Sudan University of Science and Technology

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Sero-prevalence and Epidemiological Risk Factors of Foot and Mouth Disease (FMD) in Unvaccinated Cattle in West Darfur State - Sudan

الإنتشار المصلي وعوامل الخطر لمرض الحمى القلاعية في الأبقار غير المحصنة
في ولاية غرب دارفور- السودان

A thesis submitted to the College of Graduate Studies in partial fulfillment of the requirements of the degree of Master of Preventive Veterinary Medicine (M.P.V.M.**)**

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DEDICATION

To my mother, soul of my father, brothers and sisters
who always supported and encouraged me

To my wife and children for being my inspiration and my soul mate

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ABSTRACT

A cross-sectional study conducted during May to June 2015 in West Darfur state of Sudan. The objectives were to determine the sero-prevalence of foot and mouth disease (FMD) in unvaccinated local cattle and the potential risk factors associated with the disease. Antibodies against non-structural protein of FMD virus using NSP ELISA measured as an indicator of exposure to the virus. From the total 313 blood sera tested, the overall sero-prevalence of FMD was 43.5% (n=136/313). The highest sero-prevalence was observed at Forbaranga locality 63.5% followed by Habeila 50.0%., Baidha 41.9%, Keranic 32.8% and Elgeinana locality 29.7%. A total of 11 risk factors were investigated using structured questionnaire, of which 6 were found to be associated with FMD sero-prevalence ($p \leq 0.05$). The sero-prevalence of FMD in different age groups was 70.9%, 53.8%, 60.0%, 58.8%, 21.1%, and 22.3% in-group of cattle aged older than 5 years, 5 years, 4 years, between 3 to 4 years, between 2 to 3 years and less than 2 years, respectively. Sero-positivity significantly varied with Locality site of cattle. Cattle from southern parts locality had significantly higher sero-prevalence than northern locality cattle.

In conclusion, the associated 6 factors were further analyzed multivariably by logistic regression, and finally 4 of them (locality, location, sex, and age groups) were found to be significantly associated with FMD sero-prevalence ($p \leq 0.05$).

The result of this study showed that FMD is an important cattle disease in the study area necessitating further investigation and characterization of the circulating virus serotype to apply effective control and prevention measures.

ملخص

أجريت دراسة إحصائية عرضية أثناء شهر مايو ويونيو 2015م فى ولاية غرب دارفور بالحدود الغربية للسودان لتحديد انتشار المصلي لمرض الحمى القلاعية (FMD) في الأبقار المحلية غير المحصنة وعوامل الخطر المحتملة المرتبطة بالمرض. تم قياس الأجسام المضادة ضد البروتين غير الهيكلي لفيروس الحمى القلاعية باستخدام NSP ELISA كمؤشر على التعرض للفيروس.

تم اختبار عدد 313 عينة مصل دم، كان معدل الانتشار المصلى الكلي لمرض الحمى القلاعية 43.5% (ن=313/136). وقد لوحظت أعلى نسبة إنتشار في محلية فوربارنقا 63.5% تليها هبيلا 50.0% وبيضة 41.9 % وكرينك 32.8% وأقلاها فى محلية الجنينة 29.7%. تم التحقيق من عدد 11 عامل للخطر باستخدام استبيان، وفيها وجد 6 عوامل ترتبط مع انتشار مرض الحمى القلاعية ($p\leq 0.05$). كان معدل انتشار مرض الحمى القلاعية في الفئات العمرية المختلفة 70.9 % و53.8 % و60.0 % و58.8 % و21.1 % و22.3 % في مجموعة من الماشية التى تزيد أعمارها عن 5 سنوات و5 سنوات و4 سنوات وبين 3 إلى 4 سنوات وما بين 2 إلى 3 سنوات وأقل من 2 سنة، على التوالي. تباينت الإيجابية المصلية للأبقار بشكل كبير مع موقع المحلية وكان معدل الإصابة فى الأبقار فى المناطق الجنوبية أعلى بكثير من الأبقار في المنطقة الشمالية.

تم تحليل العوامل الستة المرتبطة بالمرض بشكل متعدد المتغيرات من خلال الانحدار اللوجستي (LR)، وأخيراً تم العثور على أربعة منها (وهى المنطقة والموقع والجنس والعمر) مرتبطة بشكل كبير مع انتشار مرض الحمى القلاعية ($p\leq 0.05$).

في الختام، أظهرت نتائج هذه الدراسة أن مرض الحمى القلاعية مرض مهم في الأبقار في منطقة الدراسة، مما يستدعي المزيد من التحقيق وتحديد خصائص النمط المصلي للفيروس المنتشر لتطبيق تدابير المكافحة والوقاية الفعالة.

INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals and has great potential economic losses for it severe damages in susceptible mammals. There are seven distinct serotypes of FMD virus (*FMDV*), namely, O, A, C, SAT1, SAT2, SAT3 and Asia1. Infection with one serotype does not induce cross immunity against another (OIE, 2018).

Most of sub-Saharan African countries, FMD is endemic, except for a few countries in southern part of Africa, where the disease controlled by the separation of infected wildlife from susceptible livestock besides using vaccination. In most parts of African continent, FMD outbreaks are often underreported either because of its endemics or the fact that it is not associated with high mortalities in adult susceptible animals; as such, it not perceived as an important livestock disease among herdsmen (Lazarus *et al.*, 2012).

In Sudan, FMD is endemic in some areas with different prevalence levels and it is outbreaks occur every year; the first record of the disease in the Sudan was in 1903, four out of the seven FMD serotypes have been reported in the Sudan, namely; O, A, SAT1 and SAT2. Isolate was serotype O, then serotype SAT1 before 1952, serotype A in 1957, and serotype SAT2 in 1977. The four *FMDV* serotype antibodies detected in cattle, sheep and goat sera, but their prevalence rate was quite different between species (Habiela *et al.*, 2010).

Foot and mouth disease (FMD) is one of the OIE top-listed notifiable, transboundary and multiple animal species disease (OIE, 2019) and has local, regional, continental and global concern due to it is animal health issues and socio-economic impact (OIE; FAO, 2012). The disease was top ranked transboundary animal disease in Sudan (Baumann, 2010). The large losses were because of the death of young calves, loss of weight and decrease of milk production and an in drought power and permanent infertility (Habiela *et al.*, 2010).

Sudan is still endemic country in Africa with FMD. It occurs frequently in the winter and autumn season. The extensive livestock husbandry systems in Sudan favor the good conditions for the spread of FMD virus in the field (Habiela *et al.*, 2010).

FMD cannot differentiate clinically from other diseases of stomatitis syndromes, such as swine vesicular disease, vesicular stomatitis and vesicular exanthema. Therefore, Laboratory diagnosis of any suspected FMD case is an issue of necessity (OIE, 2018).

Objectives:

The main objectives of this study were:

1. To determine the prevalence of Foot and Mouth Disease in unvaccinated cattle in West Darfur State.
2. To identify the risk factors, which could be associated with the FMD disease.

CHAPTER ONE

Literature Review

1.1 Itinerary

Sudan is one of the richest African countries in term of natural resources particularly in livestock species, which play essential role in the livelihoods of the greater part of Sudanese nation. Livestock population number in Sudan is large and it is growing rapidly over all states particularly southern and western parts, as estimated in the year (2017), Cattle mounts to 30,926,000 head, sheep 40,752,000 head, goats around to 31,659,000 head and camels about 4,850,000 head and a considerable mass of wild livestock of diverse population (MARF, 2017). Livestock is contributes significantly to the national economy by exporting live animals and animal products to gross domestic product. Livestock has provided more than 60% of the estimated value added to this sector in recent years, and is a larger contributor to agricultural sector GDP than crop agriculture (CPALD, 2013).

Foot and mouth disease (FMD) is an extremely transmittable disease and affects more than 70 domestic and wild artiodactyls' species (Mohamoud *et al.*, 2011), it is characterized by the development of vesicles in the mouth, at coronary band and skin of inter-digital cleft (Mekonen *et al.*, 2011).

FMD is one of the OIE-listed diseases and the control regarded as high priority. Because FMD is a viral disease, there is no treatment for the sick animal, and as a notifiable livestock, disease it should be eradicated (Tapani *et al.*, 2011).

1.2 History of FMD

FMD known as a major epidemic disease frightening the livestock industry since the sixteenth century and up to date it is a major worldwide animal health problem. The history of FMD may trace to the period of Hieronymus Fracastorius, a monk who described a disease outbreak in 1546 A.D. that occurred in cattle in Italy. The first demonstration of FMD is that, a disease of animal caused by a filterable agent and ushered in the era of virology (Longjam *et al.*, 2011).

In the Sudan, the first documentation of the FMD was in 1903 (Habiela *et al.*, 2010). Virus serotyping information has been available consistently since 1952 (Abu Elzein, 1983).

1.3 FMD Virus

1.3.1 Causative Agent

The foot and mouth disease virus (*FMDV*) is belongs to the genus Aphthovirus in the family *Picornaviridae*. There are seven main viral serotypes namely, O, A, C, SAT1, SAT2, SAT3 and Asia1. Serotype O is the most widespread serotype in the worldwide. It is responsible for a pan-Asian epidemic that began in 1990th and has affected many countries all over the world. Other serotypes also cause serious outbreaks; however, serotype C is uncommon and has not been reported since 2004 (Aftosa, 2014).

Some *FMDV* serotypes are more variable than others are, but communally, they have more than 60 strains. New strains intermittently occur. While most strains affect all susceptible host species, some have a more restricted host range (e.g., the serotype O Cathay strain, which only affects pigs). Protection against one *FMDV* serotype does not protect an animal from other serotypes. The immunity from other strains within a serotype varies with their antigenic resemblance (Aftosa, 2014).

1.3.2 Virus Classification

FMDV is belongs to the genus *Aphthovirus*, one of the genera of the family *Picornaviridae*. The given name *Picornaviridae* is resulting from the Latin word 'Pico' (small) and 'rna' (RNA) which refers to the size and genome type while the genus name *'aphthovirus'* refers to the vesicular lesions formed in cloven hoofed animals (Sahle, 2004).

1.4 Strain Classification

During replication, Foot and mouth disease virus undergoes a high rate of transmutation. This is generally because of a lack of replication error checking mechanisms. Mutation through recombination might result in exchange for genetic materials that perhaps direct to the generation of new variants considered as one of the major problems in the control of FMD by vaccination (Sahle, 2004).

It has shown that genetic recombination occurs between viruses of the same serotype as well as between serotypes. Mutations through recombination could outcome with the switch of genetic material that could direct to the generation of new antigenic variants that may escape immune pressure (Sahle, 2004).

1.5 Epidemiology

Epidemiology of FMD is complex, it is a highly contagious disease and different viral, host, and environmental factors, among them, variations affect in virus virulence, particle stability in different micro environments, and chances of long-term persistence. *FMDV* multiplication and spread also depend on the host species, nutritional and immunological status, population density, animal movements, and contacts between different domestic and wild host species and animals capable of mechanical dissemination of the virus. The environment can offer geographical barriers to virus dissemination or, on the other hand, can promote virus transmission when appropriate atmospheric circumstances exist (Longjam *et al.*, 2011). In Africa, the epidemiology of FMD reviewed several decades ago. The considerable features of FMD in Africa that were highlighted include; the presence of six *FMDV* serotypes including serotypes O, A, C, Southern African Territories SAT1, SAT2 and SAT3 with only Asia1 serotype reported negative on the continent (Mwiine *et al.*, 2010).

There are seven different recognized serotypes of FMD (O, A, C, Asia1, SAT1, SAT2 and SAT3), which distributed across the world (FAO and EUFMD, 2007). Serotype O worldwide distributed, when, serotypes A and O have distributed widely in South America, Asia and Africa. Serotype Asia1 is presently limited only to Asia while SAT1, SAT2 and SAT3 are to Africa (Mekonen *et al.*, 2011). Serotypes O, A, SAT1 and SAT2 are predominant in most of sub-Saharan Africa (Rweyemamu *et al.*, 2008; Habiela *et al.*, 2010; Mishamo, 2016). Three of the South African Territories (SAT) serotypes are unique to Africa. Asia contends with four serotypes (O, A, C and Asia1), and South America with only three (O, A, C). Serotype Asia1 is limited only to Asia subcontinent and the capacity to invade free areas is common to all serotypes. Serotype O is the most widely prevalent serotype in the world flowed by serotype A. South America is genetically steady type O virus for nearly the past 5 decades under study. Serotype C appears extremely rare and disappearing from the world as a whole except for Kenya and several pockets of Brazil in South America probably because of the circulation of this particular serotype extinct in wildlife. Due to globalization, FMD epidemics changed from local and regional spread to wide worldwide spread. It should note that with globalization of trade even areas where FMD is endemic could suffer from introduction of virus strains that are exotic to the region. The risk of FMD entrance into free areas is low through authorized trade of animal and animal products from zones or countries officially recognized as FMD free by the OIE. However, animal products smuggling is of important concern and likely the main means of FMD virus introduction into free areas (Mishamo, 2016).

FMDV has commonly spread in Eurasia and Africa from the earliest times, but the Americas were most likely free of infection until introduction with European livestock during the nineteenth century. The current worldwide burden of *FMDV* infection is maintained within three continental reservoirs in Africa, Asia and South America that can be further subdivided into seven major virus pools of infection (Paton *et al.*, 2016) (Fig. 1).

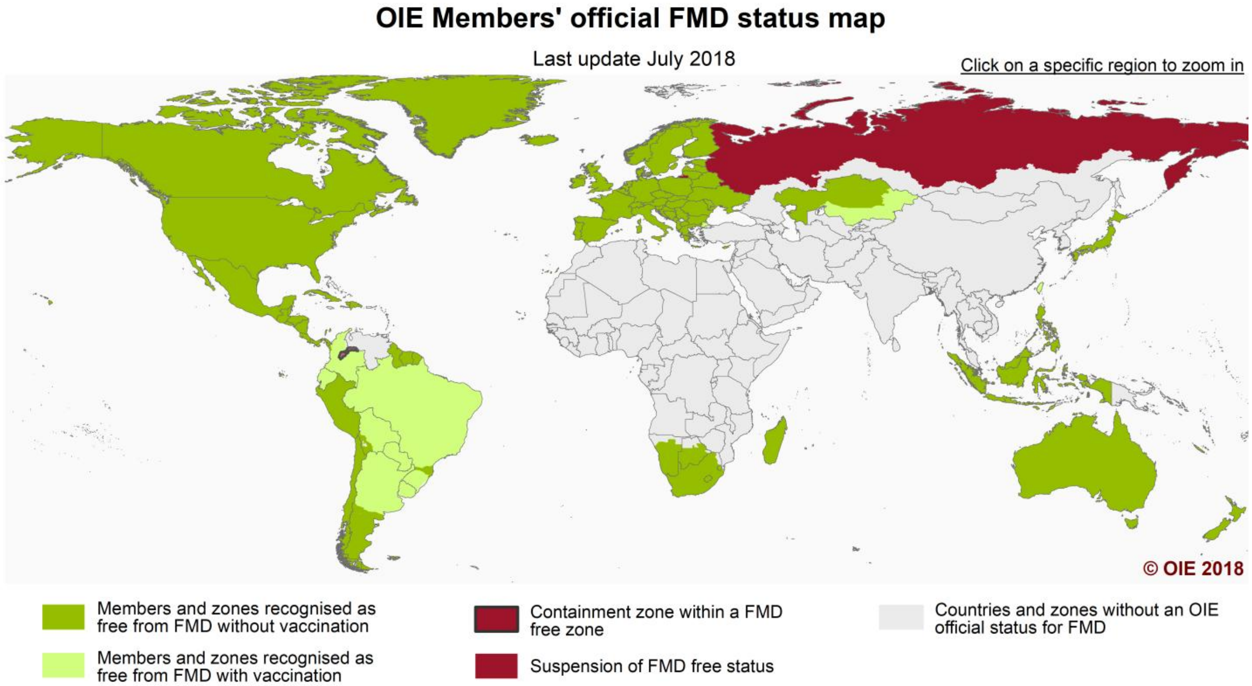

Figure 1: Geographical distribution of FMD in the World (OIE, 2018)

The introduction of molecular biology technology has enabled the genetic characterization of virus strains so the tracing of strains isolated from outbreaks can carried out with far greater precision than was possible hitherto with serological techniques. Consequently, it is currently possible to group countries or zones into epidemiological clusters according to the topotypes within each serotype that occur there. Because of globalization, the spread of FMD epidemics can change from local and regional spread to wide worldwide spread, even to remote areas as happened with the type O Pan-Asian lineage (Rweyemamu *et al*., 2008).

1.5.1 Morbidity and Mortality from FMD

The morbidity rate of FMD outbreaks of in susceptible animals can quickly come up to 100% but some strains are limited in their infectivity to particular species. However, the case fatality is in general very low, about 2% in adults and 20% in young stock. Mortality in adult animals is usually low to negligible; up to 50% of calves may die due to cardiac involvement and complications such as secondary infection. During outbreaks in endemic and developed countries, most deaths are because of a slaughter policy that usually involves all susceptible animals and herds in contact with or within a certain radius of infected herds also (Mishamo, 2016).

1.5.2 FMD Prevalence of the Disease in Sudan

In Sudan, a survey conducted between 2006 and 2008, and 1,069 sera randomly collected from cattle (469), sheep (319), goats (88) and dromedary camel (193) from seven states in the Sudan for the detection of antibodies to *FMDV*. Application of liquid phase blocking (LPB) ELISA revealed that antibodies to four serotypes were present in ruminants; namely O, A, SAT1 and SAT2. No antibodies to *FMDV* detected in camel sera. This work elucidates the current epidemiology of FMD in some parts of the Sudan. The overall sero-prevalence of FMD in the report was found to be 79.24% (CI = 95%) (Habiela *et al.*, 2010).

A cross-sectional study conducted during 2013 in Khartoum State, Sudan to estimate the sero-prevalence of Foot and Mouth Disease virus and to determine the risk factors, which could be associated with Foot and Mouth Disease of cattle. One hundred and thirty two bovine serum samples collected and tested for antibodies against FMD virus. The overall sero-prevalence of FMD in Khartoum State was found to be 53.4% (CI = 95%) (Noureldin, 2014).

1.6 Host Range of FMD

Foot and mouth disease (FMD) caused by a non-enveloped single stranded RNA picorna-virus within the *Aphthovirus* genus. It has the ability to infect all species of the order Artiodactyla including cattle, pigs, sheep and goats. Camelids not considered having an important role in transmission although they may infect in certain circumstances (Habiela *et al.*, 2010). Cattle, sheep, goats, and pigs are the main domestic species infected. The Water Buffalo (*Bubalus bubalis*) can infect and may transmit infection to other species. Camelids, experimentally infected, contract the disease but there is no evidence of transmission to other domestic livestock and there seems to be some doubt whether they play any role in the epidemiology of the disease in domestic livestock. A wide range of wild cloven-footed animals contract FMD including deer and pigs. The African Buffalo (*Syncercus caffer*) appears to particularly susceptible to infection and may act as a reservoir host (Davies, 2002). Several species of wildlife known to be susceptible to FMD virus (*FMDV*) infection and the African buffalo (*Syncerus caffer*) has implicated as a maintenance host (Davies, 2002). Although FMD known as a disease of cloven-footed animals it can occur naturally in other animals. Infection has been established experimentally in a number of other species. However, it is doubtful whether these animals play any part in the epidemiology of the disease. FMD not considered zoonotic. Although clinical cases have proven in human, these are extremely rare in relation to human exposure during outbreaks (Davies, 2002; Depa *et al.*, 2012).

1.6.1 Species Affected

The important livestock hosts include cattle, pigs, sheep, goats, water buffalo and yaks. When cattle are important maintenance hosts in most areas, but a few viruses are adapted to pigs, and some isolates might circulate in water buffalo. It is uncertain whether small ruminants can maintain *FMDV* for long periods if cattle are absent. Other susceptible species include ranched or farmed cervids such as reindeer, deer and elk. Bactrian camels can develop

FMD, but dromedary camels have slight or no susceptibility to this virus. African buffalo mainly thought to maintain the SAT serotypes, although antibodies to other serotypes have found in buffalo populations. Other species of wildlife do not seem to be able to maintain FMD viruses, and usually infected when viruses spread from livestock or buffalo. *FMDV* can also infect a few animals that are not members of the Artiodactyla. Laboratory animal models include guinea pigs, rats and mice, but these animals not thought to be important in transmitting *FMDV* in the field (Aftosa, 2014). Susceptibility was observed affecting mostly indigenous cattle (Sarker *et al.*, 2011). It is not associated with high mortalities in adult susceptible animals, there for it not perceived as an important livestock disease among herdsmen in some parts of Africa (Lazarus *et al.*, 2012).

1.6.2 Incubation Period and pathogenicity

The incubation period for FMD can vary with the species of animal, the dose of virus, the viral strain and the route of inoculation. It is reported to be one to 12 days in sheep, with most infections appearing in 2-8 days; 2 to 14 days in cattle; and usually 2 days or more in pigs (with some experiments reporting clinical signs in as little as 18-24 hours). In the other reports the incubation periods are 4 days in wild boar, 2 days in feral pigs, 2-3 days in elk, 2-14 days in Bactrian camels, and possibly up to 21 days in water buffalo infected by direct contact (Aftosa, 2014). The incubation period can be highly variable depending on host, agent and environmental factors including husbandry management factors (Senawi, 2012).

Replication of the infectious particles is extremely rapid after entry through the upper respiratory tract or lung, with viraemia seeding infection into the epithelium where secondary virus multiplication results in vesicles and shedding from the udder in milk. The virus excreted during viraemia for some days; thereafter as serum antibody develops viraemia decreases, and the animal ceases to be infectious as the lesions heal. The disease characterized by vesicular lesions on the coronary band of the hooves and in the mucosa of the mouth including the tongue and palate. Vesicles typically contain clear or straw-colored fluid before they burst and heal. There is a rise in body temperature of some 3–4°C. In sheep, the lesions are often difficult to find and may be confused with other conditions. The disease varies considerably in its severity. It may result in death or severe morbidity particularly in neonates but in areas where the infection is, endemic the disease may be mild and the few vesicles that appear may heal without further damage (Davies, 2002).

1.6.3 Immunity

The protection of a susceptible animal against FMD virus correlates highly with the antibodies level neutralization. Infection with any FMD serotype produces absolute protection against homologous FMD virus, but slight or no protection against heterogeneous viruses. Serotype specific immunity is develops 7 to 21 days after exposure to the virus. The immunoglobulin M (IgM) is most prevalent in the early convalescent serum and is less specific to the different serotypes than Immunoglobulin G (IgG), the homologous antibodies is highly specific. It has reported that healing of lesions and clinical recovery in infected animals would not occur until a few days after the IgG1 antibodies have developed. The localized antibody response, specific to anti-FMD IgM and IgA antibodies in the pharyngeal fluid of cattle develops 7 days after exposure to the virus, while IgG activity reaches pick in serum only 14-21 days after infection (Mishamo, 2016).

The age of individuals has also shown to influence the antibody response against FMD virus. Calves (age one week to six months) but deprived of maternal antibodies responded as well as, or better than 18 months old cattle to initial vaccination against FMD. Although serum antibody levels play an important role in host protection against FMD virus infection, the cellular responses mediated by T-helper and T- cytotoxic cells also play a role in the immune response to FMD virus infection (Mishamo, 2016).

1.6.4 Risk factors for FMD infection

The most important factors that statistically considerable and may well be associated with FMD are; age, sex, breed, farming system, seasonal influence, previous disease and preventive measures during examination (Sarker *et al.*, 2011). *FMDV* multiplication and spread also depends on the host species, nutritional and immunological status, population density, animal movements, and contacts between different domestic and wild host species and animals capable of mechanical dissemination of the virus. The environment can provide geographical barriers to virus dissemination or, alternatively, can promote virus transmission when appropriate atmospheric conditions prevail in this multi-factorial scenario (Longjam *et al.*, 2011).

1.6.5 Transmission

Transmission of *FMDV* is primarily from the infected animal itself, particularly during the early febrile stage. The virus is present in the tissues, body fluids and organs. Affected animals shed the virus in saliva, vesicular epithelium, milk, faces, semen, urine and vaginal fluids (Sobrino and Domingo, 2019). Transmission can be with direct contact between infected and susceptible animals, air borne spread of 12 viruses either during inhalation or through open wounds, indirect virus can be by personnel, fomites and consumption of infected animal products by susceptible animals either through water or through feed. Direct contact is the most efficient route of transmission for most livestock. However, in cattle, airborne transmission is the most common route, unlike for pigs that need higher doses and are relatively resistant to air-borne infection when compared with cattle and sheep (Senawi, 2012). During the clinical phase of FMD, the virus is present in all secretions and excretions, and it may be excreted intermittently thereafter (Alexandersen *et al.*, 2003). Spread of *FMDV* from sheep and goats to other susceptible species is more significant in clinical or sub-clinical stages than those of carrier, even though they can also act as a carrier, such as with cattle (Alexandersen *et al.*, 2003).

The transmission of FMD virus within an unvaccinated herd is usually rapid. Milk and semen from infected cattle may contain virus up to four days before the onset of visible signs. The FMD virus persists particularly in the basal epithelial cells of the pharynx and dorsal soft palate, and can recovered from some animals for over three years, although the carrier state does not usually extend beyond a year (Kitching, 2002).

The movement of infected animals usually spreads foot and mouth disease. Susceptible cattle coming into contact with an infected animal, whether goat, sheep, pig or wildlife species may be infected by the respiratory route or through an abrasion on the skin or mucous membranes. Cattle are very susceptible by the respiratory route by 10,000 times more to become infected by the oral route (Kitching, 2002).

The disease rapidly spread by movement of infected animals or mechanically on fomites such as clothing, shoes, vehicles and veterinary instruments. The reasons for the rapidity of spread to fully susceptible population are due to the highly infectious nature of the virus. Production of high titer in respiratory secretions, and the large volumes of droplets and aerosols of virus shed by infected animals, the stability of virus in such droplets, the rapid replication cycle with very high virus yields and the short incubation period (Gebregziabher and Issa, 2013).

1.6.5.1 Direct contact

In the tropical areas, the most important method of spread of FMD disease is by direct contact between animals moving freely across state and national boundaries as trade or nomadic cattle (Abunna *et al.*, 2013).

In general, livestock movements can summarized at scales ranging from transboundary imports and exports of animals to high local scales that focus on short-distance trade interactions or direct contact among herds in the context of daily foraging. Between these two extremes lie national supply chains and seasonal patterns of animal movement. Each of these scales has important implications for the spread and control of infectious diseases. For pastoralist production systems, an understanding of the contact structure among mobile herds of livestock will help overcome challenges in applying appropriate spatial risk-based surveillance and disease control, including defining appropriate control strategies for outbreaks of diseases such as FMD (VanderWaal *et al.*, 2017).

1.6.5.2 Air borne Transmission

Airborne trans-mission over long distances has implicated under certain climatic and meteorological conditions, particularly in respect to domestic pigs that exhale the highest quantities of airborne virus (Lazarus *et al.*, 2012). The disease is notoriously infectious that it can spread in so far as 50 miles downwind from one outbreak area to another (Mekonen *et al.*, 2011). Wind-borne aerosol virus produced by infected animals carried over 250 km.

Survival of virus in aerosols depends on relative humidity. Cattle mainly infected by inhalation, often from pigs, which excrete large amount of virus by respiratory aerosols and considered highly important in disease spread. Infected animals excrete large amounts of virus before clinical signs are evident and wind may spread the virus over long distances (Depa *et al.*, 2012).

1.6.5.3 Indirect contacts Transmission

FMDV may disseminated indirectly through contacts such as farmers, veterinarians, inseminators, contaminated food, trucks used for the transport of livestock etc. Other mechanisms involve the exposure of livestock to contaminated products such as meat, offal and milk. Calves drinking contaminated milk will become infected by this route (Sutmoller *et al.*, 2003) through contaminated personnel, vehicles and fomites (Lazarus *et al.*, 2012).

1.6.6 Survival on fomites

Most Foot and Mouth Disease virus strains is considered to be a moderately stable virus at a pH 7.0-8.5, particularly at lower temperatures and in humidity above 55-60% but is sensitive to heat and desiccation. The survival of FMDV also influenced by the nature of the materials as a high concentration of organic material helps the survival of the virus. The FMDV can recover from the blood, pharynx, vagina and rectum up to 97 hours prior to the onset of vesicular lesions and in mammary tissue for 3-7 weeks after infection. The highest estimated continued existence period of FMDV outside the host is about three months in regions with daily temperatures greater than 20°C (Senawi, 2012).

1.6.7 Carrier state

A carrier animal defined as one from which virus can recover four weeks or more after infection. The persistent infection has infectivity and pathogenicity to cattle and pigs, so it is very important to detect FMD persistent infected animals and remove carriers to control the outbreaks. The carrier period appears to vary between species, being in excess of 12 months in cattle, up to 9 months being in excess of 12 months in cattle, up to 9 months in sheep and goats and at least 5 years in African Buffalo. A “pseudo persistent state” may occur in pigs in which virus replicates in lymphoid tissues for a prolonged time, thereby representing a potential source of virus (Depa *et al.*, 2012).

1.7 The FMD Disease

1.7.1 Clinical Signs

The disease is often initially diagnosed based on clinical signs therefore requires vigilance by the farming community and veterinary profession and the infrastructure to allow early reporting of disease (Senawi, 2012).

The disease characterized by high fever, in appetite, salivation and vesicular eruptions on the feet, mouth cavity and teats (Gelaye *et al.*, 2009). While there is some variability in the clinical signs between species, FMD is typically an acute febrile illness with vesicles (blisters) localized on the feet, in and around the mouth, and on the mammary gland. Vesicles occur occasionally at other locations including the vulva, prepuce, or pressure points on the legs and other sites. The vesicles usually rupture rapidly, becoming erosions. Pain and discomfort from the lesions leads to clinical signs such as depression, anorexia, excessive salivation, lameness and reluctance to move or rise. Lesions on the coronary band may cause growth arrest lines on the hoof.

In severe cases, the hooves or footpads may slough. Reproductive losses are possible, particularly in sheep and goats. Deaths are uncommon except in young animals, which may die from multifocal myocarditis or starvation. Most adults recover in 2 to 3 weeks, although secondary infections may slow recovery. Possible complications include temporary or permanent decreases in milk production, hoof malformations, chronic lameness or mastitis, weight loss and loss of condition (Aftosa, 2014).

1.7.2 Pathogenesis

The respiratory system is the most important portal of infection. After inhalation, the virus can affect the pharynx and primary multiplication of the virus in the mucous membrane is transported by lymphatic and blood circulation to the sites of secondary multiplication in the lymphatic glands, epithelial tissues and in around the mouth, feet and in the mammary glands. Gross lesions develop only in areas subjected to mechanical trauma or unusual physiological condition such as the epithelium of the mouth, feet to a less extent, the teats. Bacterial complication generally aggravates the lesions, particularly those of the feet and the teat, leading to severe lameness and mastitis, respectively. In young animals, especially neonates, the virus frequently causes necrotizing myocarditis and this lesion may also be seen in adult infected with some strains of the virus particularly type O. In fatal cases, death caused either by dehydration or by ventricular fibrillation during cardiac attacks or because of bacterial complication (Admassu *et al.*, 2015).

1.7.3 Agent identification

Due to the fast spread of FMD and the serious economic consequences that can arise from an outbreak, prompt, sensitive and specific diagnosis and identification of the virus serotype is essential. Initially, presumptive diagnosis based upon clinical signs. However, confirmed laboratory diagnosis of any suspected FMD case is a matter of urgency. Furthermore, determination of the serotype involved in field outbreaks has to e established within laboratories to enable proper control of the disease. Various techniques have developed and used to diagnose FMD and to ascertain the serotype/subtype of the virus (OIE, 2018).

1.7.4 Diagnosis

The accurate diagnosis of infection with *FMDV* is of prime most importance for both control and eradication campaigns in FMD endemic areas and as a supportive measure to the stamping out policy in FMD-free areas. The history of research and diagnosis in foot-and-mouth disease falls into several distinct areas. Search for experimental laboratory animals, producing the disease culminated in the demonstration (Longjam *et al.*, 2011).

Viral diagnosis of FMD carried out on epithelial tissue or vesicular fluid from clinical samples using specific laboratory diagnostic techniques.

1.7.4.1 Virus Isolation

The isolation and characterization of the virus is the "golden standard" for the diagnosis of viral diseases. The suspensions of field sample suspected to contain FMD virus inoculated into primary pig kidney cells cultures, incubated at 37°C and examined for cytopathic effect (CPE), 24-48 hours post infection. If there is no CPE, it confirms the absence of FMDV in the samples (Admassu *et al.*, 2015).

1.7.4.2 Serological Diagnosis

Serological tests for FMD are of two types; those that detect antibodies to viral structural proteins (SP) and those that detect antibodies to viral nonstructural proteins (NSPs) (OIE, 2009).

The virus infection can diagnose by the detection of specific antibody response. The tests generally used are CFT, VN, solid phase ELISA, liquid phase ELISA and non-structural protein antibody tests such as ELISA, enzyme linked immune electro-transfer blot assay. Preferred procedure for the detection of FMD viral antigen and identification of viral serotype is the ELISA (Admassu *et al.*, 2015)

Although CFT was a fast method it needed high virus load and results sometimes affected by pro-and anti-complementary activities of the test sample (Longjam *et al.*, 2011). CFT is an alternative test for international trade (OIE, 2012). It has disadvantages, which are its relatively low sensitivity. The sensitivity and specificity of the test is also dependent upon the animal species tested and is not sufficiently sensitive to detect infection (Senawi, 2012).

1.7.4.3 Nucleic acid recognition methods

The polymerase chain reaction (PCR) techniques increasingly used for rapid identification of FMD virus and sequence analysis of any PCR positive. The reverse-transcription PCR (RT-PCR) can used to amplify the genome fragment of FMD virus in diagnostic material. Specific primers have designed between each of the seven serotypes (Admassu *et al.*, 2015).

1.8 Treatment

There is no specific treatment for FMD, other than supportive care. Treatment is likely to allow only in countries or regions where FMD is endemic (Aftosa, 2014).

1.9 Control

1.9.1 Disease reporting

A rapid response is vital for containing FMD outbreaks in free regions. Veterinarians who encounter or suspect this disease should follow their national and/or local guidelines for disease reporting. The state or federal veterinary authorities should notify immediately of any suspected vesicular disease (Aftosa, 2014).

1.9.2 Prevention

Import regulations help prevent *FMDV* introduced from endemic regions in infected animals or contaminated foodstuffs fed to animals. Heat treatment can kill *FMDV* in sluice and decreases the risk of an outbreak; however, some countries have completely banned swill feeding, due to difficulty in ensuring that adequate heat-treatment protocols followed. Protocols for the inactivation of *FMDV* in various animal products such as milk products, meat, hides and wool have published by the OIE. Global FMD control programs have recently established to reduce virus circulation and the incidence of this disease (Aftosa, 2014).

Measures taken to control an FMD outbreak include quarantines and restrictions of animal movement, euthanasia of affected and exposed animals, and cleaning and disinfection of affected premises, equipment and vehicles. Further actions may comprise euthanasia of animals at risk of being infected and/or vaccination. Disposed Infected carcasses must be of safely by burning, rendering, burial or other techniques. To prevent them from mechanically disseminating the virus Rodents and other vectors may killed. People who have exposed to FMDV may ask to avoid contact with susceptible animals for a period, in addition to decontaminating clothing and other fomites. Good bio-security measures should practice on uninfected farms to prevent entry of the virus (Aftosa, 2014).

Vaccination may used to reduce the spread of *FMDV* or protect specific animals during some outbreaks. The decision to use vaccination is complex, and varies with the scientific, economic, political and societal factors specific to the outbreak. Vaccines also used in endemic regions to protect animals from illness. *FMDV* vaccines only protect animals from the serotype (s) contained in the vaccine. For adequate protection, the vaccine strains must also well-matched with the local field strain (Aftosa, 2014).

Wildlife transmission may need to considered income locations. One important issue is the persistence of *FMDV* in wild African buffalo, which may make eradication unfeasible in some areas. In Southern Africa, transmission from African buffalo has controlled by separating wildlife reserves from domesticated livestock with fences, and by vaccination of livestock. However, wildlife fencing may not be practical in some areas, and there some disadvantages of it use. Another issue is the protection of highly susceptible wildlife species from *FMDV*. Vaccination of livestock reported to decrease outbreaks in some populations (Aftosa, 2014).

1.9.3 Eradication

It is policies and actions designed to eliminate FMD virus following an outbreak of disease. This includes both 'stamping out', defined by OIE as the slaughter of all infected and in-contact animals, together with cleaning and disinfection and all the other measures that are necessary in the event of an outbreak in an FMD-free country, region or zone. Stamping out involves: slaughter and disposal, cleaning and disinfection, movement controls, zoo sanitary measures and epidemiological monitoring (Admassu *et al.*, 2015).

CHAPTER TWO

Materials and Methods

2.1 Study area

The study was conducted in West Darfur state as shown in Figure 2, area of 79,460 km2 (30,680 sq miles) which is located on the western part of Sudan falls between Latitude: 22.762550° N-23.026222° N; Longitude: 12.80957° E-13.403430° E. West Darfur State borders North Darfur State to the north, northeast and east, Central Darfur state to the east and southeast and has share of an international border in the west with Republic of Chad. The livestock population of West Darfur state is estimated approximately at 1,088,595 cattle, 2,221,851 sheep and 1,097,452 goats (MARF, 2017) in eight localities (FSTS, 2013).

2.2 Study design

A cross sectional study carried out on traditionally managed cattle during May and June 2015 to determine the level of occurrence of FMD and to investigate the potential risk factors associated with it.

2.3 Sampling method

The study based on a data collected in a multi-stage sampling Sudan national epidemio-surveillance program of FMD in Sudan in 2015.

2.4 Sample frame

From West Darfur state five localities (Forbaranga, Habeila, Baidha, Keranic and Elgeinana) selected randomly. Five village/location selected from each locality, and then from each village/location, twenty-five unvaccinated cattle sampled.

As result, 313 cattle selected from West Darfur state as sample size.

2.5 Ageing by dentition

Number of pairs of permanent incisors was used to estimate cattle age as follows; one pair = 2 to 3 years age, two pairs = 3 to 4 years age, three pairs = 4 years age, four pairs = 5 years age and broken mouth (teeth missing or worn down) old aged.

2.6 Questionnaire

For each herd where blood samples are collected a pretested structured questionnaire (Data collection questionnaire form, Clinical examination form and Blood sample form) format with the primary objective of explains the multi-factorial background of disease was used in an interactive manner (was administered by face-to-face interview) at all selected herds level. All cattle included in this study subjected to a questionnaire, which filled out by the animal owners or herd manager. The questionnaire written in English and translated orally into the appropriate local language.

The questionnaire included individual risk factors that attribute state, locality, location, herd size, production system, housing, grazing type, water source, cattle breed, vaccination status, animal age, animal origin, elevation, housing, herd size, species, history of FMD in the herd, veterinary services, sex, and season.

Geographical Positioning System (GPS) device was used to mark the points coordinates (longitude E, latitude N). The records were saved for every herd position to build the map of study area.

2.7 Statistical analyses

All Categories of The collected data entered into a computer on a Microsoft Excel spreadsheet (Microsoft Corporation). Statistical analysis performed using Statistical Package for Social Science (SPSS) package 16 (for Windows) and checked twice before analyses. Associations between the outcome variable (status of FMD sero-positivity in cattle) and its likely risk factors first screened in a univariable analysis using 2-tailed Chi-square (χ^2) test. A multivariable model for the outcome variable was constructed using logistic regression (LR) analysis with enter method for modeling checking. FMD was considered as the dependent variable and the potential risk factors (age group, sex group, locality, location site, distance from main road, area elevation, Production type, Production system, introduction of new animals into the herd, herd size, herd composition, history of FMD in cattle herd in this year and availability of Veterinary services) as independent variables. Finally, odd ratios and 95 % confidence interval (CI) were calculated, and risk factors with a *p-value* < 0.05 were set for detecting significance results association to FMD sero-positivity.

2.8 Blood samples collection

Eligible Individual cattle those aged one year or more sampled to avoid maternal immunity. Three hundred and thirty one blood samples collected from none vaccinated cattle individuals to identify the presence or absence of antibodies against FMD in the selected study area of West Darfur State. A sterile needle injected into the jugular vein and a 10 ml plain vacutainer used to collect the blood. The blood samples allowed clotting in a cool place. Each animal assigned a number and this recorded on the form and on the vacutainer. Once the clot has retracted they were maintained inclined overnight at room temperature for serum separation. The blood vials centrifuged and serum removed into a sterile sera tube (4 ml cryo-genic vials). All serum tubes clearly labeled with date, unique herd number and unique animal number at same time during sampling a detailed record on sampling form recorded. The sera were transported in an ice box with ice packs to the General Directorate of animal Health and Epizootic Disease Control then to Central Veterinary Research Laboratory, Sudan, where, kept frozen at -20° C until analyzed for the detection of FMD antibodies.

2.9 Laboratory assay

The World Animal Health Organisation (OIE, 2018) has adopted an NSP-ELISA developed by PANAFTOSA, as its index screening method for discrimination purposes. This test complemented by a confirmatory assay there are four commercially available NSP-ELISAs and other “in-house” tests. Although each of these NSP-ELISAs has evaluated at different times and in different laboratories using different sera, none had been sufficiently validated for use in support of a vaccinate-to-livepolic.

The samples assayed according to the manufacturer’s instruction. In brief, 50 µl of pre-diluted control and sample sera dispensed into the appropriate wells of micro titer plates pre-coated with *FMD*V antigen and control antigens.

NSP ELISA for Cattle with Approximately 100% Sensitivity in unvaccinated animals and 99-100% specificity used to indicate past or present infection by any FMD serotype. Incidence of disease in the past one year to determine the suspected cases to determine if animals in the herd had been recently infected with FMD virus, thereby estimating the sero-prevalence in the herd, district or zone.

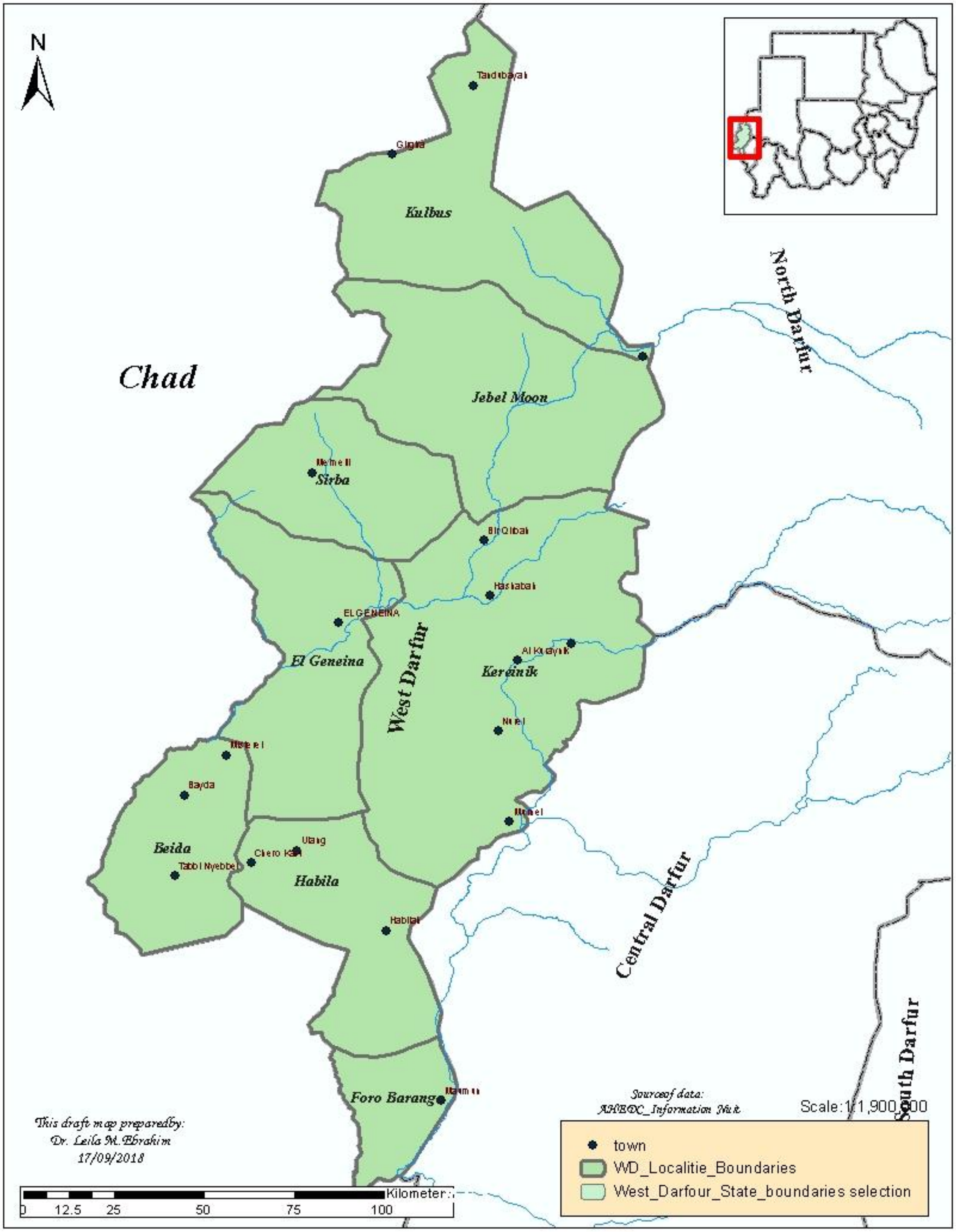

Figure 2: Map of West Darfur State, Sudan showing the study area (Drafted by GIS Unit MARF, 2019)

CHAPTER THREE

Results

3.1 FMD Sero-prevalence

From the total number of 313 sera samples collected from unvaccinated cattle of West Darfur state, tested by NSP ELISA and univariate analysis applied for risk factor associated with FMD sero-positivity using Chi-square (χ^2) test. The overall sero-prevalence was 43.5% (n=136/313) shown in (Table 1).

Table 1: FMD sero-prevalence of unvaccinated cattle (n=313) in West Darfur state, Sudan

Origin	Findings
Number of sera samples tested	(n=313)
Number of NSP ELISA positive sera samples	(n=136)
Overall FMD sero-prevalence for West Darfur state	(43.5%)

3.2 Risk factors associated with FMD sero-positivity

Univariate analysis using Chi-square (χ^2) test, thirteen risk factors were assessed using structured questionnaire for every sampled herd. Six risk factors found to be associated with FMD sero-prevalence ($p \leq 0.05$).

3.2.1 FMD Prevalence in relation to host intrinsic risk factors

3.2.1.1 FMD Prevalence among different age groups of unvaccinated cattle in West Darfur state

Regarding age groups of studied cattle herds, an increasing sero-prevalence trend was observed with increasing age and the difference was statistically significant among age groups (*p*-value=0.000) (Fig. 3).

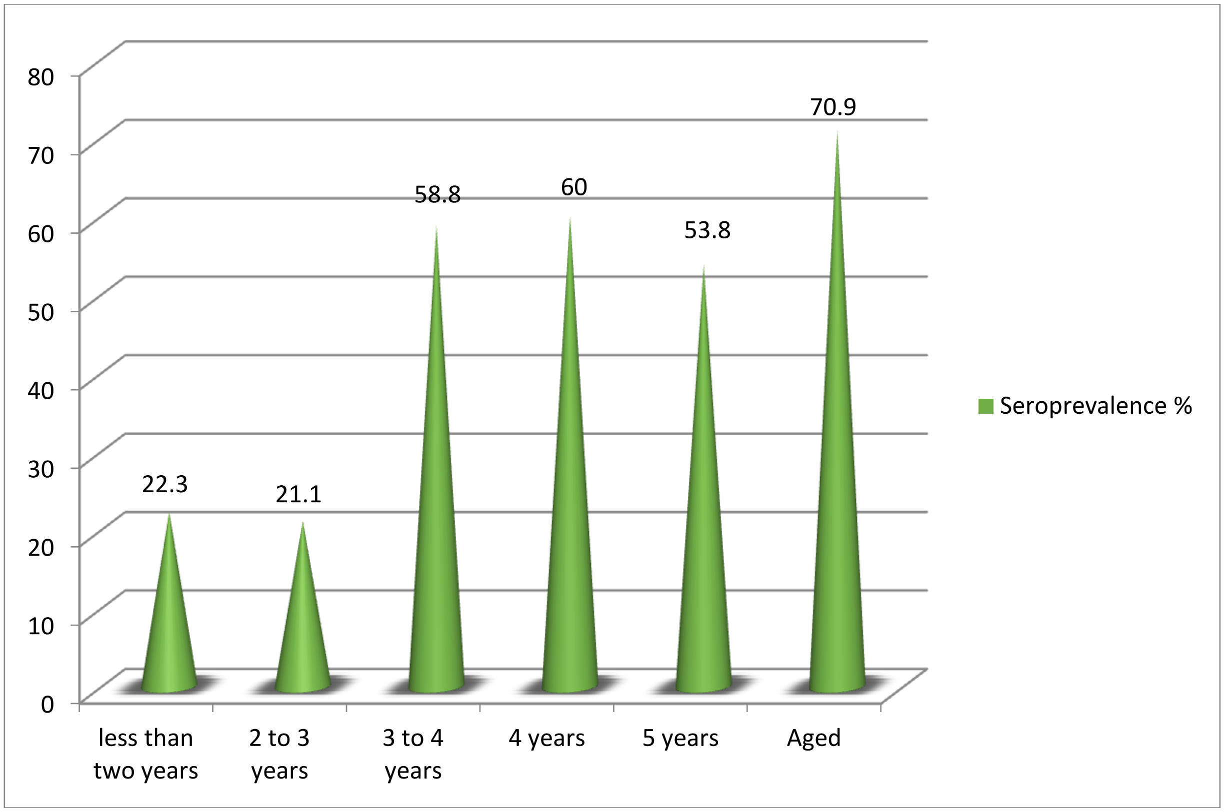

Figure 3: FMD prevalence within age groups of unvaccinated cattle in West Darfur state

3.2.1.2 FMD Prevalence between sex groups of unvaccinated cattle in West Darfur state

The sero-prevalence estimate for male and female cattle was 19.4% and 50.0% respectively, the higher prevalence was observed in females than males and the difference was statistically significant (*p*-value=0.000) (Table 2).

Table 2: FMD Prevalence within sex groups of unvaccinated cattle (n=313) within localities, West Darfur state

Sex	No. of tested animals	No. of positive samples	Sero-prevalence %	*p*-value
Male	67	13	19.4	0.000
Female	246	123	50.0	
Total	313	136	43.5	

3.2.2 FMD Prevalence in relation to host extrinsic risk factors

3.2.2.1 FMD Prevalence of unvaccinated cattle in West Darfur state in relation to Localities

The highest sero-prevalence was observed in Forbaranga locality (63.5%) followed by Habeila locality (50.0%), Baidha locality (41.9%), Keranic locality (32.8%) and the lowest prevalence was in Elgeinana locality (29.7%) and the difference was statistically significant (*p*-value=0.001) as shown in table 3.

Table 3: FMD Prevalence of unvaccinated cattle in Localities, West Darfur State

Locality	No. of tested animals	No. of positive samples	Sero-prevalence %	*p*-value
Forbaranga	63	40	63.5	0.001
Habeila	60	30	50.0	
Baidha	62	26	41.9	
Keranic	64	21	32.8	
Elgeinana	64	19	29.7	
Total	313	136	43.5	

3.2.2.2 FMD prevalence in relation to location site

According to locations Frequency and distribution of positive results of cattle for FMD sero- prevalence. The highest sero-prevalence was observed in Masmagi area 76.9% (13/10) in Habeila locality followed by Darelnaeem area 75.0% (12/9) in Forbaranga locality, Kagarna area 71.4 (14/10) in Forbaranga locality, Goz elhar 69.2% (13/9) in Habeila locality and Elgomaza sonta area 69.2% (13/9) in Forbaranga locality. Om Kharoba 58.3% (7/12), Forbaranga 58.3% (7/12), Om Elgora 53.8% (7/13), Om Tajok 50.0% (7/14), Aazirni 46.2% (6/13), Dagajoar 45.5% (5/11), Rocei 42.9% (6/14), Klalah 41.7%

(5/12), Boro Alef 41.7% (5/12), Dolmaga 40.0% (4/10), Bit Majaih 35.7% (5/14), Jokri 30.8% (4/13), Ranga 30.8% (4/13), Sembla 30.8% (4/13), Habeila 27.3% (3/11), Hager Zagawa 25.0% (3/12), Fofo 25.0% (3/12). While the lowest sero-prevalence was observed in Kasiah 18.2% (2/11) in Baidha locality, Adar 7.7% (1/13) in Elgeinana locality and Bir Madenah 7.7% (1/13) in Keranic locality. Statistically there was significant difference between locations of West Darfur state (*p*-value=0.002) (Figure 4).

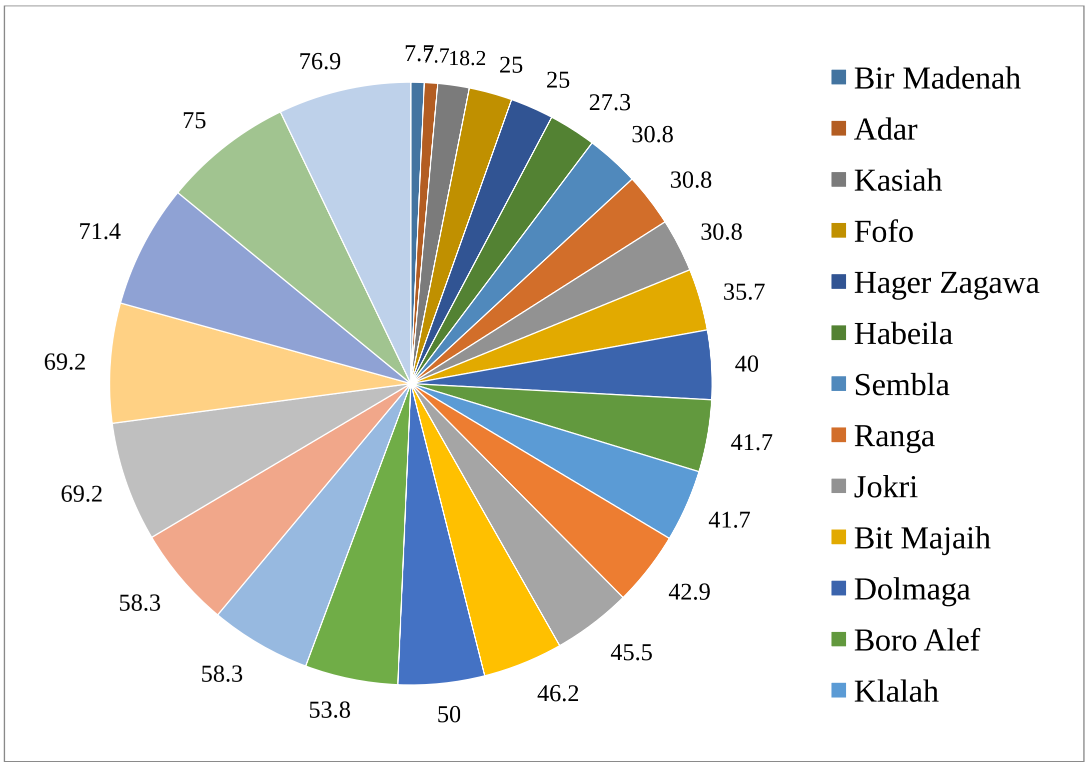

Figure 7: FMD prevalence within age groups in sampling sites of unvaccinated cattle in West Darfur

3.2.2.3 FMD prevalence in relation to distance from main road

FMD is highly prevalent in relation to long distance from main road (49.4%) compared with herds near to main roads (37.7%). and the difference was statistically significant (*p*-value =0.038) (Table 4).

Table 4: FMD prevalence of unvaccinated cattle (n=313) in relation to distance from main road in West Darfur state

Distance	No. of tested animals	No. of positive samples	Sero-prevalence %	*p*-value
Near to main road	159	60	37.7	0.038
Far from main road	154	76	49.4	
Total	313	136	43.5	

3.2.2.4 FMD prevalence in relation to area altitude (above sea level)

The sero-prevalence of FMD in relation to area elevation factor infection was higher in high altitude areas (49.6%) compared with medium (37.3%) and low-level elevation area (35.1%). The difference was statistically significant (*p*-value=0.001) (Table 5).

Table 5: Prevalence of unvaccinated cattle (n=313) in relation to area altitude (above sea level) in West Darfur state

Elevation	No. of tested animals	No. of positive samples	Sero-prevalence%	*p*-value
High	100	59	59.0	0.001
Medium	102	38	37.3	
Low	111	39	35.1	
Total	313	136	43.5	

3.2.2.5 FMD prevalence in relation to Production type

Infection was higher in cattle used for milk production (58.3%) compared with cattle used for meat and dual-purpose production (42.2%) and

(42.9%) respectively. Statistically there was no significant association difference (*p*-value=0.309) (Table 6).

Table 6: FMD prevalence of unvaccinated cattle (n=313) in relation to Production type in West Darfur state

Production type	No. of tested animals	No. of positive samples	Sero-prevalence %	*p*-value
Meat	275	116	42.2	0.309
Dairy	24	14	58.3	
Multi purpose	14	6	42.9	
Total	313	136	43.5	

3.2.2.6 FMD prevalence in relation to Production system

Infection was almost similar in both production systems of cattle; agro pastoralist 43.4% (109/251) and sedentary mixed 43.5% (27/62) compared with cattle. Results of association between production system and sero-prevalence was statistically no significant difference (*p*-value=0/986) (Table 7).

Table 7: FMD prevalence of unvaccinated cattle (n=313) in relation to Production system in West Darfur state

Production system	No. of tested animals	No. of positive samples	Sero-prevalence%	*p*-value
Agro pastoralist	251	109	43.4	0.986
Sedentary mixed farming	62	27	43.5	
Total	313	136	43.5	

3.2.2.7 FMD prevalence in relation to introduction of new animals into the herd

For the introduction of new animals into the herd, Infection was higher in that a new animal was added into the herd (51.0%) compared to that not introduced the herd (39.7%). The results of Chi-square (χ2) test showed that there was no statistically significant association (*p*-value=0.059) (Table 8).

Table 8: FMD prevalence of unvaccinated cattle (n=313) in relation to introduction of new animals into the herd in West Darfur state

Introduction new animals into the herd	No. of tested animals	No. of positive samples	Sero-prevalence %	*p*-value
Yes	104	53	51.0	0.059
No	209	83	39.7	
Total	313	136	43.5	

3.2.2.8 FMD prevalence in relation to herd size

For sero-prevalence of FMD in herd size risk factor, infection was higher in small herd size (49.6%) compared with large herd size (40.2%) and medium herd size (38.4%). The results of relationship between herd size and sero-positivity was significantly variable in the univariate analysis as shown in. Nevertheless, the difference was not statistically significant for herds of cattle (*p*-value=0.186) (Table 9).

Table 9: FMD Prevalence of unvaccinated cattle (n=313) in relation to herd size different locations in west Darfur state cattle

Herd size	No. of tested animals	No. of positive samples	Sero-prevalence%	p-value
Small	127	63	49.6	0.186
Medium	99	38	38.4	
Large	87	35	40.2	
Total	313	136	43.5	

3.2.2.9 FMD prevalence in relation to herd composition

No significant association between cattle that were kept together with small ruminants in compare with cattle alone (*p*-value=0.876) as shown in table 10.

Table 10: FMD Prevalence of unvaccinated cattle (n=313) in relation to herd composition in West Darfur state

Herd composition	No. of tested animals	No. of positive samples	Sero-prevalence %	*p*-value
Cattle+sheep	166	70	42.2	0.876
Cattle+goats	39	18	46.2	
Cattle+sheep+goats	108	48	44.4	
Total	313	136	43.5	

3.2.2.10 FMD prevalence in relation to history of FMD in cattle herd in this year

Infection was higher in cattle that have previous history of FMD (47.4%) in compared with cattle that did not have previous history of FMD for more than year (39.8%). Statistically there was no significant difference between the categories (*p*-value=0.174) as in (Table 11).

Table 11: FMD Prevalence of unvaccinated cattle (n=313) in relation to history of FMD in West Darfur State

History of FMD	No. of tested animals	No. of positive samples	Sero-prevalence%	*p*-value
Yes	152	72	47.4	0.174
No	161	64	39.8	
Total	313	136	43.5	

3.2.2.11 FMD prevalence in relation to availability of Veterinary services

Infection was higher in cattle that have access to government and private veterinary services (44.1%) in compared with cattle that access to private veterinary services only (42.0%). Statistically there was no significant difference between the categories (*p*-value=0.723) shown in (Table 12).

Table 12: FMD Prevalence of unvaccinated cattle (n=313) in relation to availability of Veterinary services in West Darfur state

Veterinary services	No. of tested animals	No. of positive samples	Sero-prevalence %	*p*-value
Govern+ private	213	94	44.1	0.723
Private	100	42	42.0	
Total	313	136	43.5	

3.3 Logistic regression analysis

Only 6 risk factors that found significant in the univariate analysis were subjected to multivariate analysis using Logistic Regression model. The result showed that out of them, 4 risk factors were found to have an association with FMD sero-prevalence with (p-value≤0.05); locality, location, Sex and Age of cattle as shown in table (13).

Table 13: Multivariate analysis for the association between FMD sero-positivity status and the potential risk factors resulting from the univariate analysis using Logistic regression of Unvaccinated Cattle in West Darfur State

Risk factor	Sero-prevalence (%)	Exp(B)	95.0% C.I.for EXP(B)		*p*-value
			Lower	**Upper**	
Locality					**.034***
Forbaranga	63.5	.044	.006	.325	.002
Habeila	50.0	.494	.060	4.091	.513
Baidha	41.9	.221	.034	1.433	.114
Keranic	32.8	.338	.039	2.954	.327
Elgeinana (*Ref.*)	**29.7**				
Location					**.003***
Darelnaeem	75	4.399	.483	40.099	.189
Boro Alef	41.7	44.052	5.673	342.082	.000
Elgomaza sonta	69.2	3.186	.394	25.742	.277
Forbaranga	58.3	1.981	.304	12.892	.474
Goz elhar	69.2	.395	.044	3.506	.404
Sembla	30.8	5.576	.716	43.438	.101
Habeila	27.3	6.490	.756	55.722	.088
Masmagi	76.9	.253	.027	2.418	.233
Kasiah	18.2	9.564	1.125	81.313	.039
Bit Majaih	35.7	2.838	.419	19.241	.285
Dagajoar	45.5	1.620	.221	11.853	.635
Om Kharoba	58.3	.410	.060	2.810	.364
Aazirni	46.2	1.902	.219	16.499	.560
Rocei	42.9	1.468	.181	11.925	.720
Klalah	41.7	1.591	.174	14.557	.681
Bir Madenah	7.7	36.545	2.405	555.389	.010
Om Elgora	53.8	.440	.065	2.989	.401
Hager Zagawa	25.0	.292	.040	2.139	.226
Adar	7.7	1.967	.139	27.925	.617
Ranga (*Ref.*)	**30.8**				
Sex					**.029***
Male	19.4	2.617	1.106	6.196	.029
Female (*Ref.*)	**50.0**				
Age					**.000***
less than two years	22.3	18.983	7.013	51.388	.000
2 to 3 years	21.1	9.627	2.879	32.194	.000
3 to 4 years	58.8	1.187	.283	4.975	.815
4 years	60.0	2.548	.607	10.699	.201
5 years	53.8	1.773	.670	4.691	.248
Aged (*Ref.*)	**70.9**				

*$p \leq 0.05$ was considered as significant; C.I. = confidence interval; Exp (B) = exponent B, representing the odds ratio

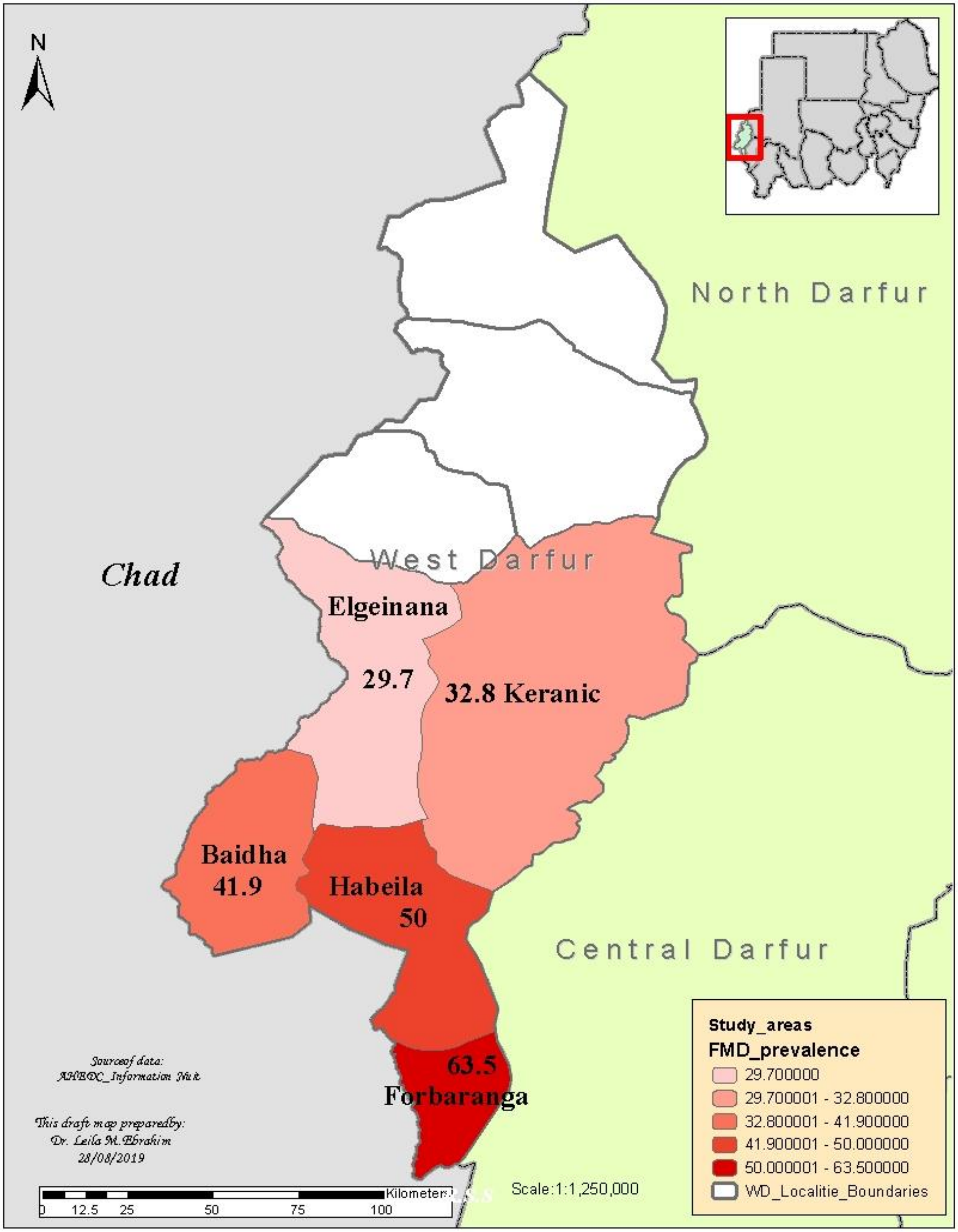

Figure 58: Map of West Darfur, Sudan showing the FMD prevalence in study localities (Drafted by GIS unit, MARF, 2019)

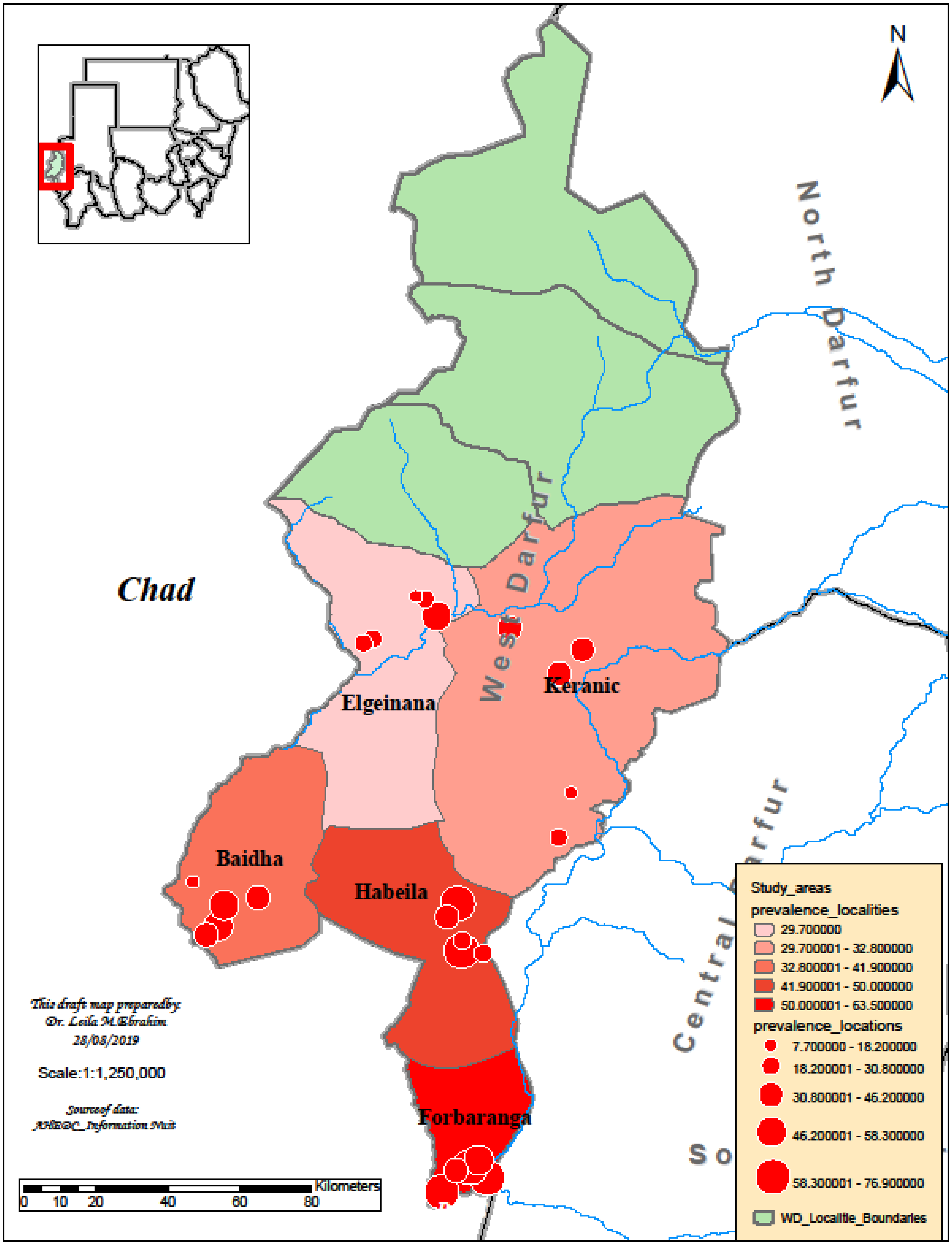

Figure 6: Map of West Darfur, Sudan showing the FMD prevalence in study localities and locations (Drafted by GIS unit, MARF, 2019)

CHAPTER FOUR

Discussion

An overall sero-prevalence 43.5% of FMD was observed in this study in West Darfur state of Sudan. This finding was in agreement with other study conducted in Ethiopia by Tesfaye *et al.* (2016) who reported 42.7%. This finding was lower than previously reported by Habiela *et al.* (2010) and Noureldin (2014) in Sudan, who reported 78.13%, 53.4% respectively. Furthermore, (Ochi *et al.* (2014) reported 56% in South Sudan and 64.73% was reported by Lazarus *et al.* (2012) in Nigeria. However, this finding is higher than the previous findings of Mishamo (2016) who reported 24.22% in Ethiopia and 19.0% in Lybia by Eldaghayes *et al.* (2017).

The risk factors showed a significant statistical association with FMD sero-prevalence in the univariate analysis were locality (*p*-value=0.001), location (*p*-value=0.002), altitude (*p*-value=0.001), distance from main road (*p*-value=0.038), sex (*p*-value=0.000) and age group (*p*-value=0.000). While production type (*p*-value=0.309), production system (*p*-value=0.986), introduction new animals into the herd (*p*-value=0.059), herd size (*p*-value=0.186), herd composition (*p*-value=0.874), history of FMD in the herd in this year (*p*-value=0.174) and veterinary services (*p*-value=0.723) showed no significant statistical association with FMD sero-prevalence.

In this study, significant variation (*p*-value=0.001) was found among areas of different altitude with a sero-prevalence of 59.0%, 37.3% and 35.1% at high, medium and low altitude, respectively. This difference may be due to the fact that in highland areas cattle ought to move freely for long distance in search of good grazing land and water, which allow interaction between livestock, which is the major factor for the transmission of the disease. The reason for the rapidity of spread to fully susceptible population is due to the highly infectious nature of the virus, the production of high titer in respiratory secretions and the large volumes of droplets and aerosol of virus shed by infected animals, the stability of virus in such droplets, the rapid replication cycle with very high virus yields and the short incubation period (Mekonen *et al.*, 2011)

Regard to distance from main road the result showed significant association (*P*-value=0.038) so the herds which located far from the main road is highly sero-prevalence than near one. Rational explanation, animal far from main road are difficult to have enough veterinary services (awareness). This was in not in agreement with other study in Tanzania conducted by Allepuz *et al***.** (2015) who reported that FMD was consistently associated with proximity to main roads.

The potential risk of herd size factor investigated in this study. For sero-positivity the herd size showed no significant statistical association (*p*-value=0.186). This is not in agreement with studies reported in Ethiopia (Mishamo, 2016). May be due to the high number of sampled animals from small herd, most of the small herd sizes kept in close at night, therefore, they facilitate frequency of direct contact and hence enhances chances of transmission and may be due to restocking of cattle from unknown sources.

There was no significant variation observed between cattle alone or with small ruminants. This is not in line with previous study conducted in Southern Ethiopia by Megersa *et al.* (2009) indicated the importance of live small ruminant on the epidemiology of FMD. Our result showed cattle herds kept with sheep and goat combination or without, suggests no role of small ruminants with FMD epidemiology.

The multivariate analysis was done using logistic regression, with confidence interval of 95% and *p*-value of ≤0.05 was used to assess the association between identified significant risk factors in the univariate analysis and occurrence FMD. In the multivariate analysis, locality (*p*-value=.034), location (*p*-value=.003), sex (*p*-value=.029) and age (*p*-value=.000), showed a significant association with sero-prevalence**.**

Two significant FMD clusters were indentified locality and location. The higher sero-prevalence observed in Forbaranga locality (63.5%) which lays in the south part of the state bordering Republic of Central Africa and Chad, followed by Habeila locality (50.0%), Baidha locality (41.9%), Keranic locality (32.8%) and the lowest sero-prevalence reported in Elgeinana locality (29.7%). The gradual decrease in the result as moved from south to north (Fig. 3), this indicates a positive relation between sero-prevalence and cattle density in south parts of the state. Present study indicated that there is statistically significant variations among administrative units (*p*-value=0.001). This is in line with previous reports in Ethiopia (Beyene *et al.* 2015). The high sero-prevalence might be due to differences in the movement and distribution of livestock, the level of contact between herds and ungulate susceptible wildlife, the grazing type in each administrative structure and the livestock contact with other herds at the international border.

The high rest sero-prevalence observed (70%) in Darelnaeem location of Forbaranga locality, that could relate to the large number of cattle population and high contact of livestock. As stated in previous study, reported in Ethiopia by (Beyene *et al.* (2015). Where, cattle have frequent contact with ungulate wildlife (Molla *et al.*, 2010). The factors behind the lowest sero-prevalence in Adar area (7.7%) may be due to the good veterinary services in the capital of the state and low density of cattle population.

In the present study, sex distribution indicated sero-positivity is highest among female (50.0%) than the male (19.4%). This finding is in agreement with a study reported in Nigeria Abubakar *et al.* (2017) and Ethiopia Desissa *et al.* (2014). A disagreement with studies reported in Kenya Kibore *et al.* (2014). These variations may be related to the effect of the higher number of females included in the sample than males due to the fact that most cattle owners usually keep few males only for breeding purpose (Mishamo, 2016).

The current study revealed a significant variation on sero-prevalence of foot and mouth disease among the six age groups. Higher sero-prevalence of FMD in over-aged and adult animals than in young and less than two years old calves is observed in this study in agreement with the previous reports of (Mohamoud *et al.*, 2011; Mekonen *et al.*, 2011 and Chepkwony *et al.*, 2012). This may be due to the cumulative exposure of cattle population to the FMD viruses (Murphy *et al.*, 1999). The relatively low sero-prevalence in younger animals may be indicative of low frequency of exposure to risk factors. Thus, adult animals might have acquired the infection from multiple serotypes and could produce antibodies against all serotypes of FMD. (Mekonen *et al.*, 2011).

CONCLUSION

Foot and mouth disease reported to be endemic in Sudan and conformed serologically widespread in West Darfur State. The disease is a main barrier to the improvement of livestock resource due to its bad effects on trade of live animal and its product.

In conclusion, by using multivariate logistic regression analysis 6 factors were appeared to be associated with the disease. Four of them (locality, location, sex group, and age) were found to be significantly associated with FMD sero-prevalence ($p \leq 0.05$).

The result of this study showed that FMD is an important cattle disease in the study area necessitating further investigation and characterization of the circulating virus serotype to apply effective control and prevention measures.

RECOMMENDATIONS

The following presented as recommendations:

- A broad regular surveillance, monitoring, reporting and serotyping of the outbreak isolates all over the country particularly in the border sites to update, verify the introduction and circulation of new serotype in the country.
- Investigate the role of wildlife in the epidemiology of the disease in Sudan.
- Sudan National Disease strategy for FMD and action plan should be strictly implemented to control movement and through regular vaccination.

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