreted

protein inhibitor of granulocyte-macrophage colony-stimulating factor and interleukin-2. *Journal of Virology*, 74(3): 1313–1320.

Delhon, G.; Tulman, E.R.; Afonso, C.L.; Lu, Z.; de la Concha-Bermejillo, A.; Lehmkuhl, H.D.; Piccone, M.E.; Kutish, G.F. and Rock, D.L. (2004). Genomes of the parapoxviruses orf virus and bovine papular stomatits virus. *Journal of Virology*, 78(No. 1): 168–177.

Dory, D.; Fischer, T.; Beven, V.; Cariolet, R.; Rziha, H.J. and Jestin, A. (2006). Prime-boost immunization using DNA vaccine and recombinant Orf virus protects pigs against Pseudorabies virus (*Herpes suid* I). *Vaccine*, 24(37-39): 6256–6263.

Dupre, A.; Auvergnat, J.; Durand, R. and Catala, D. (1978). Orf and milker's nodule. *Anneles de Dermatoloqie et de Venereologie*, 105(2): 213–214.

Esposito, J. and Fenner, F. (2001). Poxviruses. In: *Fields Virology* (*4th edition*). (Edited by Fields, B.N., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, Bernad, Straus, S.E., Knipe, D.M.) Lippincott Williams & Wilkins Publishers, Philadelphia. Chapter 85.

Fleming, S.B.; Anderson, I.E.; Thomson, J.; Deane, D.L.; McInnes, C.J.; McCaughan, C.A.; Mercer, A.A. and Haig, D.M. (2007). Infection with recombinant orf viruses demonstrates that the viral interleukin-10 is a virulence factor. *Journal of General Virology*, 88(7): 1922–1927.

Gallina, L.; Dal Pozzo, F.; McInnes, C.J.; Cardeti, G.; Guercio, A.; Battilani, M.; Ciulli, S. and Scagliarini, A. (2006). A real time PCR assay for the detection and quantification of orf virus. *Journal of Virological Methods*, 134(1-2): 140–145.

Georgiades, G.; Katsarou, A. and Dimitroglou, K. (2005). Human orf (ecthyma contagiosum). *Journal of Hand Surgery-British and European Volume*, 30(4): 409–411.

Gilray, J.A.; Nettleton, P.F.; Pow, I.; Lewis, C.J.; Stephens, S.A.; Madeley, J.D. and Reid, H.W. (1998). Restriction endonuclease profile of orf virus isolates from the British Isles. *Veterinary Record*, 143: 237–240.

Glover, R.E. (1928). Contagious pustular dermatitis of the sheep. *Journal of Complementary Pathology*, 41: 318–340.

Guo, J.; Rasmussen, J.; Wünschmann, A. and de la Concha-Bermejillo, A. (2004). Genetic characterisation of orf viruses isolated from various ruminant species of a zoo. *Veterinary Microbiology*, 99(2): 81–92.

Guo, J.; Zhang, Z.; Edwards, J.F.; Ermel, R.W.; Taylor, C. Jr. and de la Concha-Bermejillo, A. (2003). Characterisation of North American orf virus isolated from a goat with persistent, proliferative dermatitis. *Virus Research*, 93(2): 169–179.

Hagis, A.M. and Ginn, P.E. (2001). Integumentary system. In: *Thompsons Special Veterinary Pathology* (Edited by McGavin, M.D., Carlton, W.W. and Zachary, J.F.). Mosby, Inc., London.

Hausawi, F.; Abu-elzein, E. and Gameel, A. (2004). Severe ausdyk infection in one-month-old camel calves (*Camelus dromedarius*). *Veterinarski Arhiv*, 74(6): 467–474.

Hosamani, M.; Scagliarini, A.; Bhanuprakash, V.; Mclnnes, C.J. and Singh, R.K. (2009). Orf: an update on current research and future perspectives. *Expert Reviews of Anti-Infective Therapy*, 7(7): 879–893.

Huerter, C.J.; Alvarez, L. and Stinson, R. (1991). Orf: Case report and literature review. *Cleveland Clinic Journal of Medicine*, 58(6): 531–534.

Inger, K.D. (2001). Poxviruses. In: *Fields Virology* (*5th edition*). (Edited by Fields, B.N., Knipe, D.M., Howley, P.M. and Griffin, D.E.) Lippincott Williams & Wilkins Publishers, Philadelphia. pp. 2948-2975.

Inoshima, Y.; Morooka, A. and Sentsui, H. (2000). Detection and diagnosis of parapoxvirus by polymerase chain reaction. *Journal of Virological Methods*, 84(2): 201–208.

Inoshima, Y.; Shimizu, S; Minamoto, N.; Hirai, K. and Sentsui, H. (1999). Use of protein AG in an enzyme linked immunosorbent assay for screening for antibodies against parapoxvirus in wild animals in Japan. *Clinical and Diagnostic Laboratory Immunology*, 6(3): 388–391.

Jenkinson, D.M.; Hutchison, G. and Reid, H.W. (2008). The B and T cell responses to orf virus infection of ovine skin. *Veterinary Dermatology*, 3(2): 57–64.

Khalafalla, A.I.; El-Sabagh, I.M.; Al-Busada, K.A.; Al-Mubarak, A.I. and Ali, Y.H. (2015). Phylogenetic analysis of eight Sudanese camel contagious ecthyma viruses based on *B2L* gene sequence. *Virology Journal*, 12(1): 124.

Klein, J. and Tryland, M. (2005). Characterisation of parapoxviruses isolated from Norwegian semi-domesticated reindeer (*Rangifer tarandus tarandus*). *Virology Journal*, 2(79).

Kottaridi, C.; Nomikou, K.; Lelli, R.; Markoulatos, P. and Mangana, O. (2006). Laboratory diagnosis of contagious ecthyma: Comparison of different PCR protocols with virus isolation in cell culture. *Journal of Virological Methods*, 134(1-2):119–124.

Kottaridi, C.; Nomikou, K.; Teodori, L.; Savini, G.; Lelli, R.; Markoulatos, P. and Mangana, O. (2006). Phylogenetic correlation of Greek and Italian orf virus isolated based on VIR gene. *Veterinary Microbiology*, 116(4): 310–316.

Leavell, U.V.; Mac Namara, M.J.; Muelling, R.; Talbert, W.M.; Rucker, R.C. and Dalton, A.J. (1968). Orf. Report of 19 human cases with clinical and pathological observation. *Journal of American Veterinary Medicine Association*, 204(8): 657-664.

Lederman, E.; Khan, S.U.; Luby, S.; Zhao, H.; Braden, Z.; Gao, J.; Karem, K.; Damon, I.; Renolds, M. and Li, Y. (2014). Zoonotic parapoxviruses detected in symptomatic cattle in Bangladesh. *BMC Research Notes*, 7(816).

Lederman, E.R.; Green, G.M. and DeGroot, H.E. (2007). Progressive orf infection in a patient with lymphoma: successful treatment using imiquimod. *Clinical Infectious Diseases*, 44(11): e100–e103.

Li, W.; Ning, Z.; Hao, W.; Song, D.; Gao, F.; Zhao, K.; Liao, X.; Li, M.; Rock, D. and Luo, S. (2012). Isolation and phylogenetic analysis of orf virus from the sheep flock outbreak in northeast China. *BMC Veterinary Research*, 8(1): 229.

Lloyd, J.B.; Gill, H.S.; Haig, D.M. and Husband, A.J. (2000). In vivo T cell subset depletion suggests that CD4+ T-cells and a humoral immune response are important for the elimination of orf virus from the skin of sheep. *Veterinary Immunology and Immunopathology*, 74(3-4): 249–262.

Lojkic, I.; Cac, Z.; Beck, A.; Bedekovic, T.; Cvetnic, Z. and Sostaric, B. (2010). Phylogenetic analysis of Croatian orf viruses isolated from sheep and goats. *Virology Journal*, 7(314).

Lucinda, N.; Inoue-Nagata, A.K.; Kitajima, E.W. and Nagata, T. (2010). Complete genome sequence of *Brugmansia suaveolens* mottle virus, a potyvirus from an ornamental shrub. *Archives of Virology*, 155(10): 1729-1732.

Mahmud, A.K.M.F.; Rahman, K.M.Z.; Dey, S.K.; Islam, T. and Talukder, A.A. (2014). Genome annotation and comparative genomics of orf virus. *Advances in Microbiology*, 04(15): 1117–1131.

Marsland, B.J.; Tisdall, D.J.; Heath, D.D. and Mercer, A.A. (2003). Construction of a recombinant orf virus that expresses an *Echinococcus granulosus* vaccine antigen from a novel genomic insertion site. *Archive of Virology*, 148(3): 555–562.

Mazur, C.; Ferreira, I.I.; Rangel, F.F.B. and Galler, R. (2000). Molecular characterisation of Brazilian isolates of orf virus. *Veterinary Microbiology*, 73(4): 253–259.

Mazur, C. and Machado, R.D. (1989). Detection of contagious pustular dermatitis virus of goats in a severe outbreak. *Veterinary Record*, 125(16): 419–420.

Mazur, C.; Rangel, F.F.B. and Galler, R. (1991). Molecular analysis of contagious pustular dermatitis virus: a simplified method for viral DNA extraction from scab material. *Journal of Virological Methods*, 35(3): 265–272.

McElroy, M.C. and Bassett, H.F. (2007). The development of oral lesions in lambs naturally infected with orf virus. *The Veterinary Journal*, 174(3): 663 – 664.

McFadden, G. (2005). Poxvirus tropism. *Nature Reviews Microbiology*, 3(3): 201–213.

McGuire, M.J.; Johnston, S.A. and Sykes, K.F. (2012). Novel immune-modulator identified by a rapid, functional screen of the parapoxvirus ovis (orf virus) genome. *Proteome Science*, 10(1): 4

McKeever, D.J.; Jenkinson, D.M.; Hutchison, G. and Reid, H.W. (1988). Studies on the pathogenesis of orf virus infection in sheep. *Journal of Complementary Pathology*, 99(3): 317–328.

McKeever, D.J.; Reid, H.W.; Inglis, N.F. and Herring, A.J. (1987). A qualitative and quantitative assessment of the humoral antibody response of the sheep to orf virus infection. *Veterinary Microbiology*, 15(3): 229–241.

Mercer, A.A.; Ueda, N.; Friederichs, S.M.; Hofmann, K.; Fraser, K.M.; Bateman, T. and Fleming, S.B. (2006). Comparative analysis of genome sequences of three isolates of orf virus reveals unexpected sequence variation. *Virus Research*, 116(1-2): 146–158.

Mercer, A.; Fleming, S.; Robinson, A.; Nettleton, P. and Reid, H. (1997). Molecular genetic analyses of parapoxviruses pathogenic for humans. *Archive of Virology*, 13: 25–34.

Moss, B. (2001). Poxviridae: The Viruses and their replication. In: *Fields Virology 5th edition*, *Vol. One*. (Edited by Fields, B.N., Knipe, D.M., Howley, P.M. and Griffin, D.E.) Lippincott Williams & Wilkins Publishers, Philadelphia. pp. 2906–2945.

Mwanandota, J.J.; Macharia, M.; Mpelumbe-Ngeleja, C.A.R.; Yongolo, M. and Holton, T.A. (2014). Diagnosis and phylogenetic analysis of orf virus from goats in Tanzania. In: *Book of Abstracts, 32nd Tanzania Veterinary Association Scientific Conference*. (Edited by Karimuribo, E.D. and Kivaria, F.M.) 25-27 November, 2014, AICC, Arusha, Tanzania, p. 25.

Nandi, S.; De, U.K. and Chowdhury, S. (2011). Current status of contagious ecthyma or orf disease in goat and sheep–A global perspective. *Small Ruminant Research*, 96(2): 73–82.

Nettleton, P.F.; Gilray, J.A.; Yirrell, D.L.; Scott, G.R. and Reid, H.W. (1996). Natural transmission of orf virus from clinically normal ewes to orf-naive sheep. *Veterinary Record*, 139(15): 364–366.

Nitsche, A.; Buttner, M.; Wilhelm, S.; Pauli, G. and Meyer, H. (2006). Real-time PCR: detection of parapoxvirus DNA. *Clinical Chemistry*, 52(2): 316–319.

Oem, J.K.; Roh, I.S.; Lee, K.H.; Lee, K.K.; Kim, H.R.; Jean, Y.H. and Lee, O.S. (2009). Phylogenetic analysis and characterisation of Korean orf virus from dairy goats: case report. *Virology Journal*, 6(1): 167.

Pickup, D.J. (2015). Poxviruses. In: *eLS.* John Wiley & Sons Ltd, Chichester. pp. 1–13.

Rafii, F. and Burger, D. (1985). Comparison of contagious ecthyma virus genomes by restriction endonucleases. *Archive of Virology*, 84(3-4): 283–289.

Robinson, A.J. and Balassu, T.C. (1981). Contagious pustular dermatitis (Orf). *The Veterinary Bulletin*, 51: 771–782.

Robinson, A.J.; Ellis, G. and Balassu, T. (1982). The genome of orf virus: restriction endonuclease analysis of viral DNA isolated from lesion of orf in sheep. *Archive of Virology*, 71(1): 43–55.

Robinson, A.J. and Lyttle, D.J. (1992). Parapoxviruses: their biology and potential as recombinant vaccines. *Recombinant Poxviruses*, 28: 285-327.

Robinson, A.J. and Mercer, A.A. (1995). Parapoxvirus of red deer: evidence for its inclusion as a new member in the genus parapoxvirus. *Virology Journal*, 208(2): 812–815.

Said, A.A.; Mohamed, S.I.; Abd Elhamid, N.K.; Hosny, W.A. and Baheeg, E.M. (2013). Trials for isolation of contagious pustular dermatitis virus (CPDV) from sheep in Ismailia governorate. *Research in Zoology*, 3(1): 10–14.

Saravanan, P.; Balamurugan, V.; Sen, A. and Sahay, B. (2007). Mixed infection of peste des petites ruminants and orf on a goat farm at Shahjahanpur, India. *Veterinary Record*, 160(12): 410–412.

Sargison, N.D.; Scott, P.R. and Rhind, S.M. (2007). Unusual outbreak of orf affecting the body of sheep associated with plunge dipping. *Veterinary Record*, 160(11): 372–373.

Sullivan, J.T.; Mercer, A.A.; Fleming, S.B. and Robinson, A.J. (1994). Identification and characterisation of an orf virus homologue of the vaccinia virus gene encoding the major envelope antigen p37K. *Virology*, 202(2): 968-973.

Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A. and Kumar, S. (2013). MEGA 6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, 30(12): 2725-2729.

Thomas, K.; Tompkins, D.M.; Sainsbury, A.W.; Wood, A.R.; Dalziel, R.; Nettleton, P.F. and McInnes, C.J. (2003). A novel poxvirus lethal to red squirrels (*Sciurus vulgaris*). *Journal of General Virology*, 84(Pt 12): 3337–3341.

Torfason, E.G. and Gunadóttir, S. (2002). Polymerase chain reaction for laboratory diagnosis of orf virus infections. *Journal of Clinical Virology*, 24(1-2): 79–84.

Tryland, M.; Klein, J.; Nordoy, E.S. and Blix, A.S. (2005). Isolation and partial characterization of a parapoxvirus isolated from a skin lesion of Weddell seal. *Virus Research*, 108(1-2): 83–87.

Watson, P. (2004). Differential diagnosis of oral lesions and FMD in sheep. *In Practice*, 26: 182–191.

Westphal., D.; Ledgerwood, E.C.; Hibma, M.H.; Fleming, S.B.; Whelan, E.M. and Mercer, A.A. (2007). A novel Bcl-2-like inhibitor of apoptosis is encoded by the parapoxvirus orf virus. *Journal of Virology*, 81(13): 7178–7188.

Wilson, D.J.; Scott, P.R.; Sargison, N.D.; Bell, G. and Rhind, S.M. (2002). Effective treatment of severe facial dermatitis in lambs. *Veterinary Record*, 150(2): 45–46.

Yaegashi, G.; Sasaki, I.; Chiba, S. and Murakami, K. (2013). Molecular analysis of parapoxviruse detected in eight calves in Japan. *Journal of Veterinary Medical Science*, 75(10): 1399–1403.

Yeruham, D.L.; Perlb, S. and Abrahamc, A. (2000). Orf infection in four sheep flocks. *Veterinary Journal*, 160(1): 74–76.

Yirrell, D.L.; Vestey, J.P. and Norval, M. (1994). Immune response of patients to orf virus infections. *British Journal of Dermatology*, 130(4): 438–443.

Zarnke, R.L.; Dieterich, R.A.; Neiland, K.A. and Ranglack, G. (1983). Serological and experimental investigations of contagious ecthyma in Alaska. *Journal of Wildlife Diseases*, 19(3): 170–174.

Zhang, K.; Shang, Y.; Jin, Y.; Wang, G.; Zheng, H.; He, J.; Lu, Z.; and Liu, X. (2010). Diagnosis and phylogenetic analysis of orf virus from goats in China: a case report. *Virology Journal*, 7(78).

Zhang, K.; Liu, Y.; Kong, H.; Shang, Y. and Liu, X. (2014). Comparison and phylogenetic analysis based on the *B2L* gene of orf virus from goats and sheep in China during 2009-2011. *Archives of Virology*, 159(6): 1475-1479.

APPENDICES

Appendix 1: Field data. Detailed information about flock's history and samples collected grouped according to sampling locations by district.

ANIMAL	SEX	AGE	TEMP. °C	SAMPLE NUMBER	SAMPLE CODE	SAMPLE ID	SAMPLE TYPE	REGION SAMPLED	FARMER/ SOURCE	NUMBER OF ANIMALS	SICK ANIMALS	FIRST CASE DATE	DEAD ANIMAL	NEWLY INTROD. ANIMALS	VILLAGE	DISTRICT	SAMPLING DATE
				1	A1	FAMM1-W	Swab	Oral mucosa									
Goat	F	Adult	39.5	2	A2	FAMM1-WM	Swab	Wound around mouth	Muharami Manyakala	28	9	May, 2015	2	No			
				3	A3	FAMM1-C	Scab	Lips									
Goat	M	Kid	39	4	D	MKMM4-W	Swab	Oral mucosa									
				5	F1	MAMT1-W	Swab	Oral mucosa									
Goat	M	Adult	38.5	6	F2	MAMT1-WR	Swab	Rib wound							Kwambwera	BAGAMOYO	23 May, 2015
				7	F3	MAMT1-C	Scab	Lips									
				8	J1	MKMT5-W	Swab	Oral mucosa	Maulidi Mtoro	8	5	April, 2015	1	No			
Goat	M	Kid	39.9	9	J2	MKMT5-WM	Swab	Wound around mouth									
				10	J3	MKMT5-C	Scab	Lips									
				11	K1	MKMD1-W	Swab	Oral mucosa									
Goat	M	Kid	39.4	12	K2	MKMD1-WM	Swab	Wound around mouth									
				13	K3	MKMD1-C	Scab	Lips									
				14	K4	MKMD1-C	Skin Scrap.	Facial area									
				15	L1	MKMD2-WM	Swab	Wound around mouth	Madaraka Alex	5	5	April, 2015	0	No	Kimanga	ILALA	26 June, 2015
Goat	M	Kid	39.5	16	L2	MKMD2-WH	Swab	wound around Facial area									
				17	L3	MKMD2-CM	Scab	Lips									
				18	L4	MKMD2-CN	Skin Scrap.	Neck									
				19	Q1	FANY2-W	Swab	Oral mucosa									
Goat	F	Adult	39.2	20	Q2	FANY2-C	Scab	Lips	Nyange	9	4	April, 2015	0	No	Mapinga	BAGAMOYO	17 May, 2015

ANIMAL	SEX	AGE	TEMP. °C	SAMPLE NUMBER	SAMPLE CODE	SAMPLE ID	SAMPLE TYPE	REGION SAMPLED	FARMER/ SOURCE	NUMBER OF ANIMALS	SICK ANIMALS	FIRST CASE DATE	DEAD ANIMAL	NEWLY INTROD. ANIMALS	VILLAGE	DISTRICT	SAMPLING DATE
Goat	F	Adult	38.1	21	R1	FANYA3-W	Swab	Oral mucosa	Massawe	37	8	April, 2015	14	Yes, 10 from Kisarawe and all died			
				22	R2	FANY3-C	Scab	Lips									
Goat	F	Kid		23	S1	FKMS1-W	Swab	Oral mucosa									
				24	S2	FKMS1-C	Scab	Lips									
Goat	F	Adult	40.2	25	V1	MAMS4-W	Swab	Oral mucosa									
				26	V2	MAMS4-C	Scab	Lips									
Goat	F	Adult	38.8	27	Z1	MAMS8-W	Swab	Oral mucosa									
				28	Z2	MAMS8-C	Scab	Lips									
Goat	F	Adult		29	CC	FATS1-CE	Skin Scrap.	Ear	Tesha	5	1	April, 2015	0	No	Mbezi	KINONDONI	27 May, 2015
Goat	M	Adult		30	DD	MAMU-CE	Skin Scrap.	Ear	Mama Urasa	5	1	April, 2015	0	No			
Goat	F	Adult	38.6	31	EE1	FAAN1-W	Swab	Oral mucosa	Anna Musa	36	17	April, 2015	4	No	Mwanzomgumu		
				32	EE2	FAAN1-C	Scab	Lips									
				33	EE3	FAAN1-CN	Skin Scrap.	Neck									
Goat	F	Adult	38.5	34	HH1	FAAN4-W	Swab	Oral mucosa									
				35	HH2	FAAN4-CE	Skin Scrap.	Ear									
Goat	F	Adult	39.6	36	JJ	FAAM1-W	Swab	Oral mucosa									
Goat	M	Adult	39.1	37	KK1	MAAM2-W	Swab	Oral mucosa									
				38	KK2	MAAM2-CM	Scab	Lips									
Goat	F	Adult	38.9	39	LL1	FAAM3-W	Swab	Oral mucosa	Amanyise	27	9	April, 2015	0	No	Dondwe	KISARAWE	06 June, 2015
				40	LL2	FAAM3-CHL	Skin Scrap.	Hind leg									
Goat	F	Adult	39	41	MM1	FAAM4-W	Swab	Oral mucosa									
				42	MM2	FAAM4-CTT	Skin Scrap.	Teat									
Goat	F	Adult	37.6	43	OO1	FASA1-W	Swab	Oral mucosa	Shabani Ally	74	2	03-06-15	0	Yes, from Kibaha	Msongola		
				44	OO2	FASA1-C	Scab	Lips									
Goat	F	Adult	39.8	45	QQ1	FAAD1-W	Swab	Oral mucosa	Adamu Diwani	25	11	April, 2015	3	No	Kakubiro		
				46	QQ2	FAAD1-CB	Skin Scrap.	Back									
Goat	M	Kid	39.1	47	TT1	MKAD4-W	Swab	Oral mucosa									
				48	TT2	MKAD4-C	Scab	Lips									
							Total			**259**	**72**		**24**		**8**	**4**	

Appendix 2: Nucleotide sequences alignment of Tanzania ORFV. The alignment of nucleotide sequences was done using MEGA6 software and exported to excel sheet.

Domain: Data Coding Codon Start: 1

TZ/MM2/15-ORFV-B2L-gene	A	T	G	T	G	G	C	C	G	T	T	C	T	C	C	T	C	C	A	T	C	C	C	C	G	T	G	G	G	C	G	C	C	G	A	C	T	G	C	C	G	C	G	T	C	G	T	G
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TZ/MM2/15-ORFV-B2L-gene	A	C	G	C	T	G	C	T	C	G	T	G	G	A	C	G	T	G	C	A	G	A	G	C	A	A	G	G	A	C	A	A	G	G	A	C	G	C	G	G	A	C	G	A	G	C	T	G
TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/R2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/MM2/15-ORFV-B2L-gene	C	G	C	G	C	G	G	C	G	G	G	C	G	T	C	A	A	C	T	A	C	T	A	C	A	A	G	G	T	C	A	A	G	G	T	G	T	C	C	A	C	C	A	A	G	G	A	G
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TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/MM2/15-ORFV-B2L-gene	T	G	G	T	A	C	G	T	G	G	G	C	A	G	C	G	C	C	T	C	G	C	T	C	A	C	G	G	G	C	G	G	G	T	C	C	G	T	G	T	C	C	A	C	C	A	T	C
TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/MM2/15-ORFV-B2L-gene	T	T	C	C	A	C	C	T	C	A	A	C	C	A	C	T	C	C	G	G	G	G	G	C	G	G	C	G	T	A	T	T	C	T	T	C	T	C	G	G	A	C	T	C	G	C	C	G
TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TZ/TT2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TZ/K3/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/R2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/MM2/15-ORFV-B2L-gene	G	A	G	C	G	C	T	T	C	C	T	G	G	G	C	T	T	C	T	A	C	C	G	C	A	C	G	C	T	C	G	A	C	G	A	G	G	A	C	C	T	C	G	T	G	C	T	G
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TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/OO2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TZ/HH2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

TZ/MM2/15-ORFV-B2L-gene	C	G	C	G	T	G	A	T	C	A	T	C	A	C	C	G	A	G	T	G	G	A	A	G	A	A	C	G	C	G	G	A	C	C	C	G	C	T	G	T	C	G	G	T	C	T	C	G
TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/K3/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/OO2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/MM2/15-ORFV-B2L-gene	G	T	G	C	G	C	A	A	G	T	T	C	G	T	G	G	T	G	C	C	C	G	G	G	C	G	G	G	A	C	G	A	C	G	C	T	G	C	G	A	A	C	A	A	C	A	C	C
TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TZ/TT2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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Appendix 3: Amino acid sequence alignment of Tanzania ORFV. The alignment of amino acid sequences was done using MEGA6 software and exported to excel sheet.

Domain: Data

TZ/MM2/15-ORFV-B2L-gen	M	W	P	F	S	S	I	P	V	G	A	D	C	R	V	V	E	T	L	P	A	E	V	A	S	L	A	Q	G	N	M	S	T	L	D	C	F	T	A	I	T	E	S	A	K	K	F	L	Y	I
TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/MM2/15-ORFV-B2L-gen	S	T	N	K	H	L	A	W	D	L	M	N	R	Y	N	T	F	Y	S	M	I	V	E	P	K	V	P	F	T	R	L	C	C	A	V	V	T	P	T	A	T	N	F	H	L	N	H	S	G	G
TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TZ/TT2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TZ/K3/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TZ/L3/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/R2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/HH2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

TZ/MM2/15-ORFV-B2L-gen	G	V	F	F	S	D	S	P	E	R	F	L	G	F	Y	R	T	L	D	E	D	L	V	L	H	R	I	E	N	A	K	N	S	I	D	L	S	L	L	S	M	V	P	V	I	K	H	A	G	A
TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/OO2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TZ/HH2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

TZ/MM2/15-ORFV-B2L-gen	V	E	Y	W	P	R	I	I	D	A	L	L	R	A	A	I	N	R	G	V	R	V	R	V	I	I	T	E	W	K	N	A	D	P	L	S	V	S	A	A	R	S	L	D	D	F	G	V	G	S
TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TZ/TT2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/HH2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

TZ/MM2/15-ORFV-B2L-gen	V	D	M	S	V	R	K	F	V	V	P	G	R	D	D	A	A	N	N	T	K	L	L	I	V	D	D	T	F	A	H	L	T	V	A	N	L	D	G	T	H	Y	R	Y	H	A	F	V	S	V
TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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TZ/HH2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

TZ/MM2/15-ORFV-B2L-gen	N	A	E	K	G	D	I	V	K	D	L	S	A	V	F	E	R	D
TZ/LL2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TZ/TT2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TZ/K3/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TZ/L3/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TZ/BB/13-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TZ/R2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TZ/V2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TZ/OO2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TZ/HH2/15-ORFV-B2L-gene	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.