ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

RETROSPECITVE AND CROSS-SECTIONAL INVESTIGATION OF RIFT VALLEY FEVER IN SMALL RUMINANTS IN PASTORAL AREAS OF ETHIOPIA

BY
MELESSE BALCHA GHELAN

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DEBRE ZEIT, ETHIOPIA

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A thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University in the partial fulfilment of requirements of Master of Science in Tropical Veterinary Medicine

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LIST OF ABBREVIATIONS

Ab	Anti body
Ag	Antigen
ABTS	2, 2`-Azino di-ethyl benzothiazoline sulfonic acid
Anti-RVFV	Anti Rift Valley Fever Virus
CCD	Cold Cloud Density
CDC	Center for Disease Control and Prevention
DIC	Disseminated Intravascular Coagulation
ELISA	Enzyme Linked Immunnosrbant Assay.
EPAIAT	Ethiopian Participatory Applied Impact Assessment Team
EMPRES	Emergency Preparedness System
ETB	Ethiopian Birr
FAO	Food and Agriculture Organization
FEWS	Famine Early Warning System
HRPO	Horse-Radish Peroxidase
IEP	Inter-Epizootic Period
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IQC	Internal Quality Control
L-Segment	Long segment
MMWR	Morbidity Mortality Weekly Report
MoA	Ministry of Agriculture
M-segment	Medium segment
NAHRC	National Animal Health Research Center
NDVI	Normalized Difference Vegetation Index
NICD	National Institute for Communicable Diseases
OD	Office International des Epizootics
PA	Peasant Association
PBS	Phosphate Buffered Saline
Pp	Percentage positivity
RSSD	Remote Sensing Satellite Data
RVF	Rift Valley Fever
RVFV	Rift Valley Fever Virus

S-segment	Short Segment
SPS	Sanitary Phyto Sanitary
TCP	Technical Cooperation Project
TMB	Tetra-methylbenzidine.
WHO	World Health Organization
WTO	World Trade Organization

ABSTRACT

Rift Valley Fever Disease is a peracute or acute, febrile, mosquito-borne, zoonotic disease characterized by high rates of abortion and neonatal mortality. It occurs primarily in sheep, goats and cattle. It is a zoonotic disease causing an unexpectedly very high numbers of cases and deaths. It is caused by Rift Valley Fever Virus of the *Bunyaviridae* family and genus Phlebovirus.

Due to the epidemics in Saudi Arabia and Yemen, in September, 2000 export trade of live animals from east African countries, including Ethiopia, was banned by the Gulf countries due to fear of importing risk of Rift Valley Fever. In some of the countries the disease was identified but in others like Ethiopia there was no evidence of the disease, except suspicion and inability of the country to prepare scientific evidence on the situation of the disease. In response to this and to safe guard its population a National surveillance strategy had been launched in 2001 and is being implemented, though it hadn't generated an official report. The ban affected the country as a whole but there were parts of the community affected more, the pastoralists and investors in the sector. The pastoralists provide about90% of the export animals particularly small ruminants. Therefore this survey was proposed in September 2003, with the aim to support the surveillance programme by generating a scientific report from the pastoral areas through determining the sero-prevalence of RVF in the small ruminants and detecting the risk factors associated with.

Across-sectional and retrospective survey was conducted on 1176 sheep and goats sera to detect anti-Rift Valley Fever Virus IgG and IgM antibodies using indirect ELISA. All the samples were collected in 1999 and the year 2003/2004 from Afar regional state and Borana zone. Of the total 900 sera tested, for cross-sectional survey, 29 (3.22%) were found positive to IgG antibodies; but 30 samples including the 29 IgG positives, tested for the detection of IgM were with no any positive. Because the serum samples used for the retrospective survey were tested in the year 2000 with an ELISA kit that have many limitations that resulted in high false positives; therefore, a retest of 246 sera including 142 previously positives resulted in only 32 (22%) positives reducing the previous prevalences of the total sera from 17.9% (142/820) to 3. 9% (32/820). The recalculated respective prevalence of Afar and Borana was 2.6% (13/500) and 5.9% (19/320). The respective prevalence by the previous kits was 12.8% and 24.4%.

The cross-sectional survey prevalence is 3.2%, 4% and 2% for the total sero, Afar region and Borana zone, respectively. Prevalence showed increase in Afar while there was a decrease in Borana. The risk factors found significantly with sero-positivity were species of animal, living in irrigated land and areas found near permanent water sources and with flooding problems by univariate analysis of risk factors but only animal species, distance from permanent water sources and flooding. The age-specific sero-prevalences analyzed based on positives predictive value of the test revealed that there was no any indication of viral activites within the last four years. The geographic distribution of the sero-positives was mapped using a georeferenced data and the sero-prevalence data.

The predicative value of the test was less than 50%, ranging form 33.4% to 50%, therefore, it will be erroneous to give any conclusion on the situation of the infection or disease in the country based on this serology with out any supportive confirmatory diagnosis of the causal agent either from vector or vertebrate host.

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1. INTRODUCTION

One of the different uses of livestock in Ethiopia is generation of income from export trade of live animals, meat and mutton, and hides and skin. The contribution of this sector is estimated to be 15 per cent and 33 per cent to the national and agricultural GDPs, respectively. Hides and skin are also known to contribute 17 per cent of the country's annual export earning while other livestock products and live animal export fetch about 1-2 percent (Hailemariam, 2000 and Haile Miriam and Demise, 2002). The average annual income generated from export trade from 1990-1999 is 153 million ETB (Hailemariam 2000). The contribution of livestock in the export sector was seen to drop from 24.7 percent in 1974 to 7.78 percent in 1999 (Tibbo *et al.*, 2001). This is due to many factors, which can be directly related to the trade or indirectly, affecting it. These include: poor livestock management; inadequate veterinary services; absence of market oriented livestock production reflected by lack of trade routes to the market and use of animals for meat production secondary to draught power prevalence of rampant livestock diseases, high rate of smuggling of livestock from high production areas of the pastoralists and recurrent severe drought.

There are many diseases interfering in the livestock trade of the country. Of these diseases RVF is currently very important to the countries in the Horn of Africa including Ethiopia.

Rift Valley Fever Disease is a peracute or acute, febrile, mosquito-borne, zoonotic disease characterized by high rates of abortion and neonatal mortality. It occurs primarily in sheep, goats and cattle. The susceptibility of different breeds of animals to RVF viruses considerably differs. Some indigenous African animals may have only inapparent infections, while infection in exotic breeds results in severe clinical disease with mortality and abortion. Although susceptible, older animals and some other species usually do not show signs of the disease.

Humans acquire the infection by handling infected materials (blood or tissue), exposure to infected (mosquito) vector or to aerosols (OIE, 2000; CDC, 2000). In areas where there is small animal population but a dense human settlement, infection of humans by vectors is a striking feature, even in such areas the disease may be recognized first in humans (OIE, 2000). In the epidemic-epizootic form economic losses can be high due to neonatal deaths and abortion as well as certain level of infertility, which retards the replacement of animals. The

last outbreak of the disease in the North Eastern Kenya and Southern Somalia in 1997/98, for instance caused livestock losses of about 70 per cent and infection of 89,000 and deaths of 300 human (Woods *et al*., 2002).

A trade ban was imposed, by the gulf countries, on nine countries in the horn of Africa in which where problems of RVF had been identified and not. Ethiopia was one of those countries with no identified problem of the disease but victimized for its proximity to the countries with the problem and for it has no any scientific evidence proving the nonexistence of the disease.

The trade share of the countries of the Horn of Africa was interrupted due to the export trade bans imposed on them by the countries of the Gulf States after RVF outbreaks occurred. These are the one lasted from February 1998 to April 1999 by Saudi Arabia and since mid of the year 2000 imposed on eight countries by six countries of the Gulf States. Among affected countries Ethiopia, Eritrea and Somalia suffered seriously. The result from the first ban leading to a decrease by more than 30 per cent and by more than 92 percent decrease of export from October to December 2000 in Somalia only (Tibbo *et al.*, 2001). The impact from the second ban was higher due to lack of alternative markets and it was followed by periods of shortages of rain. The annual export loss for Ethiopia due to the ban was more thon 18 million ETB (Hailemariam, 2000).

There are parts of the community suffering more. Pastoralists who were totally dependent on livestock production and income generated from the sector were more affected. The pastoralists in regional states of Afar, Somali and Borana supply 90% and 70% of the export animals from Ethiopia and Somalia, respectively (FEWS, 2001 and Asfaw 2002a). There was a report of decrease of export income of ELFORA Enterprise from ETB3, 239,730 (1998/99) to ETB 834,533 (2000) (Dagnachew, 2001). In addition to the trade ban there are other global situations urging the need to establish surveillance strategy of such transboundary diseases. These include:

- The increasing global awareness to wards these economically and zoonotically important diseases;
- Increased conservative approaches towards the countries who are not undergoing surveillances on such diseases;
- The WTO-SPS agreements provide extra values from the world markets different from those who have not established the control strategy.

Therefore in the context of the trade bans and their impacts as well as considering the changing global situations the Government of Ethiopia has established a nationwide RVF surveillance strategy in 2001 in collaboration with the regional Technical Cooperation Program (TCP) of FAO on trade related transboundary livestock diseases (MoA, 2003).

This study was designed to do sero-surveillance on RVF in small ruminants of the pastoral areas of Afar and Borana. The study sites were selected based on their relevance to the export trade, they being potential epizootic areas and high risk areas (due to their natural environment and being at the proximity with countries known to be with the direct disease problem), and are contributing to high proportions of the needy people in the country relative to their population size (Tadesse, 2002).

To achieve this it was designed to conduct both retrospective and cross-sectional survey using serum samples in serum bank in National Animal Health Research Center (NAHRC) and with sera collected from active field survey, respectively. 820 sera from bank and 900 from active survey were tested using indirect Enzyme Linked Immunosorbant Assay (ELISA) manufactured in the by the National Institute for Communicable Diseases (NICD), Johannesburg, for the detection of anti-RVF virus immunoglobulin G (IgG) and M (IgM).

The survey included both field and laboratory works; the field work being for the collection of serum samples and questionnaire and other disease related data and the laboratory work for the examination of the sera for the detection of anti-RVFV antibodies. The field activities covered 17 districts (10 Afar and 7 Borana) and 33 Peasant associations (PAs) (19 Afar and 14 Borana). The Sampling schedule was designed to fix to the after rainy season of each study site. The Laboratory investigation was conducted at NAHRC at Sebata located 25 Km southwest of Addis Ababa on the Addis-Jimma main road.

The survey was conducted in collaboration with Ministry of Agriculture, NAHRC, and Addis Ababa University, Faculty of Veterinary Medicine for the shared supply of facilities and inputs

Expected outcomes from this study will be a real picture of the situation of the disease in both study areas with determination of the problems and risk factors playing roles. It also increases the contribution of Faculty of Veterinary Medicine Addis Ababa University in collaboration with the MoA and NAHRC.

The beneficiaries from this study will be the country as a whole and in particular those social groups of the community who are fully dependent on the income from the sector, the pastoralists and the investors.

The objectives of this were:

- To determine the sero-prevalence of Rift Valley Fever in small ruminants in pastoral areas of Afar region and Borana zone of the regional state of Oromya.

- To compare the sero-prevalence of RVF during the years 1999 (Post Epizootic Period) and 2003/2004 (Inter-Epizootic Period) in the respective site.

- To identify risk factors in the respective study sites.

2. LITERATURE REVIEW

2.1 Aetiology

2.1.1 Taxonomy

Rift valley fever is caused by *Rift Valley Fever Virus* (RVFV). This RNA virus belongs to the family of *Bunyaviridae* and under the genus of *phlebotomus* (Sall *et a*l., 1999; Schmaljohn and Hooper, 2001; Woods *et a*l., 2002; DEFRA, 2002; Miller *et al*., 2002). Family *Bunyaviridae*, which comprises over 200 viruses (serotypes, subtypes and lineages), has been isolated from various arthropods and vertebrates in all continents of the World, except Antarctica (Schmaljohn and Hooper 2001). Based on the sharing morphologic, morphogenic and antigenic properties, the family was subdivided into 4 animal genera (Bunya virus, Hantavirus, Nairovirus, and Phlebovirus) and one- plant genus (Tospoviru*s*) (WHO, 1982; Schmaljohn and Hooper, 2001).

The Phlebotomes genus viruses are more diverse in terms of arthropod vector than those of other arthropod - borne genera of the family. At present this genus comprises nine species and 16 ungrouped viruses (Schmaljohn and Hooper, 2001). Most virus members are associated with Phlebotomus sand flies, hence the genus name Phlebovirus. However, there are some exceptions such as RVF of a medical and agricultural important virus in Africa, which is primarily associated with A*edes* species of mosquitoes (WHO, 1982; and Nichol, 2001).
RVF virus is a pathogen identified as potential human bioterrorism agent by the National Animal Health Reporting System (NAHRS) and Center for Disease Control and Prevention (CDC) (Bruce and Francois, 2003 and CIDRAP, 2003)

2.1.2 Structure of Rift Valley Fever Virus

Rift Valley Fever viruses are spherical or ovoid particles of about 90 to 100 nm in diameter and display surface glycoprotein projections of 6 to 7.5nm, which are embedded in a lipid bilayer envelope of approximately 5-7 nm thick. The surface spikes generally consists of heterodimers of the viral glycoproteins G1 and G2 (about 160 per RVF particle) which interact to form surface morphologic units arranged regularly and that vary among viruses in different genera. Viruses in the phlebovirus genus have roundly closely packed morphologic units, approximately (Fig.1) (Schmaljohn and Hooper, 2001)

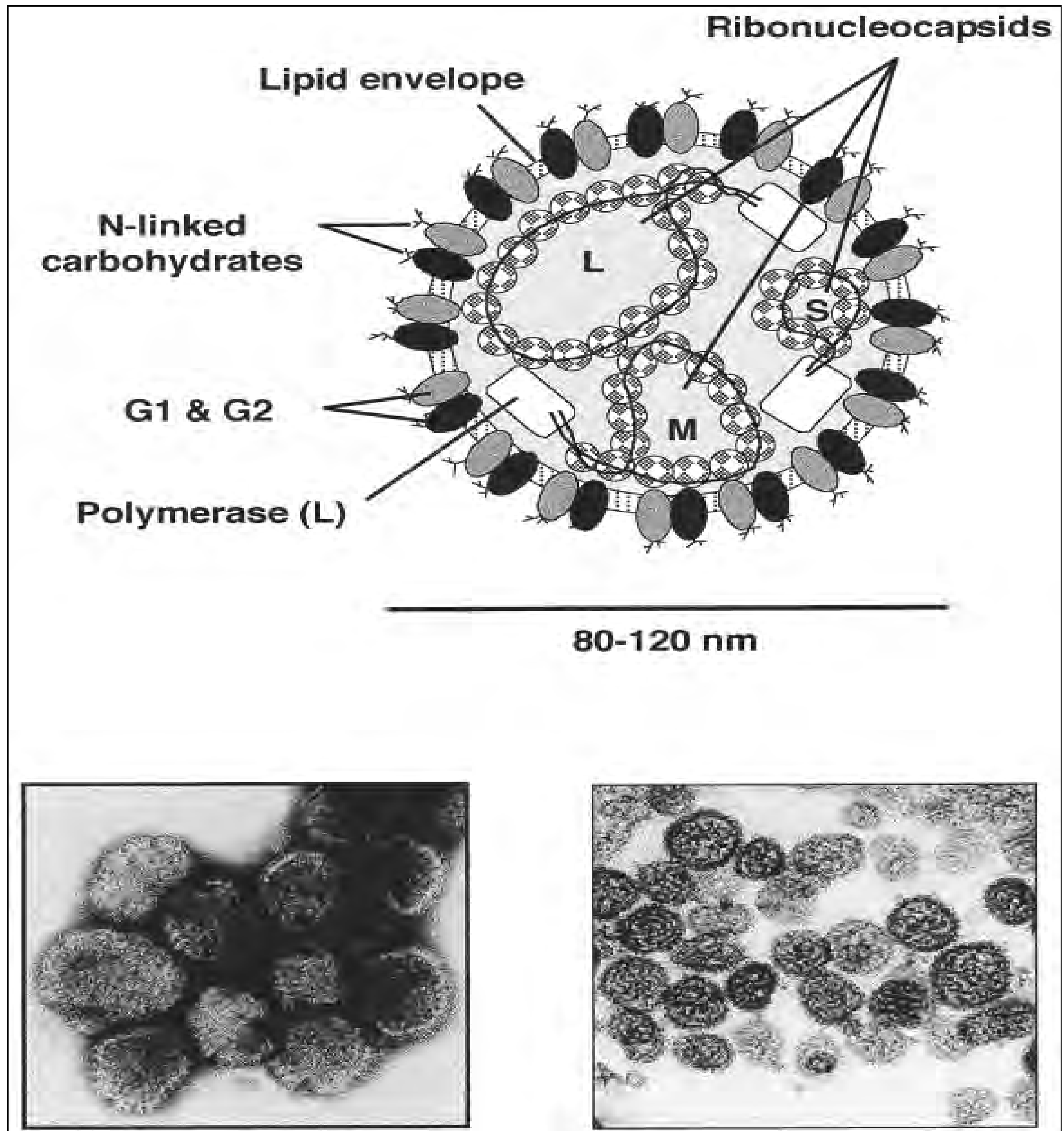


Figure 1**:** Morphology of *Bunyaviridae* Virions*.* Schematic cross-section of a virus.

NB: The three RNA genome segments (S, M and L) are complexed with nucleocapsid protein to form ribonucleocapsid structures. The nucleocapsids and RNA-dependent RNA polymerase are packed within a lipid envelope that contains the viral glycoproteins, G_1 and G_2. Note that there is no matrix protein. (Source: Schmaljohn and Hooper, 2001).

2.1.3 Types of Rift Valley Fever Virus

Up to now there are 21 isolated strains of Rift Valley Fever virus including the new isolate from Saudi Arabia designated as Ar SAO1 with strain name SA01 1322 (Miller *et al.,* (2002).

The 21 strains are of arthropod, animal or human origins and they are serologically the same. But isolates differ in virulence (Sall *et al*., 1999; EMPRES/FAO, 2000 and Miller *et al*., 2002).

The phylogenic analysis study on the 20 isolates from Africa confirmed the distribution pattern of RVFV to be within three lineages. These are Central-East Africa, West Africa and Egypt designated as Ia, Ib and II, respectively. The study also confirmed that some of the strains in the Central-East Africa and Egypt groups to be closely related, suggesting the existence of endemic-enzootic maintenance cycle of virus in these areas. Host specificity was observed indicating frequent exchange of viruses between different hosts (Sall *et al;* 1999).

2.1.4 Genomic Structure and Organization

The viral genome that contains the information necessary for virus's perpetuation and multiplication is the nucleic acid(s) is formed during the synthetic multiplication phase of the invading virus (Joklick, 1988; Levinne, 2001). The genome of Rift Valley Fever virus consists of three negative sense, single stranded RNA segments designated L (large), M (medium) and S (small) (Schmaljohn and Hooper, 2001). They are seen as three different sized circular RNAs, their termini forming non-covalently bounded panhandle structures. They encode the structural proteins (N, G_1/G_2 and L) in the L, M and S segments respectively (Nichol, 2001).

The RNA segments are complexed with structural protein (N) to form individual L, M and S nucleocapsids, which appear to be helical. At least one of each of the L, M and S nucleocapsids must be contained in a virion for infectivity; however, equal numbers of them may not be packaged in mature virions leading to individual size difference (Schmaljohn and Hooper, 2001). Necked nucleic acid of RVFV is not infective unlike many animal viruses.

Therefore, this part of the virus is the one with the greatest determining potential for species specification, host range, perpetuation etc. characteristics that are responsible for all the virus' effects.

2.1.4.1 Natural Genomic Segment Reassortment and Its Implications

Recombination describes a process by which nucleic acid sequences from two genotypically different parental viruses are exchanged so that the progeny contain sequences derived from both parents (Levinne, 2001). For RNA viruses containing segmented genomes,

recombination occurs through reassortment of individual parental genome segments in to progeny (Levinne, 2001). RVFV is one of the RNA viruses with this capacity.

This evolutionary natural phenomenon (reassortement) in RVFV has the following implications:

- The virus will have epidemic potential with the changing environmental and global situations as it was experienced from the *Influenza virus* (Barry, 1988; Sall *et al.,* 1999; Nathanson, 2001)
- Viruses with segmented genomes offer a special opportunity to analyze their pathogenic potential and perhaps construct vaccine strains; (WHO, 1982)
- The use of live attenuated vaccines of RVFV in epidemic cases where active viral circulation is questionable (Sall *et al.,* 1999; DeFilippis and Villarreal, 2001).
-

2.2 Epidemiology

2.2.1 Host Range and Susceptibility

Rift Valley Fever Virus infects many species of animals and humans (Table1). Neonatal lambs, kids, calves are highly susceptible (EMPRES/FAO, 2000; OIE, 2000; Nichol, 2001; DEFRA, 2002; Miller *et a*l., 2002; Mebus, 1997). Other authors put only cattle, sheep and human are highly susceptible (EMPRES/FAO 2000) and others include goats (OIE, 2000; Nichol, 2001; DEFRA, 2002). But previous studies in East Africa showed that sheep and goats are equally susceptible and a relatively higher tolerance of the African breeds of cattle was manifested by shorter viremia duration and with absence of clinical manifestations during epizootics (Davies, 1998; Asfaw, 2002b and AGA/ FAO, 2002). But EMPRES/ FAO (2000) generalized by indicating that clinical diseases are observed in sheep, cattle and goats, domesticated buffaloes, camels and humans. The susceptibility of wild antelopes was also explained in Hubert *et al*., (1975). The susceptibility of different laboratory animals such as mice, and baby hamster has been mentioned by (Hubert *et a*l., 1975; OIE, 2000). Donkey is mentioned as host of RVF once by Boden (1998). It has not proved possible to infect avian species; they are thought to be accidental hosts fed upon by RVF vector mosquitoes. The wild ruminant species found in the enzootic areas are not disease hosts, no clinical RVF is seen

when disease occurs in domestic ruminants in the same habitat. However; many develop antibody to RVF (AGA/FAO, 2002)

Therefore, all these authors' reports on the host indicate as there is difference in susceptibility to the virus and the severity of the disease based on the species, breed, age and physiologic situations.

Table 1: Rift Valley Fever host range and disease severity

Morality in 100% Severe illness	*Abortion Mortality Severe illness*	*Viremia Abortion*	*Infection Viremia*	*Refractive to infection*
Lambs	Sheep	Monkeys	Horses	Guineas pigs
Calves	Cattle	Camels	Cats	Rabbits
Kids	Goats	Rats	Dog	Pigs
Puppies	Water buffalo	Gray squirrels	Monkeys	Hedgehogs
Kittens	Humans			Tortoises
White mice				Frogs
Hamster				Chickens
Field mice				Canaries
Door mice				Pigeons
				Parakeets

(Source: Mebus, (1997)

2.2.2 Occurrence and distribution

Rift Valley Fever was first recognized as a disease in1930/1931. Since its isolation by Doubney and his colleagues, (1930) as cited in Seller (1981) from sheep in the Rift Valley of Kenya, large RVF epizootic in various areas of sub-Saharan Africa had been noted and clinically compatible outbreaks had been retrospectively identified as far back as 1912 (Schmaljohn, 2001). Until the isolation of Rift Valley Fever Virus (RVFV) from September 2000`s unexplained hemorrhagic fever in humans and associated illness in livestock along the South western border of Saudi Arabia and neighboring Yemen, the disease was known to be the problem of Africa only (Miller *et al*., 2002; Wood *et al*., 2002).

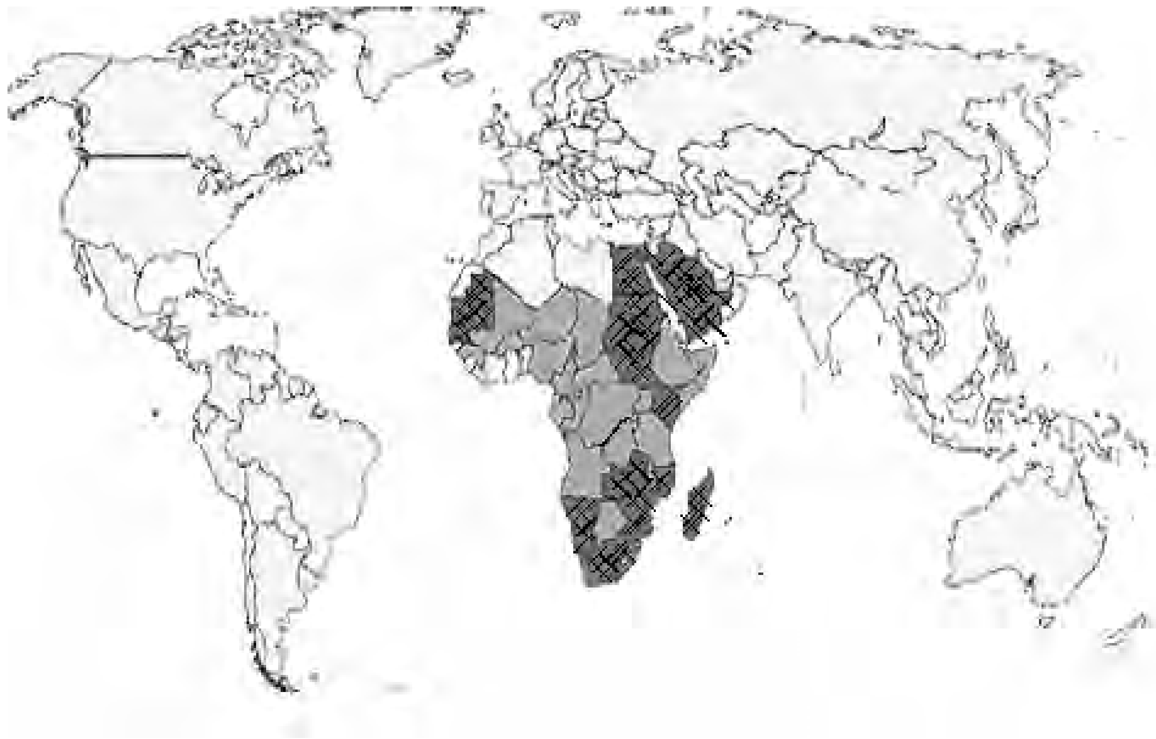
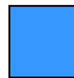


Figure 2: Rift Valley Fever Distribution Map



Countries with endemic disease and Substantial outbreaks of RVF: Gambia, Senegal, Mauritania, Namibia, South Africa, Mozambique,Zimbabwe, Zambia, Kenya, Sudan, Egypt, Madagascar, Saudi Arabia, Yemen

Countries known to have some cases, Periodic isolation of virus, or serologic Evidence of RVF: Botswana, Angola, Democratic Republic of Congo, Congo, Gabon, Cameroon, Nigeria, Central African Republic, Chad, Niger, Burkina Faso, Mali, Guinea, Tanzania, Malawi, Uganda, Ethiopia, Somalia.(Source Special Pathogens Branch/CDC, 2003)

2.2.2.1 Rift Valley Fever in Africa.

The geographic distribution of RVF covers much of Africa, from Senegal to Madagascar and from Egypt to South Africa, with most repeated epizootics in livestock being reported in East and Southern Africa (Schmaljohn and Hooper, 2001).

Since its first isolation during 1930-31 in east Africa, Kenya the isolation from different countries continued, in 1944 from mosquitoes in Ugandan Semliki forest (Sall *et al*., 1999). The disease was next recognized between an extensive epizootic occurred among sheep and cattle during the summer of 1950/1951 on the Inland Plateau region of South Africa which is the main sheep rearing area of that country. It had been repeatedly occurring since that year

1950 but the extremely, high loss among sheep and cattle (100,000 deaths and many human cases was dramatic. Out breaks repeatedly occurred in 1952 and 1956 in the same area suggesting that the virus had survived several winters on the plateau. Thereafter it occurred after 13 years; an apparent absence concurrently with an extensive epizootic occurred in cattle of Rhodesia and sheep of southern Mozambique (Hubert *et al*., 1975; WHO, 1982; EMPRES/RAO, 2000).

Until 1973 it had not been recorded in northern Africa beyond the usual sub-Saharan range but that year it reached Sudan and then Egypt, where major epizootic occurred in 1977-1978 (Linthicum *et al*., 1988a and 1988b; Mebus, 1997; DEFRA 2002). The spread of the disease to Egypt was first of its kind due to higher human cases and then is considered as a turning point in the history of RVF. Because of its importance as a potential zoonotic and it represented the 1st identified extension beyond the range of sub-Saharan Africa. The fact that the epizootic rapidly became established in a riverine or delta/irrigation system totally different from the African epizootic areas further south must serve as a warning of the potential for RVF to become established elsewhere, particularly the Mediterranean. The higher level of human involvement has been suggested to be due to the feeding habit of the mosquito vector, *Culex pipiens* and increased close contact of humans and animals in density populated areas of the country round the water channels (WHO 1982; EPRES/FAO, 2000; and CDC, 2002).

Even if the disease was reported in 1978 in West Africa from the Severe epidemic affected the Senegal River basin in Mauritania and Senegal, but there is indication of the persistence of the disease before as retrospective studies of the serum indicates (Linthicum *et al*., 1988 a; EMPES /FAO, 1998, Tibbo *et al*., 2001).

Generally according to this review RVF had been recognized in more than 26 countries of Africa and two countries outside Africa (Saudi Arabia and Yemen). Ethiopia was found in four literatures (WHO, 1982; Davies and Nunn, 1998; Tibbo *et al* 2001) being suspected to have the disease but with no confirmatory activities either from the country or the other side.

The reports by different groups on the epidemics interval differs between 5-15 years (WHO, 1982) 5-20 Years by EMPRES/FAO (1998), 5-25 years by Davies and Nunn (1998) and 15-30 years in Tibbo *et al*., (2001).

The Knowledge on the mechanism by which spread to and/or introduction into new epidemic/epizootic areas is not with concrete evidence or not more than speculation as it can be seen in the following discussions.

2.2.3 Modes of Transmission

There are two major patterns of transmission into which most viruses may be classified:

1. Viruses maintained in a single host species and
2. Arthropod borne viruses that alternatively infect insect and vertebrate hosts (Nathanson, 2001). RVFV is transmitted both by arthropods and none-vector borne routes of transmission.

2.2.3.1Vector Transmission

Rift Valley Fever Virus is primarily spread amongst animals by the bite of infected mosquitoes (WHO, 2000). Subsequently, the isolation of RVF from many species of mosquitoes in East and South Africa has substantiated this observation, and epizootic spread is invariably associated with the presence of abnormally high populations of mosquitoes (WHO, 1982; and Linthicum *et al*., 1988b; Woods *et al*., 2002).

A wide variety of mosquito species may act as the vectors for the transmission of the viral agent; and in different regions different species of mosquitoes may prove to be the predominant vector species (WHO, 2000). Potential mechanical vectors, epidemic vectors and true vectors are given in Table 2.

Table 2: Different species of vectors of RVF virus

Potential mechanical vectors	*Epidemic vectors*	*True vectors*
Midges	*Culex*	*Aedes*
Stomoxis	*Aedes*	*Eretmepodites*
Phlebotoms	*Anophèles*	
	Mansonia	

(Source : EMPRES/FAO, 2000)

2.2.3.2 None Vector Transmission

The direct transmission is simply the non-vector transmission but for the purpose of discussion we classify it in to two:

(a) Direct infection
(b) Potential spread by animal products

a) Direct infection: Most cases of RVF in humans in Africa during epizootic have followed close contact with live and dead animals. This is in addition to mosquito transmission. Humans are easily infected by contact with the body fluids of infected animals through contact with abraded skin, wounds or mucous membranes or by inhalation of aerosols generated. Thus the slaughter of infected animals, necropsy procedures and laboratory manipulations of tissues and isolated viruses are activities carrying a high risk of disease transmission (WHO, 1982; Sall *et al*., 1999; WHO, 2000; Woods *et al*., 2002). WHO (1982) suggests that traditional habit of slaughtering animals by the throat cutting to contribute to the increase of human cases during epidemics.

(b) Potential spread: by animal products: The animal products which are considered include fresh, chilled and frozen meat (beef mutton), milk and milk products, wool, bones, skins hides, and manure. All unprocessed fresh animal products that originate from infected areas during an epizootic should be regarded as potentially infective and treated accordingly (WHO, 1982).

2.2.4 Ecological dynamics for RVF epizootic emergence and inter-epizooticPersistence of the virus

2.2.4.1 Incidence of the diseases

a) Epizootic Period: A high prevalence of antibody may be detected in hosts, notably, cattle, sheep and goats, following periods of epizootic RVF activity. The highest figures are usually obtained from cattle, where 60-95% have been found to have IgM against RVF virus (AGA/FAO, 2002); but study during the recent outbreak in Yemen showed many flocks with prevalence of 30-90% to IgM antibody (Geiger, 2002). He mentioned serological studies for IgG antibodies during outbreaks of RVF in western African countries to have exhibited herd

prevalence 50% up to 100% to a higher proportion of sera having detectable levels of IGM antibodies. Similar results have been shown in sheep but generally a lower prevalence is found amongst goats and camels. There are differences between epizootics in the same areas. For example, 90% sera-conversion may occur in one epizootic and them 15-25% in another. These figures are related to the level of challenge by infected vectors & prevailing epidemiological factors at the time (AGA/FAO, 2002).

b) Inter-epizootic periods (IEP): Epizootic persists for 1-3 years before virus activity dies out; virus activity may then not be detected for very long periods. In enzootic zones such as centre or coastal forest, there may be some virus activity most years depending upon the rainfall and mosquito populations. Sporadic cases in humans have been reported in inter-epizootic periods and 1-5% of domestic animals in such areas may be found to sero-convert. In the bushed and wooded grasslands where epizootics are most severe, no virus activity may be detected for many years during IEP. There may be some emergence of RVE infected *Aedes* mosquito in most years in areas of high rainfall in Africa.

2.2.4.2 Generation of Epidemics and Inter-epidemics Persistence

a) Inter-epidemic Persistence of RVF virus. Recurrent occurrences of RVF virus activity in localized areas of Southern and Eastern Africa during most years has been observed. This provides one of the keys to understanding virus survival during inter epizootic periods.

The other key factor to persistence of the virus lies on the biology of certain flood water-breeding *Aedes* mosquitoes (*A. vexan)* and *A. lineatopennis* (Linthicum, 1988). These *Aedian* mosquitoes endure dry periods as eggs, which can survive for long periods, possibly several seasons, in dried mud. It is obligatory for these eggs to be subjected to a period of drying before they can hatch. RVF virus is transmitted transovarially in certain *Aedian spp*. of mosquitoes and infection persists for life. Ideal conditions for the breeding of *Aedian* mosquitoes (and other mosquito species) are found in low-lying shallow depressions termed "Dambos" which flood when abnormally heavy rainfall occurs and this raises the water table sufficiently. In occasions explosive increase in the A*edian* mosquito vector populations follows with increased transmission of RVF virus mainly to cattle (amplifying and surviving as source for uninfected *Aedians* and others such as *Culicins* and *Anopheles species* of mosquitoes that can disseminate the disease (Linthicum *et al*. 1988b; Davies and Nunn, 1998; EMPRES/FAO, 2000 and AGA/FAO, 2002). The depressions, which may be identical with or

related to the geological formations known as “Dambos”, are very common (abundant) in Savannah, bushed and woody grass land of South Kenya, where repeated out breaks occur (Linthicum *et al*., 1988b).

The eggs of secondary or epidemic vectors of RVF (*Culicids* and *Anopheline*) mosquitoes do not survive the drought but they colonize during flooding of the “Dambos” coming from near by rivers or dams (EMPRES/FAO, 2000). So it helps for the multiplication of the secondary group - vectors. *Aedine* mosquito species (true vectors) are thought to maintain a low level of viral transmission to livestock, with epidemics triggered by amplification of viral transmission from the increased interaction of the cattle-mosquito cycles (Tibbo *et. al*., 2001 and AGA/FAO, 2002). This may be indicator of the advantageous critical control point of the vector. On top of these, the inter-epidemic epizootic gap ranges from 5-27 years. The above suggestions are summarized by the hypothesized RVFV life cycle (Figure 3) in sub- Saharan Africa (Linthicum *et al*., 1988b).

b) Generation of epidemics. According to EMPRES/FAO (2000), the generation of epidemic seems to be associated with the simultaneous intensification of vector and viral activity over large areas within which the virus is already present in some sites, rather than lateral spread from cryptic endemic foci. Considerable number of virus must be introduced or need time for amplification and dispersal of the already existing minimum number. No carrier state is known in any species.

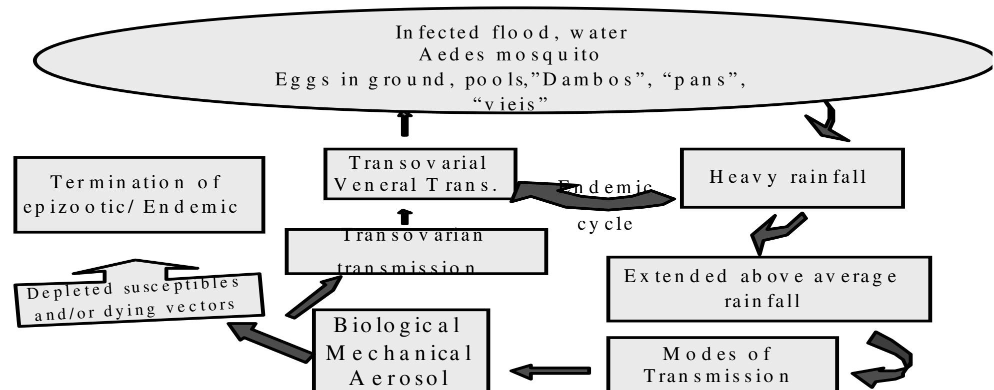


Figure3: Hypothetical RVF virus life cycle in S.S. Africa**.** (Source: Linthicum *et al*., (1988b)

Therefore the interaction of the ecological dynamics for the climate and weather, the vector and vertebrate host will be important.

a) Ecological dynamics of climate & weather: Factors that play major role in determining the emergence of the infected mosquito populations and amplification of virus (critical features) are the development of greater cloud cover and density accompanied by regular and significant precipitation. This increases the level of the water table and creates the pre-epizootic conditions, which can be monitored & identified by RSSD. This rise in the water table results in an increase in vegetation quantity and green density, which can be quantified by RSSD as the Normalized Differential Vegetation Index (NDVI), which is based upon the ration of brown over green vegetation density readings from the satellites. The critical NDVI value which was found to be associated with the emergence of RVF Infected mosquitoes in Kenya & Zambia was 0.45 (AGA/FAO, 2002). This may not apply in other biotypes. Flooding occurs as the water table rises in Dambos and water pans or as a result of water spillage from river and water-sources to cover the riverine floor plains. This flooding, if it persists, allows the emergence of the RVF reservoir, the mosquito vectors.

b) Ecological dynamics of the vector. The flood water breeding *Aedes* mosquitoes emerge as larva within 2-4 days of flooding and as adults 6-8days later, according to the prevailing temperatures. The range of movement from the site of emergence is dependent largely upon the prevailing winds. The overall dispersion for males and females is about 0.15km. An emerging population survived for approximately 45 days in an experimental study, but this may be greater at times of an RVF epizootic, when the cloud cover is greater and the vegetation and humidity create a habitat, which favours longer survival. Huge populations of *Aedes* and other mosquito species may be seen in association with the flooded areas, and these persist whilst the flooding remains, although the species population structure changes. The *Aedes spp*. oviposit in the water and vegetation associated with the flooded areas. Most other species oviposit at the waters edge or in small isolated flooded pockets.

c) Ecological dynamics for the vertebrate host. The genotype of an animal is a major determinant of out come of an RVF infection. RVF virus activity in areas where the domestic animals are resistant may only be signaled by human cases and quite extensive virus amplification may be revealed by serology. The distribution of the resistant genotypes is thought to be within the limits of enzootic virus activity in wetter forest derived areas and grasslands. In the epizootic areas to the north in some semi-arid and arid-zones in the Sahel,

and possibly also in the south of the continent, proportions of susceptible genotypes are thought to increase (Linthicum, *et al.*, 1998b AGA/FAO, 2002).

2.2.5 Profile of RVF in Ethiopia

Following epidemics of RVF in Kenya and Somalia in 1997/98, import of livestock and their products was banned from nine countries of the Horn of Africa including Ethiopia by the Gulf Arab countries; Saudi Arabia being the worlds leading importer of sheep and goats and camels (Davies and Nunn, 1998; Asfaw, 2002 and Tadesse, 2002).

From the context of the livestock trade and subsequent socioeconomic implications the Ethiopian Government had given a very high priority for the establishment of RVF surveillance preparedness to react swiftly to newly occurring outbreaks and re-emerging foci of the disease. For this national RVF system has been established 2001 and is working in its full effort at NAHRC. The project was established in shared costs with the government of Ethiopia and Regional Technical Cooperation Programme of FAO, TCP/ETH/0168 project.
Since its establishment the project has attained many achievements, even if some short comings are still existent. Of the achievements:

- Preparation of preliminary document on the surveillance strategy with the details of suitability indices and risk maps (fig.4 and 5) and its implementation was the major.
- Provision of materials, equipment and all the reagents needed,
- Training of professionals at NAHRC and Regional Laboratories,
- Establishment of 30 sentinel herds in 20 areas suspected to be high risk,
- Establishment of information network in the Ministry of Agriculture and under way to those regions relevant to the export trade (Regional states of Somali, Afar and Borana Zone of Oromya). These achievements of the project have transferred the country from not able to undergo surveillance to a country able by itself.

Antibody Prevalence: sero-surveillance result of 7705 sera of four shoots, cattle & camel species tested at NAHRC in the year 2000, was analyzed by Tadesse 2002 in University of London; according to the analysis 8.1% of all species tested demonstrated presence of RVFV specific IgG antibodies in their blood. The details of antibody prevalence of all species being 1.9%, 2.6%, 11.9% and 15.7% for camels, cattle, goats and sheep, respectively. The report even states that the disease had occurred before 1998. The result of this survey was mentioned to be with a higher rate of false positivity in a letter sent from FAO to the Federal Ministry of

Agriculture of Ethiopia explaining that the ELISA test kit used in that survey was with many limitations. And it recommends a retest of these sera. Some of the limitations of the ELISA kit were mentioned in the letter as follows:

- Because of an inadequate test protocol no control antigen was used for the test sera collected in Ethiopia leading to an inadequate correction of the net OD values and consequently leading to test results in all sera with any background reactivity.
- It used raw OD values as cut-off, i.e. OD ≥0.4 being considered as positive, this is also problematic, since sera with OD values even greater than 0.6 were found to be negative as well as less than 0.4 positive, during the recent survey.
- The chromogen which was used in the test was TMB which is highly reactive and therefore in several plates the OD values of the positive control exceeded 3.0, this would have also resulted in excessive colour in the test sera and consequently resulted in high false positives.
- This combined with the fact that the test OD results uncorrected resulted in a high number of probably false positive results from survey.

Combining the facts described above (testing the sera in 01/100 dilution, using raw OD 0.4 as a cut off, use of the uncorrected gross OD value, high colour in most of the plates, the rest will have a high sensitivity but the lone specificity (MoA, unpublished). This was proved by the retrospective test result of this particular survey, which ended with only 22% of the previously positive sera to retest positive with the better quality recent ELISA test kit.

Furthermore, the test of 840 camel sera was conducted with no availability of camel IgG specific conjugate. These all decrease the reliability of that result and make it not dependable for reporting with out a supporting retest result by more reliable test.

2.2.6 Economic Importance

The direct effect of the disease causes a huge economic loss resulting in loss of up to more than 70% of livestock observed in the recent out break of in Kenya (1997/98). Types of loss are detailed in section 2.3 which mentions morbidity and mortality rates. The other impact of the disease in its consequence on export trade of the developing counties that led to banning with the consequent loss of income particularly in those people whose lively-hood is based on livestock production, and trade; particularly the pastoralists of East Africa (FEWS, 2001) (Figure 6). Three countries of East Africa Ethiopia, Somalia and Sudan lost their 54.4% share

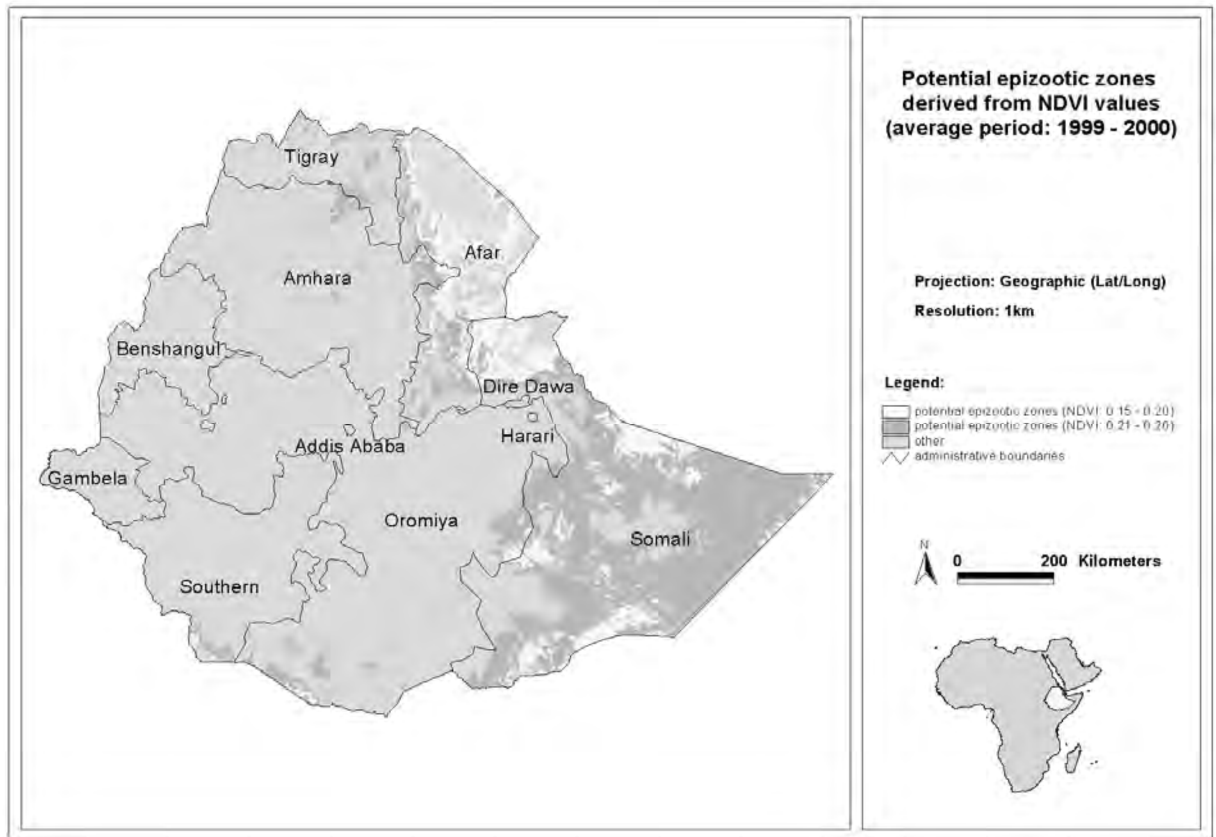
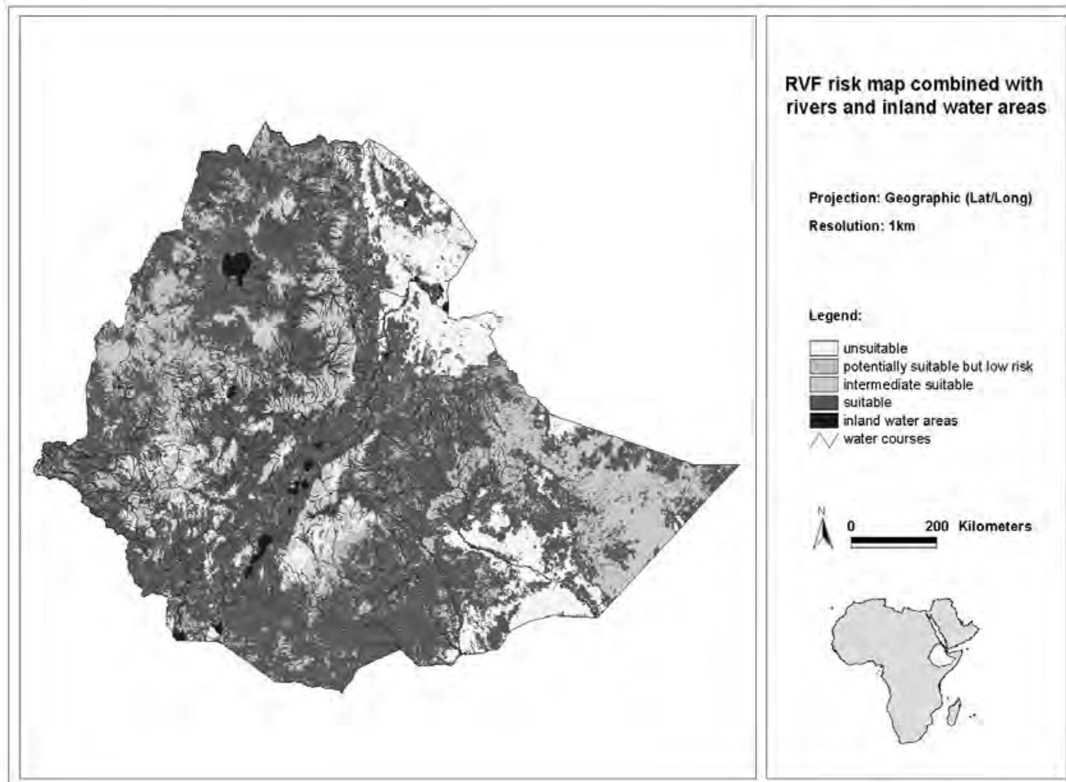


Fig. 4: Potential RVF Epizootic Zones of Ethiopia**. (**Source: Asfaw *et al*., 2002)

Figure 5: RVF risk map and water bodies (Source: Asfaw *et al*., 2002)



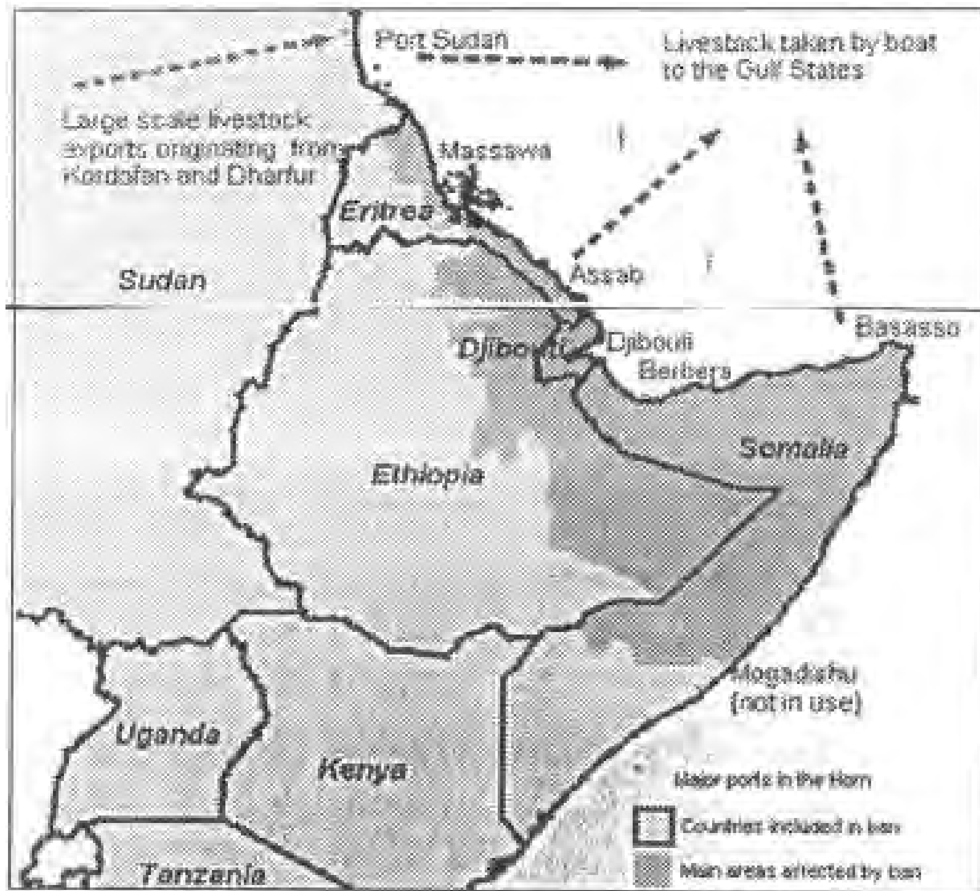


Figure 6: Areas most affected by the trade ban**.** (Source: FEWS, 2001)

of yearly supply of small ruminants of Saudi Africa since the ban (Asfaw, 2002a). OIE regulation recommends a ban of 3 years from areas where RVF epizootics were detected.

The other irresistible loss is the public health impact. Several epidemics resulted in huge cases and many deaths of human. The Egyptian epizootics of 1977 known for its highest was accounted in 18,000 cases and 598 deaths and the recent outbreak in Kenya in 1997/98 resulted in 27,500 cases and 171 reported deaths (Woods *et al.,* 2002). But in East Africa impacts of most epidemics are aggravated by following drought leading to hunger and famine (FEWS, 2001 and Tibbo *et al.,* 2001).

2.3 Clinical appearance of Rift Valley Fever

It is probable that many specific symptoms of virus infection that have negative effects on host fitness may persist because they are associated with an increase in viral transmission (DeFilippis and Villarreal, 2001).

2.3.1Animals

Incubation period: Experimentally, the incubation period in newborn lambs, kids, calves, and puppies is about 12 hours. In adult sheep cattle, goats and dogs incubation period may be as long as 3 days. In humans incubation period is 4 to 6 days (Mebus, 1997).

Clinical Signs: Signs of the disease tend to be nonspecific, rendering it difficult to recognize individual cases. Most, if not all, infected pregnant sheep, goats, cattle (and most likely domestic Asian buffaloes) and camels abort affected fetuses at any stage of pregnancy usually under going autolysis (OIE, 2000). The most severe reactions occur in newborn lambs and kids, which die within hours of infection, rarely surviving more than 36 hrs. A biphasic fever of up to 40°Cmay develop and the fever remains until sharply subsides before death. Affected animals are listless, decline to move or feed and respiration is rapid. Mortality reaches 90 percent or more in animals less than one week of age (EMPRES/FAO, 2000).

Animals older than 2 weeks may die peracutely, acutely or may develop an inapparent infection. In peracute diseases death occurs before the development of notable signs. Acute disease death is characterized by high fever for 1 to 3 days, anorexia weakness, listlessness and rapid respiration. Some animals regurgitate rumen content and exhibit blood stained mucopurulent nasal discharge, fetid diarrhea and melanea. Jaundice may develop. Death occurs after about three days of illness. The mortality rate is lower than in week old lambs but can still reach 50 percent or more (EMPRES/FAO, 2000).

The disease in calves resembles that in lambs essentially fever, weakness, in appetence and diarrhea, which may be blood stained but jaundice is more frequent. Death occurs in 2 to 8 days and the mortality rate is generally low it is around 20 percent.

Adult cattle exhibit clinical signs infrequently, but some may develop acute disease with fever for 2 to 3 days anoxia, lachrymation, hyper salivation, nasal discharge, dysgalactia and diarrhea which may be blood stained. Frequently abortion is the only manifestation in this

species. The mortality rate does not usually exceed 10 percent but can be higher. A prolonged course of 10 to 20 days with marked jaundice has been described in the Sudan.

Asian water buffalos are known to be susceptible from the Egyptian epidemics. Abortion & deaths occur.

In camels antibodies against RVF virus have been detected and RVF virus has been isolated during epidemics. Deaths and abortions almost certainly occur, but the disease has been little studied in this species**.**

Wild animals: Antibody surveys and experimental infection studies had demonstrated that many species of wild ruminants (African buffaloes and numerous antelope species) sustain infection; yet, the results of that infection have not been clearly described. It is highly likely that both abortions and mortalities occur in at least some wild life species during epidemics (OIE, 2000; EMPRES/FAO, 2000).

2.3.2 Rift Valley Fever in human

Uncomplicated RVF in human characteristically manifests itself as an acute influenza like illness with transient fever, rigor (shivering), headache, severe muscle and joint pain, photophobia and anorexia sometimes with a petecchial rash, nausea, vomiting and epistaxis. The course is 4 to 7 days leading to full recovery in 2 weeks (WHO, 200).

The most frequent complication is retinitis, usually bilateral occurring 1 to 3 weeks after the primary febrile illness. Permanent loss of central vision is suffered by some 50 percent of those affected; there may be permanent or bilateral blindness. Often fatal, complications have been more prominent in the epidemics in West Africa and Egypt. In a proportion of RVF cases a biphasic fever is seen with encephalitis developing during the second febrile phase. Patients suffer confusion, hallucinations and vertigo choreiform movements sometimes leading to coma. The case mortality rate is generally low but full recovery may be prospered and long-term neurological complications have been reported.

A hemorrhagic diathesis with hepatitis is a relatively new form of the disease, first described in 1975 in South Africa (EMPRES/FAO, 2000). It is an acute febrile illness of 2-4 days duration followed by jaundiced and wide spread hemorrhages in mucosae and subcutaneously. Bleeding occurs as needle puncture sites, from the gums and nose and there may be haematemesis and diarrhea with melaena. Death usually occurs within 3-6 days and a

few patients recover after a long slow coinable scene (EMPRES/FAO, 2000 and WHO, 2000).

2.4 Pathology

The pathogenesis of RVF results from the spread of the virus from the site of introduction to the body and initial replications sites to the metabolically essential organs such as the spleen, liver, and brain**.** After inoculation of the virus by mosquito bite, the virus is transported by lymphatic drainage to the regional lymph node where local replication takes place (EMPRES/FAO, 2000). Virus spills over into the circulation, causing the primary viremia and spread of the virus to the major organs. Replication in the lymph nodes, spleen, liver, adrenals, lung and kidney tissues results in high viremia. In severe cases, hepatic necrosis is prominent, and necrotic foci can be observed in the brain of cases exhibiting the less frequent encephalitic form of the disease. RVF virus replication in cells is highly cytotoxic suggesting that most cellular destruction in acute illness is likely due to direct virus killing of host cells (EMPRES/FAO, 2000; WHO, 2000; DEFRA, 2002).

Pathologic feature of the disease differs considerably. Leucopoenia is frequently seen during the first 3 or 4 days of infection when fever and vireamia are usually at their highest level. Altered serum enzyme levels (Aspartate aminotransferase and sorbitol dehydrogenase) indicative of hepatocyte destruction often occurs during the acute phase. Leukocytes often occur in the early phase of recovery. Thrombocytopenia and fibrin thrombi in several organs suggest the disseminated intravascular coagullopathy (DIC). This may be a feature of severe disease in livestock as is seen in hemorrhagic infections in humans (EMPRES/FAO, 2000).

The most severe lesions are found in aborted sheep fetuses and newborn lambs. The liver is usually enlarged, soft, friable and yellowish-brown to dark in colour. Irregular congested patches and hemorrhages of varying size are often present in the substance of the liver together with pale foci. Jaundice is seen in only relatively small proportion of sheep lambs. In older sheep, the hepatic lesions are generally not so severe but jaundice may be more marked. Pale areas of cell necrosis combined with large hemorrhages give a mottled appearance to the liver. Hemorrhages and edema of the gall bladder are common and the bile may contain blood. Elsewhere, in newborn lambs, petecchial and ecchymotic hemorrhages

are found in the abomasal mucousae and the contents are often dark brown (EMPRES/FAO, 2000; WHO, 2000).

The mortality in young animals frequently takes place during the vireamia stage of the disease with no specific lesions. Most animals that die from RVF show evidence of liver necrosis, which may be extensive hemorrhage.

In humans most deaths occur as a result of hemorrhagic syndrome. That is a feature of certain other similar virus infections. However, the liver necrosis may serve to distinguish the cases of RVF (Hubert *et al*., 1975; WHO, 1982).

2.5 Diagnosis

Diagnosis is conducted mostly as part of a survey. Effective diagnosis is detrimental in deciding on the control of viral diseases. Since RVF outbreaks occur in concomitant with other viral diseases, it is appropriate to suspect case of RVF, whenever the following symptoms are observed: high abortion rates (possibly approaching 100 percent in ewes, cows and goats), high mortality (possibly approaching 100 percent) in lambs and calves less than 7 days old and lower rates of disease and mortality in older animals and an influenza like illnesses in humans (Davies an Nunn 1998; Nichole, 2001).

2.5.1 Specimens Taken for Laboratory Examinations

If RVF is suspected extra precaution should be taken during collection, shipment and transportation, since aerosol infection of laboratory professionals may take place during manipulation of samples from inflected host. Specimens should include heparinized blood, clotted peripheral and heart blood and tissue samples from liver, spleen, kidney and lymph nodes collected preferably at the height of viremia. Samples from aborted fetuses should include brain. Where delay is anticipated in samples reaching a laboratory or where samples have to be transported at ambient temperature, tissue samples can be preserved in glycerol saline solution (50:50) (EMPRES/FAO, 2000; OIE, 2000; Nichol, 2001). Viral diagnosis is usually quite simple given the high viraemia is present throughout the acute phase of illness.

2.5.2 Virus isolation and Identification

This is achieved by growing the virus in cell culture or by performing intraperitoneal or intracranial inoculation of weaning mice or hamsters. 2 day-old lambs may be used for primary virus isolation as well. The virus can be readily being cultured on cell cultures of different types of laboratory animals such as African Green Monkey kidney (VERO) cells, baby hamster kidney cell (BHK-21) or primary cells of sheep or cattle origin. Confirmation is made using immunofluorescence or immunoperoxidase staining (WHO, 1982; OIE, 2000 and EMPRES/FAO, 2000). Cytopathic effect is indicative.

2.5.3 Antigen Identification

It is sometimes faster to demonstrate the antigen to RVF in passage material/or in primary field specimens than to isolate the virus. This can be attained by gel diffusion immunofluoresent or immunoperoxide staining of frozen section, complement fixation and ELISA; Immunofluorescent staining could be made from impression smears of the liver spleen, brain or infected cell culture, (OIE, 2000 and EMPRES/FAO, 2000).

2.5.4 Detection of virus specific antibodies

This can be achieved by virus neutralization and ELISA tests but not the haemagglutination inhibition (HI) test, which is non-specific; these are used mainly retrospectively to determine the extent of an epidemic. For diagnostic confirmation, recent or current infection must be distinguished from pre-existing immunity. Paired samples collected during the acute phase and again 2 to 3 weeks later provide evidence of recent infection. M-capture ELISA allows diagnosis of recent infection to be made on a single serum sample (EMPRES/FAO, 2000). Infected animal develop specific antibodies, which may be demonstrated by virus neutralization test as early as 3 days following infection and after 6 – 7 days by ELISA and heamagglutination test. Serological tests used less often include immunofluoreascence, complement fixation and immuno diffusion (OIE, 2000).

2.5.5 Detection of virus RNA

Detection of virus RNA is conducted by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) (EMPRES/FAO, 2000).

2.5.6 Histopathology

Histopathology performed on formalin- fixed sections of liver; lesions are distinctive but immune peroxides staining of viral lesions add specificity.

Issues to get consideration in the diagnosis of RVF:

1. Cross reactions occur between RVF and other viruses in the phlebotomus sero group. These are most accentuated, when using the haemaglutination inhibition (HI) and immunofluorescence (IF) test (WHO, 1982).
2. In RVF-free countries diagnostic tests should be limited to those requesting inactivated antigen. This can be obtained from the OIE Reference Laboratory (OIE, 2000).

3. To be suitable for sero - surveillance of RVF in regions not infected with RVF, a serologic test must be inexpensive, relatively simple, reliable, applicable to both human and domestic animal sera; and available equipment and reagents must be used (WHO, 1982). In addition, the test should be sensitive, relatively specific, although specificity is not essential due to availability of reference laboratories. According to this criterion, ELISA/ELFA/Enzyme Linked Fluorescent Assay is more economical with respect to the antigen, sensitivity and specificity.

4. Areas and climatic conditions favouring RVF may also favour other diseases with which RVF may share clinical manifestations and hosts. Therefore, RVF should be differentiated from other diseases showing similar clinical signs (EMPRES/FAO, 2000 and Woods, et al., 2002). These include: Wesselsbron disease, Nairobi Sheep Disease, intoxication with poisonous plants and bacterial diseases such as Pasteurellosis, Salmonella and Anthrax (EMPRES/FAO, 2000). These require the application of epidemiological, clinical and toxicological investigations, appropriate micro biological and serological examinations.

2.6 Prevention and Control

2.6.1 Immunity

2.6.1.1 Natural

While the age of an animal increases, its susceptibility to RVF virus infection decreases. Innate immunity Varies between breeds and some breeds of sheep and goat appear to be relatively resistant. Herd immunity levels are high after epidemics the immunity appears to be life-long. Immune dams transfer immunity to their offspring via colostrums and this affords some protection for up to five months in lambs (EMPRES/ FAO, 2002). Therefore the only dependable way to control and prevent RVF will be using vaccination and vector control.

2.6.1.2 Vaccination

Rift Valley fever can be prevented and controlled by sustainable program of animal vaccination. Both live attenuated and killed vaccines have been developed.

The mouse-adapted Smith Burn Strain of RVF virus derived vaccine is highly immunogenic. It induces durable, probably life-long immunity since seven days post-vaccine with a single dose of vaccine (EMPRES/FAO, 2000) cattle may not be fully protected. Large quantities can be produced readily. However, since the virus is only partially attenuated there is a high risk of virulence due to reversion or genetic reassortment (WHO, 2000; EMPRES/FAO, 2000; Nichole, 2001) or fetal damage and prolonged gestation in a proportion of population of animals.

With regards to export trade, according to OIE article 2.1.3.7 a country is considered to be not free of RVF or (infected) if vaccination with a live vaccine has been carried out since 3 Years (OIE, 2000). The use of such vaccine is therefore not advisable in countries where no RVF has been proven.

The vaccine from the in activation of wild strain of RVF virus with formalin or J-Propriolectone give low antibody response with out previously mentioned unwanted results. But repeated inoculate on after an initial double vaccination with an interval of 2 to 4 weeks is

required to maintain immunity which is short- lived. They are expensive to produce (Mebus, 1997; EMPRES /FAO, 2000; OIE 2000; WHO, 2000; Nichole, 2001).

It is advisable to under go vaccination and vector control in combination with the information from Meteorology and Normalized Difference in Vegetation Index (NDVI/) and Cold Cloud Density (CCD) obtainable from Remote Sensing Satellite Data, since it can predict up six months in advance (Linthicum *et al.,* 1988b; Davies and Nunn 1998).

2.6.2 Control in RVF free Areas

In these areas control should focus on prevention and control of virus entry, establishment and/or dissemination using

- Slaughter of infected and animals in contacts
- Quarantine
- Survey of high risk areas

2.6.3 Control in Human

Hence, there is no licensed vaccine for human other protective measures can be applied. These include:

1. Protection from insect bites by
   - Wearing light colored clothing
   - Insect repellants
   - Net protected beddings
2. Avoiding contacts with known infected and/or suspected samples and materials. (CDC, Traveler's Health Information on RVF, 2000).

3 MATERIAL AND METHODES

3.1 General Description of the Study Areas

The study areas are Afar regional state and Borana zone of Oromya, regional state, which are located in the northeast and south of Ethiopia, respectively. In both areas both pastoral and agro- pastoral farming systems are practiced, while the former being the most common. They move seasonally based on availability of pasture and water as well as flooding in some of the localities of Afar e.g. Gewane, Dubti and Burumudaitu thus the flocks and stocks from different areas are mixed. Species splitting is a common practice in both areas, leaving lactating cows, calves, goats and sheep to move only short distances or in the settlement areas (Hoggs, 1993; Biffa and Chaka, 2002; EPIAT, 2003).

Their locations give them the proximity to neighbouring countries Djibouti and Eritrea (Afar) and Kenya and Somalia (Borana). Among these countries Kenya, Somalia and Djibouti are particularly known to have the problem of the disease Rift Valley Fever. Therefore, there is risk of introduction of the disease through both study sites.

The altitude ranges from 1500—1000m.a.s.l with gradual decreases towards neighbouring countries (Negasa, 1993). There are plateaus near by side of small high lands. Rainfall ranges from about 600/700mm per year in the higher altitude areas to 300/400mm in more low lands (Negasa, 1993; Biffa and Chaka 2002).

There are four Seasons in Afar locally known as *Sugum* (February--April), which represents short rainy season with irregular pattern; *Cagay* (May—July) is the hot dry season, *Karma* (August—September) is the long rainy season and *Jilaal* (November—January) represents the dry cold season (Tibbo *et al.,* 2001). The main rain season in Borana (*Ganna*) extends March to May and short rain season (*Hagaya*) from September to October (Biffa and Chaka, 2002). The remaining months are dry.

The vegetation in both areas is savannah type grassland and woody bush land. Water sources in both areas are big rivers, tributaries as well as man made water wells and ponds. The river known in Afar is Awash, which crosses many districts of the region, and there are three rivers crossing Borana from north to west; permanent rivers Ghenalle in east and Dawa centrally and the seasonal river Segen in the west.

Livestock is an essential part of the people living in both areas. They are produced as source of food; for the purpose of income generation; social prestige to be used as compensatory gift in cases of marriage and murdered person. Species diversification is practiced to secure the family during drought. The dominant species based on the population size is cattle followed by small ruminants. Both are used for income generation and as milk and meat sources (Biffa and Chaka, 2002 and EPIAT, 2003). There is shifting to the increased holding of the browsing species small ruminants in Afar (EPIAT, 2003) and camel in Borana (Biffa and Chaka, 2002) due to increasing human population and shrinkage of grazing land aggravated by the repeatedly occurring drought.

Table 3: Livestock population in of Afar regional state and Borana zone in Oromya Regional State.

Region/	*Livestock Population (in 1000s)*				
Zone	*Cattle*	*Sheep*	*Goats*	*Camel*	*Equine*
Afar	703.4	953	2,014.4	288.6	16.8
Borana	1,771.6	717.1	1,274.1	669.9	64

Source: Afar Region Agriculture Bureau (Afar, 1998) and Biffa and Chaka (2002).

Illegal livestock trade with neighbouring countries is commonly practiced in both areas (Tadesse, 2001).

These two areas were selected for this study because:

- They are relevant to the export trade,
- Their ecologies are conducive (natural and man made flooding areas, woody grasslands etc.) to the multiplication of vectors,
- Seasonal mixing of flocks and herds of different origin at the communal watering and grazing areas,
- There are also different irrigation activities,
- They are at proximities with countries that are suspected and confirmed to have the problem of Rift Valley Fever.

- Small ruminants were preferred to other species for this study due to indications of previous preliminary studies on more susceptibility of these

species to the disease. They are also more important source of income both from export trade and local markets (Asfaw, 2002b; Tadesse, 2001). The export ban on export trade due to the last RVF outbreaks in Saudi Arabia and Yemen, in the year 2000, had particularly affected these communities (Figure 5).

On top of these the population of the pastoralists areas are the ones suffering more due to the trade ban imposed on the country since the year 2000.

3.2 Study Design

Both cross-sectional and retrospective serological surveys were conduced with field and laboratory investigations. Serum samples were collected from the animals selected randomly, questionnaire data were collected by interviewing owners and attendants to gather information related to the disease. Other information including georeferenced data and photographic information were collected.

All the laboratory work was conducted in the National Animal Health Research Canter (NAHRC) at Sebeta, located south west of Addis Ababa at 24km on the Addis-Jimma road. Serum sample from the active survey and serum bank were tested with Indirect Sandwich ELISA for anti-RVFV IgG antibodies. Serum samples found positive for IgG were retested with IgM ELISA to detect anti-RVFV IgM antibodies.

3.2.1 Study Animals

The reference population in this survey was the 4.96 million small ruminants from both study sites (Table 3). These animals are managed under pastoral and agro- pastoral farming systems mixed with other species. These were the source population.

3.2.2 Sources of Sample

3.2.2.1 Retrospective Serology

The sera used for retrospective analysis were obtained from serum bank, NAHRC. These sera were collected during the post-epizootic period of RVF in 1999 and were found stored at -20°C in a deep freezer. The teams from Kombolcha and Mekele and Assela Regional Laboratories collected the serum samples from Afar region and Borana zone, respectively. All are properly labeled with accompanying information regarding the source areas and animals. Samples tested positive in the previous sero-survey by NAHRC staff were sorted out and retested using the new test protocol of RVF ELISA kits, from Institute of Communicable Diseases, Johannesburg, for anti-RVFV IgG antibodies. The previous kits were reported to detect high false positive reactors, thus decision was made to retest all the positives. Some negative sera were included in the retest to check if some negatives give that result by this test also.

3.2.2.2 Cross-Sectional Serology

Serum samples were collected from small ruminants randomly selected from both study areas. The samples were collected both from sheep and goats (>=6 months of age) of both sexes, which are considered as homogenous population (Asfaw *et al.*, 2002). The samples collected from active surveillance were tested for the detection of anti-RVF IgG antibodies, and those samples tested positive for IgG were retested by anti-RVFV IgM antibodies.

To facilitate the serum sample collection activities treatments were given free of charge, as incentives to animals with visible clinical symptoms. During the sampling treatments were given to 708 (403 Afar and 305 Borana) animals that belonged to 196 households. The treatments were of anthelemintics, antibiotics, and acaricides and wound dressing sprays.

3.2.3 Sampling Frame and Sample Size Determination

The sampling frame consists a list of 353 and 490 PAs of Afar regional state and Borana, respectively had been obtained from the epidemiology unit of the Federal Ministry of Agriculture (MoA). Retrospective serum samples were obtained from a list of 820 sera (500 Afar and 320 Borana) stored in the NAHRC at Sebata. List of Villages/ flocks were not available.

3.2.3.1 Retrospective Sero-survey

The terminology retrospective here is only on the context of using retrospective sera, but not from effect to causal study. For retrospective investigation 246 were selected purposively out of the total 820 serum samples in the bank. Of these 142 had been IgG positive, tested by the ELISA kit developed before the current test. The IgG test conducted on these sera in the year 2000 survey detected 8.00% (64/500) and 24.36% (78/320) in Afar and Borana, respectively; the average being 12.68% (NAHRC unpublished document). These 142 positive sera and 103sera from the negative were retested with IgG ELISA; and these samples were 144 from Afar and 102 from Borana (Table4).

3.2.3.2 Cross-sectional Survey

To determine the sample size for the active sero-survey a two-stage sampling, which followed random sampling at different hierarchical levels, was used. Peasant Associations (PAs) and villages or herds were determined as primary and secondary sampling units, respectively. The parameters were based on those designed for the national RVF surveillance proposed in 2002 (Asfaw *et al*., 2002). The list of the villages and/or flocks was not available and they were randomly selected during the field survey. For the determination of the number of PAs to be sampled the sampling fraction of previous survey 0.065 and 0.033 for PAs in Afar and Borana, respectively were used. To randomly select the PAs and determine the sample sizes Survey Tool Box Computer Software was used. The list of villages was obtained from the district agriculture offices and administrative offices of each randomly selected PA. Based on these parameters 900 (553 Afar and 347 Borana) animals were sampled during the active sero survey. And this animals were from 33 (19 Afar and 14 Borana) PAs located in 17 (10 Afar and 7 Borana districts (Woredas). To select randomly these PAs from each study area Survey Tool Box was used. The country was divided into two ecological zones (strata) mainly

highland and mainly lowland districts. This was based on Normalized Distribution Vegetation Index (NDVI) and Cold Cloud Cover (CCC) data. The sample sizes from highland and lowland PAs was 14 and 29 sheep and goats respectively (Asfaw, 2002 b). According to this information it had been decided to sample 14 and 29 sheep and goats from each area. From the randomly selected PAs only four PAs were from highland, which all were from Borana and twenty-nine from lowland (19 from Afar and 10 from Borana). Sheep and goats of both sexes were sampled assuming that they are homogenous since previous studies indicated so (Asfaw, 2002b). Sheep and goat <6 months of age were rejected from sampling frame (Asfaw, 2002b). PAs, which are not suitable for the disease occurrence, were not included in the sampling frame. PAs drawn by the Survey Tool Box random selection but found inaccessible were replaced by other PAs that have similar ecology and accessible. Details of the sites sampled are summarized in Table 5. No any active case was found during the serum collection period.

3.3 Data Collection

3.3.1 Serum Sample Collection and Submission

Since RVF is zoonotic all the necessary hygienic measures were considered. Serum samples for serology were collected and submitted according to the following procedure:

- About 10ml blood samples were collected from in plain vacutainers and needles;
- It was allowed to clot for 1-3 hours at room temperature and then after stored horizontally overnight at 4^oC;
- The sera were separated by centrifugation at 2000-3000 rpm for 10-15 minutes and serum was transferred into a single sterile cryovial, and kept cool and frozen for transport and labelled and stored at -20^oC until tested.

Table 4: Distribution by the administrative strata of the sera collected from Afar and Borana for

Rift Valley Fever survey in 1999.

Region	*Zone*	*District*	*PA*	*N° samples*	*Ovine*	*Caprine*	*N° Previos Positive*	*N° of retested sera*
Afar	One	Dubti	Dembel	20	20	0	4	20
			Beyahile	20	20	0	1	20
		Chifra	Mesgid	40	20	20	2	2
			Sedehada	20	20	0	2	2
	Two	Megale	Faro	40	20	20	12	12
			Adu	40	20	20	0	0
		Berahle	Demale	40	20	20	3	3
		Erepti	Erepti	20	20	0	4	4
		Ab`ala	Ab`ala	20	20	0	5	5
			Hidam	40	20	20	11	0
	Three	Burumudaytu	Debel	20	20	0	4	20
			Daferom	20	20	0	0	20
			Geleallo	20	20	0	0	0
		Amibarah	Melkawerer	20	20	0	5	5
			Gedigedaro	20	20	0	2	2
		Gewane	Gewane	20	20	0	7	7
			Gelildura	20	20	0	2	2
	Five	Fursi	Hadal ela	40	20	20	0	0
			Artuma	20	20	0	0	0
Total Afar	**4**	**10**	**19**	**500**	**380**	**120**	**64**	**144**
Oromya	Borana	Dire	Godichica romso	40	20	20	25	40
			Daasi	40	20	20	31	40
		Arero	Wachile	40	20	20	3	3
			Allona	40	20	20	4	4
		Teltele	Mermero	40	20	20	8	8
			Horbale	40	20	20	0	0
		Hageremariam	Finchawa	40	20	20	3	4
			Derudenfele	40	20	20	4	3
Total Borana	**1**	**4**	**8**	**320**	**160**	**160**	**78**	**102**
Grand Total	**5**	**14**	**28**	**820**	**540**	**280**	**142**	**246**

Table 5: Summary of Serum Samples collected for the Cross-Sectional Survey of Rift Valley Fever, October2003-March, 2004..

Region	*Zone*	*District*	*PA*	*Samples plan*	*Sample achieved*	*Species*		*N° of Flocks sampled*
						Cap.	*Ovine*	
Afar	One	Aysaita	Galifagi	29	29	8	21	4
			Bokaytu	29	31	28	3	4
		Dubti	Beyahle Sahil	29	29	0	29	7
		Mille	Bekerideara	29	29	19	10	4
			Werenson	29	29	12	17	4
	Two	Koneba	Alhiena	29	29	23	6	4
		Afdera	Aligenda	29	29	29	0	4
			Kosorawda	29	29	27	2	4
	Three	Burumudaitu	Debel	29	29	24	5	5
			Tulie	29	29	17	12	4
		Gewane	Biliforo	29	29	11	18	6
			Urafita	29	29	7	22	5
		A/Fentale	Deho	29	29	20	9	8
			Duddub	29	29	19	10	5
	Five	Dewey	Wediragi	29	29	1	28	2
			Derseda	29	29	21	8	7
		Telalak	Hemaleysn	29	29	16	13	2
			Weydelele	29	29	16	13	4
			Amedidas	29	29	7	22	4
Total	4	10	19	551	553	305	248	87
Oromya	Borana	Moyalle	Buledi	29	29	17	12	4
			Medo Migo	29	29	27	2	6
			Tuqua	29	29	22	7	4
			Arbelle	29	29	27	2	5
		Yavello	Kobo	14	14	12	2	3
			Cholquassa	14	15	4	11	6
		Teltelle	Bilao	29	29	16	13	4
			Sarite	29	29	14	15	4
		Arero	Ranji	29	29	18	11	6
			Geleba	29	29	16	13	8
		H/Mariam	Dawa Bichu	29	29	21	8	4
			Dukisa Chebiti	14	14	8	6	4
		OdShakiso	Bupo	14	14	14	0	2
		Abeya	Dibicha Laluncha	29	29	27	2	4
Total	1	7	14	346	347	243	104	64
Grand Total	5	17	33	897	900	548	352	151

3.3.2 Questionnaire survey

During survey of RVF information regarding the occurrence of abortions (cluster of abortion), mortality on neonates, occurrence of flooding, vector distribution, irrigation, human and animal movements were collected through questionnaire. A total of 132 (4 per PA) respondents were interviewed from all the 33 PAs. The information obtained from this survey was transferred to excel and the analysis results were used to associate the geographic or environmental information to the serological findings.

3.3.3 Gathering georeferenced data

Georeferenced data such as geographic location and altitude were collected from each sampling site. The data from this is used to map the distribution and extent of the disease to the respective study sites. Other supportive photographic information on water lodged areas and others were gathered during the serum collection period.

3.4 Laboratory Analysis

During the laboratory work a total of 1176 samples from retrospective and cross-sectional surveys were tested using Indirect ELISA technique for the detection of anti-RVFV specific IgM and IgG antibodies. The sera for cross-sectional study were tested for both anti-RVFV IgM and IgG but those from retrospective survey were tested for IgG only. Out of these only 30 were tested for anti-RVFV IgM.

3.4.1 Types and Purposes of ELISA Tests

Both IgM and IgG ELISA were used to detect the presence of anti-RVFV immunoglobulin-M and G, respectively. These tests are used to detect recent and past infections of RVF virus, respectively.The techniques and procedures of the test were according to the protocols recommended, by the supplier, National Institute for Communicable Diseases, Johannesburg (NICD, 2003), with slight modification to fit to software package EDI (ELISA Data Interchange) used during the test.

3.4.2 Test Protocols

3.4.2.1 Rift Valley Fever IgG ELISA test protocol and Principles

The ELISA for IgG is based on an indirect sandwich format in which the plates are coated with mouse anti-RVFV serum and then reacted with antigen. Test sera are applied and specific anti-RVFV IgG antibody is detected with an anti-bovine IgG HPRO conjugate and ABTS substrate. The reaction between HPRO conjugate plus ABTS substrate is responsible for the development of colour, which heralds the positivity. The intensity of colour developed is read with multi channelled spectrophotometer at 405nm filter, the same reading principle holds true for IgM also.

3.4.2.1 Rift Valley Fever IgM ELISA test protocol and Principles.

The ELISA for IgM is based on a capture format in which the plates are coated with rabbit anti-sheep IgM capture antibody and then reacted with test sera. Anti-sheep capture antibody can be used for detection of IgM in sheep and goats. The captured IgM antibody is reacted with RVFV antigen, and the bounded antigen is then detected with mouse anti-RVFV serum and anti-mouse IgG HRPO conjugate reacting with ABTS. Preparations of reagents and the dilutions of the two are different (sections 3.4.3.2 and 3.4.3.3).

The dilutions stated in test procedure of both ELISA tests were as tested at the National Institute for Communicable Diseases (NICD) and not re-optimized. The reagents had been irradiated to inactivate the RVF virus and were safe.

3.4.3 Test Proper

Preparation of reagents and working dilutions

a) Buffers
The buffers used were: Coating buffer (PBS 1x), blocking buffer (PBS 1x + 5% NFSMP), diluent

buffer (PBS 1x + 2% NFSMP) and washing buffer (PBS 1x + 0.1% $Tween_{20}$)

b) Reconstitution of Reagents

The reconstitution of reagents was carried out with precaution and those that need fresh preparations were prepared accordingly. The details are as mentioned in Table 6.

Table 6: Reconstitution of reagents and preparation of working dilutions of the ELISA test.

Reagent	*Kit identification*	*Reconstitution Volume*	*Working Dilution/Buffer*
Capture antibodies	Special pathogens Mouse anti-RVF	0.06 ml	1/5000 in PBS
Antigen 1	Special pathogens Control antigen	0.3 ml	1/400 In Diluent Buffer
Antigen 2	Special pathogens RVF antigen	0.3 ml	1/400 In Diluent Buffer
Negative serum control	Sheep anti-RVF negative control (C-)	0.2 ml	1/400 In Diluent Buffer
Weak positive serum control	Sheep anti-RVF Low positive (C+-)	0.2 ml	1/400 In Diluent Buffer
Strong positive serum control	Sheep anti-RVF Strong positive (C++)	0.2 ml	1/400 In Diluent Buffer
Conjugate	Special pathogens Anti-Sheep HRPO	0.150 ml	1/5000 In diluent buffer
Substrate	ABTS Peroxidase Substrate	100 ml	Ready to use

3.4.3.1Test procedure and plate layout

There is slight difference between procedures and plate layouts of the two ELISA test. The details are as follows:

I. Test procedures of IgG ELISA

Between each step there was a three times wash except after addition of conjugate that needed rather six times. There was no washing after addition of substrate or at the step before reading.

1. Plates were coated with Capture antibodies 100 micro litre per well and incubated at +4°C overnight. Wash plate.
2. Each well was blocked with 200 micro litre of blocking buffer per well and incubated 1 hour at 37°C, without shaking. Wash plate.

3. In the rows A, C, E, and G 100 micro litre of RVF antigen per well were added while the same volume of control sera were added in to wells B, D, F, and H then incubation for 1 hour at 37°C, under continuous shaking was conducted. Wash plate.
4. Test sera and control sera were added at 100 micro litres per well volumes in to wells with RVF and controls in duplicate. The dilution rate of test and control sera was 1/400. Control sera after diluted 1/400 in separate tube in a volume enough for all plates (e.g. for 1 plate 1micro-litre of control was added in to 400micrlitre of diluent buffer) and then 100 micro liter per well was added to their respective wells in the test plates. The conjugate controls receive 100 micro litre of diluent buffer. Incubation after this is also 1 hour at 37°C under continuous shaking. Wash plate.
5. The conjugate was prepared, 1/5000 in diluent buffer (for 1 plate, 2 micro litre of conjugate was added to 10 ml DB), distributed 100micro litre per well in all wells and was incubated at 37°C for 1 hour under continuous shaking. Wash plate.
6. And finally 100 micro litre of substrate (ready to use) per well was added put protected from light and waited for 30 min until colour develops and then read with multi channel spectrophotometer at 405 nm filter . Plates with C++ OD values are less than 1.000 can be read every 10 min.

II. Plate layout

The plate layout as shown in Figure 8 for both ELISA test configuration was the same, but the one used for IgG was modified based on the software programme used to read the optical density, EDI (ELISA Data Interchange) and since it uses 8 wells for the positive control and omitted the conjugate control.

Cc Conjugate control (Diluent)
C++ High positive control serum
C+ Low positive control serum
C- Negative control serum
1-40 Test sera
Rows A-C-E and G 1-12 RVFV Ag
Rows B-D-F and H 1-12 Control Ag

Figure7: the modified plate layout used for IgG test during this Survey

A	Cc	Cc	1	5	9	13	17	21	25	29	33	37
B	**Cc**	**Cc**	**1**	**5**	**9**	**13**	**17**	**21**	**25**	**29**	**33**	**37**
C	C++	C++	2	6	10	14	18	22	26	30	34	38
D	**C++**	**C++**	**2**	**6**	**10**	**14**	**18**	**22**	**26**	**30**	**34**	**38**
E	C+	C+	3	7	11	15	19	23	27	31	35	39
F	**C+**	**C+**	**3**	**7**	**11**	**15**	**19**	**23**	**27**	**31**	**35**	**39**
G	C-	C-	4	8	12	16	20	24	28	32	36	40
H	**C-**	**C-**	**4**	**8**	**12**	**16**	**20**	**24**	**28**	**32**	**36**	**40**

III. Test procedure for IgM

Unless otherwise stated volumes used were added 100 micro litre per well and all washes are performed were 3 times for 15sec using 300micro litre of wash buffer per well using multi channel manual washer.

1. Test plates were coated with 100 micro litre rabbit anti-sheep IgM diluted 1:4000 in PBS and plates were incubated at 4°C overnight. Plates were washed.
2. 200 micro litre per well of blocking buffer and incubated for 1h in moist chamber at 37°C. Plates were washed.
3. Addition of 100 micro litre of test and control sera diluted 1:400 in diluent buffer into wells were followed by incubation of the plate for 1h in moist chamber at 37°C. Plates were washed.
4. 100 micro litre per well of RVFV Ag and control Ag each diluted 1:1000 in diluent buffer were added to rows A-D 1-12 and rows E-H 1-12, respectively (plate layout) and the plates were incubated for 1h in moist chamber at 37°C. Plates were washed.
5. 100micro litre per well of mouse anti-RVFV serum diluted 1:5000 in diluent buffer were added and incubated for 1h in moist chamber at 37°C. Plates were washed.
6. 100 micro litre per well of anti-mouse IgG HRPO conjugate diluted 1:8000 in diluent buffer was added and incubated for 1h in moist chamber at 37°C. Plates were washed.

Finally 100 micro litre of ABTS per well was added and the plates were kept for 30 min at room temperature (22-25°C) in dark, and read using multi channel spectrophotometer at a filter of 405nm.

IV. Plate layout

The plate layout for IgM was not modified and reading was using ProComm Software package.

Figure 8: the unmodified plate layout used for IgM ELISA Test

A	C++	C++	1	5	9	13	17	21	25	29	33	37
B	C++	C++	2	6	10	14	18	22	26	30	34	38
C	C+	C+	3	7	11	15	19	23	27	31	35	39
D	C-	C-	4	8	12	16	20	24	28	32	36	40
E	**C++**	**C++**	**1**	**5**	**9**	**13**	**17**	**21**	**25**	**29**	**33**	**37**
F	**C++**	**C++**	**2**	**6**	**10**	**14**	**18**	**22**	**26**	**30**	**34**	**38**
G	**C+**	**C+**	**3**	**7**	**11**	**15**	**19**	**23**	**27**	**31**	**35**	**39**
H	**C-**	**C-**	**4**	**8**	**12**	**16**	**20**	**24**	**28**	**32**	**36**	**40**

(Source: NICDb, 2003)

C++ High positive control serum
C+ Low positive control serum
C- Negative control serum
1-40 Test sera
Rows A-D 1-12 RVFV Ag
Rows E-H 1-12 Control Ag

V. Test interpretation

The amount of colour developed is proportional to the amount of IgM antibody that has been captured. Net optical density (OD) values were first recorded for each serum as the value determined with RVFV Ag minus the value determined with control Ag. Tests in which the OD values of both C++ RVF antigen were higher than 0.8 (1.00 is better) and not more than 2 (1.85 is better) were good. OD values of C++ Ag 0.8 and 1.85 were the lower and upper control limits. The OD values of C+ and C- control antigen should be roughly the same (It is better they be lower than 0.2). Plates that did not fulfill these they were rejected and samples were retested. The variation between two duplicates should not exceed 15%, as

recommended by the manufacturer. And the amount of colour developed in IgG ELISA is similar to the amount of IgG detected.

Cut off determined by the manufacturer was Percentage positivity of 18.1% and above and 11.1% and above are considered positive for IgG for goat and sheep, respectively. But since the manufacturer recommended recalculating the threshold for every population it has been recalculated to be at 15%.

VI. Standardization of the Test

The threshold value to be used by the NAHRC has been determined based on the analysis of 2956 sheep and goat sera samples from highland areas expected to be from negative areas. The cut off at different levels of specificity were determined and set at 15% percentage positivity (Pp) at 98% Specificity. The Sensitivity of the test was 99.1%. The test kit quality was checked and assured by a laboratory technologist working for FAO in RVF reference laboratory Senegal, Dakar, while this survey had been undergoing.

3.5 Data storage and Analysis

The following Soft ware programmes had been used to store and analyze the data:

- Survey Tool Box; in sampling and calculation of true prevalence
- EDI (ELISA Data Interchange) and ProComm to read test results from ELISA reader,
- Excel and Stata to analyze the data

3.5.1 ELISA data transfer

EDI (ELISA Data Interchange), ProComm software packages made available by FAO for disease surveillances were used to read the IgG and IgM test results. The Multi channel Spectrophotometer ELISA reader was connected with a computer and printer set for this purpose. Then the test results were put with their sample descriptions and OD (Optical Density) readings with RVF antigen and control Ag sera. The readings of the control sera

C++ (strong positive) C+ (low positive) and C- (negative control) sera were put separately on the same page. The data were manipulated to calculate the percentage positively (PP) values and determined the status as positive or negative for the test sera. The values were stored after being transformed in to MS Excel program (Microsoft Corporation).

3.6. Sero-prevalence estimation

The sero-prevalence was determined by considering the total number of animals tested and positive rectors in each stratum (age, species, Peasant Associations and others) using the formula (Thrushfield, 2003) and extrapolating it.

$$\textbf{Prevalence} = \frac{\textbf{number of positive reactors}}{\textbf{Total numbers tested}}$$

The sex-specific sero-prevalence of RVF was not calculated for it causes bias because almost 98% of a flock is composed of female animals.

The age-specific sero-prevalence was calculated for each age group tested. This value has been used to calculate the annual mean sero-conversion rates of the areas with the respective age group. This value was calculated to predict the probability of an animal of each age group to get infected with RVFV within one year and to see the impact of culling out sero-converters on the sero-prevalence in the respective areas.

The formula used to calculate this was according to that in (Thrusfield, 2003): -

$$\textbf{P=1- antilog ((log (1-Py)/Y)}$$

Where, P = Probability of becoming infected in one year (i.e. rate of sero-conversion)
Py = proportion of population that have become infected by age Y (sero-prevalence at age Y).
Y = Age in years.

A 95% confidence interval (CI) estimation was made with the upper and lower limits, using the STATA statistical software that gives the exact confidence interval for binomially distributed data species and each sites surveyed and for the whole sera samples. It was calculated with formula:

CI = Mean Prevalence $\pm$1.96 Standard Error.

A 95% confidence interval estimation for the true prevalence was calculated using the Survey Tool Box software package which calculates true prevalence and its confidence interval simultaneously based on the specificity and sensitivity of the test and the apparent prevalence.

True prevalence was calculated from the apparent prevalence based on the sensitivity and specificity of the test, 99.1% and 98% respectively using the formula (Pfeiffer, 2002 and Thrustfied, 2003)

$$\textbf{True prevalence} = \frac{AP + (SP-1)}{SE + (SP-1)}$$

where AP is test prevalence, SP& SE stand for specificity and sensitivity, respectively. To calculate this Survey Tool Box soft ware programmed was used.

Estimation of Predictive Value of the Test: Always in doing either serological tests or other screening tests to determine the presence of a disease in a population it is important to know the probability that an animal, positive according to the test, is actually positive; alternatively that a test-negative animal is a true negative. These probabilities are the predictive value of the test (Pfeiffer, 2002 and Thrustfield, 2003). A negative test result, however, virtually always will correctly identify uninfected animals when prevalence of infection remains low but the parameter most often quoted as the predictive value of a test is the positive (as opposed to the negative) test result (Thrustfield, 2003).

The positive predictive value of the test results was calculated using the following formula

$$\frac{P \times SE}{P \times SE + (1-P) \times (1-Sp)}$$

Where P = estimated prevalence of the disease in a population, SE is sensitivity and Sp stands for specificity. The estimated prevalence of RVF in Ethiopia is 1-2% (Asfaw et al., 2002),

which was based on that of Mauritania; sero-prevalence was recalculated based on this range of prevalence to estimate the actual situation of the disease or infection in the population.

The logistic regression statistical test was used to test the risk factors associated with sero-positivity based on the information gathered during the survey for the categorical nature of the data. Factors assumed to be risks for RVF vector multiplication and RVFV infection were tested, first with univariate logistic regression and those with P value less than 0.2 were included in a multivariate analysis. For the multivariate model factors were dropped from the model one by one based on the higher P value until all significant variables were included.

Spatial data analysis of RVF in both study areas was made using the geographic information system (GIS) software ArcView for windows Version 3.2. For this the sero-prevalence obtained from laboratory analysis of the sera was used. The sero-prevalence was overlaid over zone boundary map led by the information on the location of each PA sampled. The information to this was collected using GARMIN 76 GPS during the field work.

Then, the point prevalence of RVF was overlaid on the satellite image map of Ethiopia to visually inspect the potential relationship between the disease and environmental variables.

4. RESULTS

4.1 Descriptive Statistics of Study population

During the months of sample collection for Rift Valley Fever sero-prevalence investigation, a total of 352 sheep and 548 goats were examined. Further data were collected on demographic characteristics of the animals, management and other factors regarded to be associated to the spread of RVF in animals.

The maximum and minimum age of the animals was 10 and 0.5 years, respectively. Ages were categorized into three groups as animals ≤ year, ≥ 1 year & ≤ three years, and those animals greater than 3 years. The respective number of animals in each group were 162 (18%), 470 (52.2%) and 268 (29.8%) animals. The proportion of animals in different sex groups was 155 (17.7%) males and 745 (82.3%) females. During the survey animals sampled from 151 (91 from Afar regional state and 60 Borana zone) sheep and goat flocks were included.

4.2 Sero-prevalence of Rift Valley Fever in sheep and goats

4.2.1 Sero-prevalence based on IgG antibodies detection (cross-sectional survey)

The sero-prevalence of RVF in Afar Regional State and Borana zone is shown in Table 7. Out of 900 samples tested with the indirect sandwich ELISA technique, 29 (3.2%) were positive for IgG groups of antibodies. The respective prevalence in these two areas at zone, district and PA level are shown in Tables 11, 12 and 13. All the five zones; 12 (70%) of the 17 districts and 16 (48.5%) of the 33 PAs were sero-positive for anti-RVF virus IgG antibodies. It was greater in Afar Regional State at 4.0% (22/553) than in Borana zone at 2.0% (7/347) and the highest being that of Zone one of Afar at 6.8% (10/147).

Although animals having IgG antibodies were widely dispersed geographically, the highest prevalence was found in Aysaita district accounted for (11.7%; 7/60) followed by its neighbours Dubti and Afdera districts with (10.3%, 3/29) and (6.9%, 4/58), respectively. The lowest prevalence was in Borana in Moyalle district (0.9%, 1/116) which is bordering Kenya.

Out of 33 PAs surveyed, Galifagi of Aysaita accounted for the highest sero-prevalence (13.8%, 4/29) and was followed by Beyahile Sahil in Dubti and Hemaleysen in Telalak at 10.3% each (3/29) and Bokaytu in Aysaita at 9.7% (3/31). In Borana zone, the highest prevalence was observed in Ranji PA of Arero district at 6.9% (2/29). Among all the highest prevalence rates at all levels were found in Zone one of Afar Regional State (Tables 8 and 9).

The sero-prevalence of RVF in sheep and goats was 5.4% (19/352) and 1.8% (10/548), respectively. In sheep, it was 5.2% (13/248) and 5.8% (6/104) for Afar and Borana, respectively. The respective sero-prevalence in goats was 3.0% (9/305) and 0.4% (1/243) (Table 8). The highest sero-prevalence by age group was for sheep and goats of 5-6 years of age (9.1%; 4/44) and is followed by age groups 6-7 years, 4-5 years and 3-4 years at (5.9%; 1/17), (4.1%; 6/146) and (3.0%; 8/217), respectively. No IgG antibodies were detected s in the sera out of 35 and 11 animal age groups less than 1 year and greater than 7 years, respectively. The highest annual mean sero-conversion was observed in age groups 5-6 years of age (1.9%) followed by in age group 1-2years and 3-4 years at1.4% and 1.1%, respectively. The sero-conversion rate in age groups 4-5years and 6-7years were almost equal 0.95% and 0.93%, respectively. There animals at age group 5-6 years were the ones with the highest sero-prevalence and sero-conversion rates (Table 11).

The distribution of the positive animals based on their location's distance from permanent water point (DFPWP) showed that (58.62%;17/29) and (62%; 18/29) of the total positive animals were found to be within a distance less than or equal to 0.5Km and 1Km respectively; only 37.93% (11/29) of the positives were living within distances greater than 1Km. Only one positive animal out of the 29 positives was found in an area that is located at a distance greater than 5 km. And no any positive animal was found at a distance beyond 8Km, while the longest distance from permanent water in the sampling was 20 km. The relative sero-prevalence of animals living at different distance from permanent water sources is detailed in Table 16; the highest sero-prevalence (7.3%; 17/234) was in animals living at distance less than or equal to 0.5Km.; while the lowest (0.4%; 1/261) was in distances farther than 5Km. Ten of the sites were with relatively long lasting flooding (Table 16).

4.2.2 Sero-prevalence based on IgG antibodies detection (retrospective survey)

Out of 820 sera that were collected in 1999 after the 1997/98 epizootics RVF in east Africa and tested using a similar test system with different test protocol, 142 (17.3%) were reported to be positive, though doubts about the protocol of the test were surfacing. Therefore, retest with the currently available test kit with 98% specificity and 99.1% sensitivity was recommended. For this retest of 246 sera (including all previous positives) was conducted. 32 (13 from Afar and 19 from Borana) or 22.8 per cent of the 142 previous positives were found sero-positive during the retest. The total prevalence which was reported to be 17.3% (142/820) or 12.8 % (64/500) in Afar and 24.4% (78/320) in Borana was reduced with the new test kit to 3.9% (32/820), 2.6% (13/500) and 5.9% (19/320), respectively (Table 12).

The distribution of the sero-positivity in different districts showed that 50% of the districts in Afar and all in Borana were found positive in 1999. Higher prevalence rates were observed in Dubti (17.5%, 7/40), Abe'ala (10%, 2/20) and Burumudaitu (5%, 2/40) of Afar regional state, as well as in Dire district (12.5%, 10/80), Arero (6.3%, 5/80), Teltele (2.5%, 2/80) and Hageremariam (2.5%, 2/80) districts.

Out of 29 (21 from Afar and 8 from Borana) PAs that were surveyed for RVF in 1999, 14 of Afar and 8 of Borana PAs were retested. From retested sera, 13 PAs (7 from Afar and 6 from Borana) were sero-positive. The results at PA level are given in Table 10 with the highest in Afar being that of Behayile Sahil (25%) followed by Dembel in Dubti district, Debel in Burumudaitu district and Abe'al with 10%each. In Borana PAs, Dassi (15%) was the highest followed by Godichicharomsa and Allona (each 10%).

There was a decrease in RVF sero-prevalence in Borana from 5.9% in 1999 to 2.0% in 2003, while an increase was observed in Afar from 2.6% to 4.0%. While the total sera of the two periods investigated, decreased from 3.9% to 3.2% was observed in the respective retrospective and cross-sectional investigations (Table 7 & 12).

Among districts investigated during both surveys, Dubti and Burumudaitu from Afar and Hageremariam from Borana were found to be sero-positive in both periods. Teltele district, which was previously sero-positive was found to be negative during the cross-sectional investigation; while Gewane, a district in Afar, that was negative in 1999 was found to be sero-positive in 2003/2004 (Tables 8 & 14).

4.2.3 Sero-prevalence based on IgM antibodies detection (cross-sectional survey)

With the objective of detecting the presence of recent infection in the study sites, an IgM ELISA based on capture format was used. No any animal was sero-positive using this test.

Table 7: Sero-prevalence of Rift Valley Fever in Borana zone and Afar Regional State; by Indirect Sandwich ELISA in Sera collected from October 2003 to March 2004.

Sample	Zone (Afar Region)				Borana	Total
	Zone one	Zone two	Zone three	Zone five		
Total sera	147	87	174	145	347	900
Positive sera	10	4	4	4	7	29
Prevalence (%)	6.8	4.6	2.3	2.8	2.0	3.2

Table 8: Sero-prevalence of Rift Valley Fever in different strata in sheep and goats in Afar Regional state and Barana zone, November 2003 to April 2004

	Number of samples tested			Positive (number & %)		
Strata	Afar	Borana	Total	Afar	Borana	Total
Area/site						
Zone	4	1	5	4 (100)	1 (100)	5 (100)
District	10	7	17	7 (70)	5 (71.4)	12 (70.6)
PA	19	14	35	10 (52.6)	6 (42.9)	16 (48.5)
Flock	87	64	151	9 (10.3)	7 (10.9)	16 (10.6)
Species						
Ovine	248	104	352	13 (5.2)	6 (5.8)	19 (5.4)
Caprine	305	243	548	9 (3.0)	1 (0.4)	10 (1.8)
Sex						
Female	472	273	745	19 (4.0)	6 (2.2)	25 (3.4)
Male	81	74	155	3 (3.7)	1 (1.4)	4 (2.6)
Total	553	347	900	22 (4.0)	7 (2.0)	29 (3.2)

*Numbers in brackets stand for percentages.

Table 9: Summary of Apparent and True sero-prevalence with respective Estimates of 95% Confidence Intervals, cross-sectional survey (2003/2004).

Variables	*Total Sample Tested*	*N^o of Positive*	*Sero-Prevalence (%)*	*Binomial Exact (95% Confidence Interval).*	*Estimated True Prevalence (%)*	*95%Confidence Interval for the True Prevalence*
Afar Region	553	22	3.98	2.51—5.96	2.04	1.21—2.866
Zones						
Zone One	147	10	6.8	3.31—12.15	4.94	2.88—7.01
Zone Two	87	4	4.6	1.27 — 11.36	2.68	0.44—4.91
Zone Three	174	4	2.72	0.63—5.78	0.74	0.00—1.97
Zone Five	145	4	2.76	0.76—6.91	0.78	0.00—2.14
Borana Zone	347	7	2.02	0.81— 4.11	0.02	0.00—0.77
Districts						
Aysaita	60	7	11.67	4.14—22.57	9.96	5.84—14.08
Dubti	29	3	10.34	2.19—27.35	8.59	2.97—14.21
Mille	58	0	0	0	0.00	0.00—0.00
Koneba	29	0	0	0	0.00	0.00—0.00
Afdera	58	4	6.7	1.91—16.73	4.84	1.58—8.10
Burumudaitu	29	1	3.45	0.42—11.91	1.49	0.00—4.86
Gewane	58	2	3.45	0.42—11.91	1.49	0.00—3.88
AwashFentalle	58	0	0	0	0.00	0.00—0.00
Dewey	58	1	1.72	0.72—9.75	0.00	0.00—1.70
Telalak	87	3	3.45	0.72—9.75	1.49	0.00—3.44
Moyalle	116	1	0.86	0.02—4.71	0.00	0.00—0.85
Yavello	29	1	3.44	0.09—17.77	1.48	0.00—4.85
Teltele	58	0	0	0	0.00	0.00—0.00
Arero	58	3	5.17	1.08—14.38	3.27	0.37—6.16
HagereMariam	43	1	2.33	0.06—12.29	0.34	0.00—2.63
OdoShakisso	14	0	0	0	0.00	0.00—0.00
Abeya	29	1	3.44	0.09—17.77	1.48	0.00—4.85
Species						
Ovine	352	19	5.40	3.28—8.30	3.502	2.30—4.69
Caprine	548	10	1.82	0.57—3.33	0.00	0.00—0.57
Total Sample	900	29	3.22	2.17—4.59	1.26	0.67— 1.84

Confidence interval estimations were conducted both for apparent- and true-sero-prevalence based on the cross-sectional and retrospective surveys. The estimation made for the total sera showed the confidence interval range to be from 2.17-4.59% and0.67-1.84% for apparent and

true prevalence, respectively. The highest estimate from the zones was that of Zone one of Afar which ranges from 3.31-12.15% and 2.88-7.01% for apparent and true prevalence, respectively, while that of Borana zone was 0.81-4.11 and 0.00-0.77%. From districts Aysaita and Dubti were with higher estimates. Estimates of the species showed 3.28-8.30% and 2.30-4.69% for ovine while that of caprine were lower (Table 18).

Table 10: Distribution of Sero-positives of Rift Valley Fever in Peasant Associations of Afar Regional State and Borana zone; based on cross-sectional survey result

District	*PAs*	*N^o^ of Samples*	*N^o^ of Positive*	*Prevalence (%)*
Aysaita	Gali Fagi	29	4	13.79
	Bokaytu	31	3	9.68
Dubti	Beyahile	29	3	10.34
Afdera	Aligenda	29	2	6.9
	Kosorawda	29	2	6.9
Burumudaitu	Debel	29	1	3.44
	Tulie	29	1	3.44
Gewane	Urafita	29	2	6.9
Dewey	Wediragi	29	1	3.44
Telalak	Hemaleysen	29	3	10.34
Moyale	Buledi	29	1	3.44
Yavello	Cholkassa	15	1	6.67
Arero	Ranji	29	2	6.9
	Geleba	29	1	3.44
H/ Mariam	Dawa Bichu	29	1	3.44
Abaya	Dibicha Laluncha	29	1	3.44

Othe 17 PAs located in 11 districts were with sero-prevalence of zero (Annex 1)

Table 11: Age-specific RVF Sero-prevalence and Annual Mean age-specific sero-conversion Rate, Afar regional state and Borana Zone (2003/2004)

Age group (Yrs)	*Number of samples*	*N° of positives*	*Prevalence (%)*	*Annual Mean Sero-conversion Rate (%)*	*Distribution of Sero-positive*
<1	35	0	0	0	
1-2Yr	195	4	2.05	1.37	Urafita & Buledi & Wediragi
2-3Yr	235	6	2.55	1.03	Cholkassa, Beyahile, Bokaytu, Kosorawda, Hemaleysen & Dawabichu
3-4Yr	217	8	3.69	1.07	Ranji & Dibicha Laluncha Hemaleysen, Galifagi &Debel
4-5Yr	146	6	4.11	0.93	Bokaytu, Galifagi, Kosorawda, Tuli & Geleba
5-6Yr	44	4	9.09	1.89	Beyahile, Galifagi, &Aligenda
6-7Yrs	17	1	5.88	1.0	Galifagi
>=7	11	0	0	0	
Total	900	29	3.22		

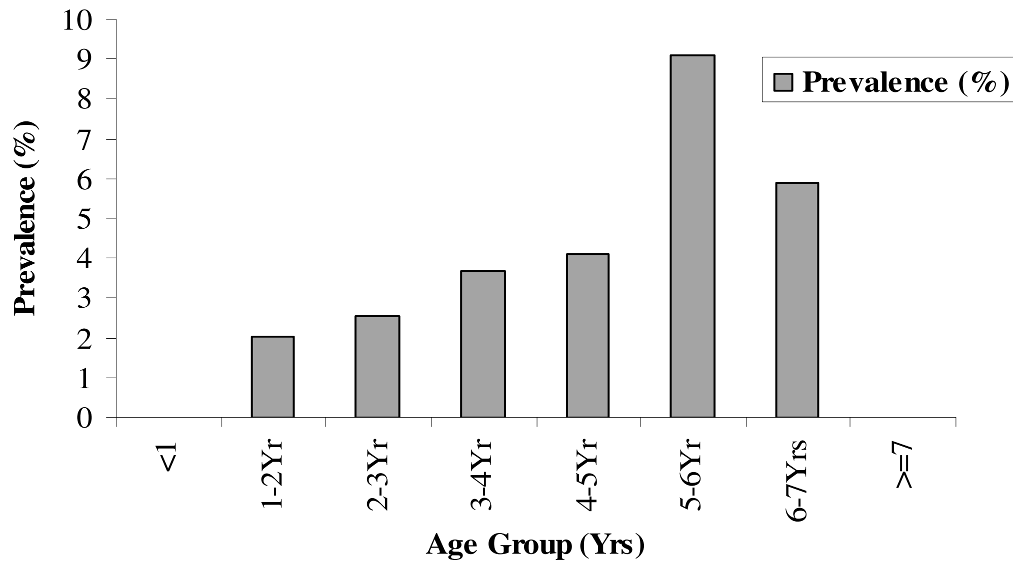


Figure 9: Distribution Pattern of Rift Valley Fever Antibody prevalence in age groups.

Table12: Sero-Prevalence of RVF in Afar and Borana areas, based on the Retrospective Sero Survey Result.

Sites	*N^o samples tested*	*N^o of positive*		*Prevalence (%)*		*No. Of Samples retested from the 1999*
		2000	*2004*	*2000*	*2004*	
Afar	500	64	13	12.80	2.60	144
Borana	320	78	19	24.38	5.49	102
Total	820	142	32	17.32	3.90	246

Table 13: Sero-prevalence of Rift Valley Fever in Afar and Borana Zone(s) from the serum Samples collected in 1999.

Zone	*Zone One*	*Zone Two*	*Zone Three*	*Zone Five*	*Borana*	*Total*
N^o of Samples	100	200	140	60	320	820
N^o of Positives	8	3	2	0	19	32
Sero-prevalence (%)	8.00	1.50	1.43	0	5.94	3.90

Table14: Distribution of Sero-prevalence of Rift Valley Fever in different strata of Afar Regional State and Borana Zone from serum samples collected in 1999.

Site	*District*	*PA*	*N^o of sample*	*Ovine*	*Caprine*	*Positive (number & %)*	
						2000	*2004*
Afar	Dubti	Dembel	20	20	0	4 (20.0)	2 (10)
		Beyahile	20	20	0	1 (5.0)	5 (25)
	Chifra	Mesgid	40	20	20	2 (5.0)	1 (2.5)
		Sedehada	20	20	0	2 (10)	0
	Megale	Faro	40	20	20	12 (30)	0
		Adu	40	20	20	0	0
	Berahle	Demale	40	20	20	3 (7.5)	1 (2.5)
	Erepti	Erepti	20	20	0	4 (20)	0
	Ab`ala	Ab`ala	20	20	0	5 (25)	2 (10)
		Hidamo	40	20	20	11 (27.5)	NRT
	Burumudayt	Debel	20	20	0	4 (20)	2 (10)
		Daferom	20	20	0	0	0
		Geleallo	20	20	0	0	0
	Amibarah	Melkawerer	20	20	0	5 (25)	0
		GediGedaro	20	20	0	2 (10)	0
	Gewane	Gewane	20	20	0	7 ((35)	0
		Gelildura	20	20	0	2 (10)	0
	Fursi	Hadal ela	40	20	20	0	0
		Artuma	20	20	0	0	0
Total	10	19	500	380	120	64 (12.8)	13 (2.6)
Borana	Dire	Godichicha	40	20	20	25 (62.5)	4 (10)
		Daasi	40	20	20	31 (77.5)	6 (15)
	Arero	Wachile	40	20	20	3 (7.5)	1 (2.5)
		Allona	40	20	20	4 (10)	4 (10)
	Teltele	Mermero	40	20	20	8 (20)	2 (5)
		Horbale	40	20	20	0	0
	H/mariam	Finchawa	40	20	20	3 (7.5)	0
		Derudenfele	40	20	20	4 (10)	2 (5)
Total	4	8	320	160	160	78 (24.38)	19 (5.94)
Grand Total	14	28	820	540	280	142 (17.32)	32 (3.90)

Numbers in parentheses stand for percent

Table 15: Distribution of Sero-positivity to Rift Valley Fever in relation to Intrinsic Risk Factors

Intrinsic Risk Factors	*Samples Tested*	*N° of positives*	*Prevalence (%)*	*Proportion to the total positive (%)*
Species				
Ovine	352	19	5.4	65.52
Caprine	548	10	1.8	34.48
Age category (Yrs)				
<=1	162	3	1.85	10.35
>1 and <= 3	470	15	3.19	51.72
>3	268	11	4.10	37.93
Sex				
Female	745	25	3.4	86.97
Male	155	4	2.6	13.79

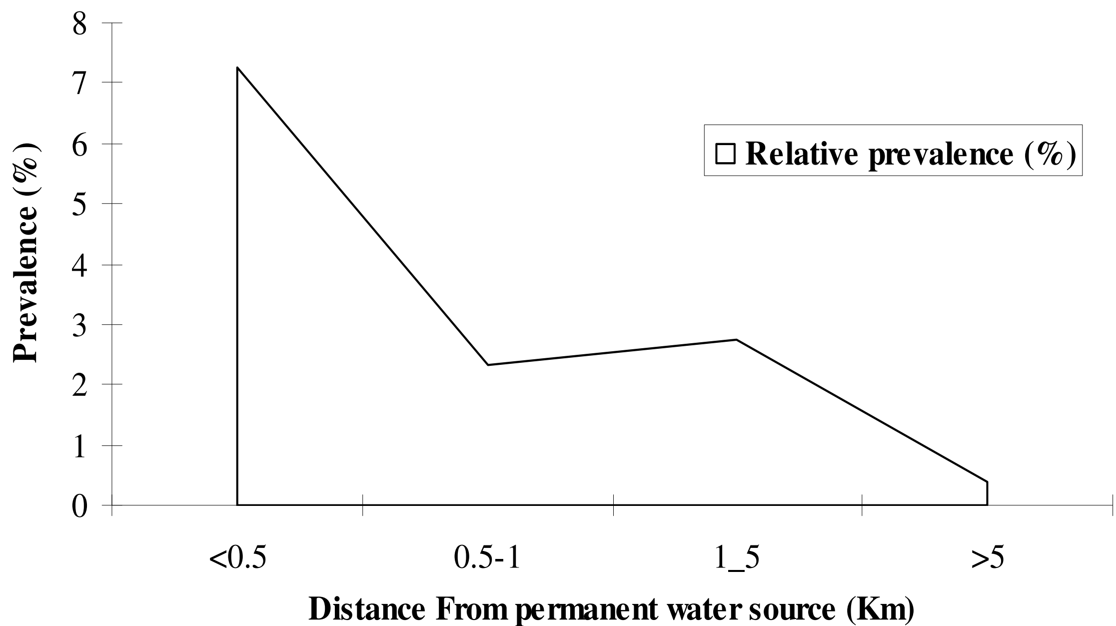


Figure 10: Pattern of Rift Valley Fever Sero-prevalence in relation to distance from Permanent

Water source

Table 16: Distribution of Sero-positivity to Rift Valley Fever in relation to Extrinsic Risk Factors

Extrinsic Factors	*Samples tested*	*No of positives*	*Prevalence (%)*	*Proportion to the total positive (%)*
Abortion history (flocks)				
Absent	531	20	3.76	68.97
Present	369	9	2.44	31.03
Flooding				
Absent	362	5	1.38	17.24
Short lasting	376	9	2.39	31.04
Long lasting	162	15	9.26	51.72
Irrigation				
Absent	724	15	2.07	51.72
Present	176	14	7.96	48.28
Biting insect Problems				
Absent	741	25	3.37	86.21
present	159	4	2.52	13.79
DFPWS* (Km)				
<= 0.5	234	17	7.26	58.62
>0.5 & <=1	43	1	2.32	3.45
>1 & <= 5	362	10	2.76	34.48
>5	261	1	0.38	3.45

* DFPWS (Km); Distance from permanent water source in kilo meter.

4.3 Predictive Value of the Test

The predictive value estimates of the test at different prevalence of infection and /or disease ranging from 0.1% to 80% was calculated and the details are put in Table 21. The sero-prevalence recalculated from the positive predictive value of the test showed the animals assumed to have had infection or diseased were 12 (1.38%) on average and 9 (1.71%) and 3 (0.87%), in Afar and Borana, respectively. Of the age groups highest numbers assumed to have had ifection or disea were 3 from age groups 2-3years and 3-4. While, the number of animals at age group 1-2 were only 2.This calculation was based on the predictive value at 1.5% expected prevalence rate (Table 18).

Table 17: Estimated Positive Predictive Value of the IgG ELISA Test Used in the Survey.

Size of Population	*Estimated Infection/Disease Prevalence (P) (%)*	*Sensitivity (%)*	*Specificity (%)*	*Positive Predictive Value of the test (%)*	*No False Positive*
1000	0.1	99.1	98	4.73	19.98
1000	0.5	99.1	98	19.95	19.9
1000	1	99.1	98	33.36	19.8
1000	2	99.1	98	50.28	19.6
1000	5	99.1	98	72.28	19
1000	10	99.1	98	84.61	18
1000	20	99.1	98	92.53	16
1000	50	99.1	98	98.04	10
1000	70	99.1	98	99.15	6
1000	80	99.1	98	99.50	4

Expected prevalence in the lowlands of Ethiopia is 1-2%.

Table 18: Estimated Prevalence of RVF Infection or Disease in small ruminants of Afar Region

and Borana Zone; based on positive predictive value of the test; sera collected in 2003/2004.

Character	*Samples tested*	*No of Positive*	*Apparent prevalence (%)*	*Estimates based on Predictive value of test*	
				N° positive	*Prevalence (%)*
Total Sample	900	29	3.22	12	1.38
Afar	553	22	3.98	9	1.71
Borana	347	7	2.02	3	0.87
Species					
Ovine	352	19	5.4	8	2.32
Caprine	548	10	1.82	4	0.78
Age groups (Years)					
1-2	195	4	2.05	2	0.88
2-3	235	6	2.55	3	1.1
3-4	217	8	3.69	3	1.59
4-5	146	6	4.11	3	1.77
5-6	44	4	9.09	2	4
6-7	17	1	5.88	0.43	2.39

4.4 Risk Factor Analysis

Assessment and univariate and multivariate analysis using logistic regression statistical test in certain intrinsic (individual animal characters) and extrinsic (herd and environmental factors) showed association to high or low prevalence.

4.4.1 Univariate Analysis

Univariate analysis results of risk factors using logistic regression are detailed in Table 19. Difference in sero-positivity to RVF IgG antibodies was seen between some animal characteristics; there was significant difference between ovine and caprine species with P

=0.005 (OR 3.07 and 95% CI 1.4 - 6.7) i.e. $P < 0.05$ ovine being with the higher sero-positivity. Comparison among age groups showed there was no significant difference between animals less than or equal to 1 year and animals from 1 year to 3 years of age p= 0.383 and between animals $\leq$ 1 year and older than 3 years with P= 0.214 and CI 0.5 – 6.1 and 0.6 - 8, respectively i.e. $p > 0.05$ of the extrinsic factors. Significant difference in sero-positivity was seen between animals living in areas where there was long lasting flooding and no flooding between animals living in irrigated and not irrigated areas and between animals living nearer to permanent water source and in those living at a distance farther than 1Km with $P < 0.05$. But even if there were differences seen, it was not significant between females and males, between animals living within a distance < 0.5 Km and 0.5-1km from permanent water sources with $p > 0.05$.

Table 19: Summary of the univariate analysis by Logistic Regression Test Results of the Different Characters; for the total Sera.

Status	*Odds Ratio*	*Std. Error*	*P Value*	*95% CI*
Species	3.07	1.22	0.005	1.4 -6.7
Sex	0.76	0.42	0.62	0.26- 2.22
Abortion	1.57	0.64	0.271	0.7-3.48
Biting insects	1.35	0.74	0.58	0.46-3.94
Irrigation	3.32	1.26	0.002	1.57-7.0
Age categories				
Between1&2	1.75	1.12	0.383	0.5-6.1
Between1 & 3	2.27	1.5	0.214	0.62-8.26
Flooding				
Between 1& 2	1.75	0.99	0.32	0.58-5.28
Between 1 & 3	7.29	3.83	0.000	2.6-20.41
DFPWS				
Between 1& 2	0.3	0.32	0.253	0.04 -2.34
Between 1& 3	0.36	0.15	0.013	0.16-0.81
Between 1& 4	0.04	0.05	0.004	0.01-0.37

Number of observation = 900

4.4.2 Multivariate Analysis

Significant associations were seen with sero-positivity and some of the intrinsic and extrinsic factors during multivariate analysis. A highly Significant association of sero-positivity to anti-RVFV antibodies was seen with animal species the same as that in univeriate analysis with P < 0.05. Even if there was a very high association with variables related to water sources (flooding, irrigation distance from permanent water source) with P < 0.05 but there was high collinearity between these characters. Generally species and distance from permanent water source or flooding were found fit to the final model of the logistic regression identified as the risk factors that were highly associated with sero-positivity to RVF antibody sero-positivity in Afar region and Borana zone, with P < 0.05 (Tables 20 and 21) .

Table 20: Logistic Regression Analysis Model of the Risk Factors Associated with Sero-positivity

Status	*Odds Ratio*	*Std. Err.*	*P>|z|*	*[95% Confidence Interval]*
Species	2.625325	1.057818	0.017	1.191817--5.783042
Category. DFPWS*	5303936	.0916067	0.000	.3780797 .7440691

Number of observation = 900, Probability > chi2= 0.0000, LR chi2 (2) =23.67 notes: flooding dropped due to collinearity. DFPWS, Distance from permanent water Source.

Table 21: Regression coefficient of the risk factors Associated with Sero-positivity to RVF

Status	*Coef. of regression*	*Std. Err.*	*P>|z|*	*[95% Confidence Interval]*
Species	0.97	0.40	0.017	0.18 --1.75
Cate. DFPWP	- 0.63	0.17	0.000	- 0.97--0.3
_Constant	-2.450278	.4758321	0.000	-3.382892-- -1.517664

Number of observation =900, LR chi2 (2)= 23.67 and Probability > chi2=0.0000 notes: flooding dropped due to collinearity.

A DOT MAP SHOWING SERO-PREVALENCE OF RIFT VALLEY FEVER IN AFAR R/STATE & BORENA ZONE OF OROMIA R/STATE IN 2003

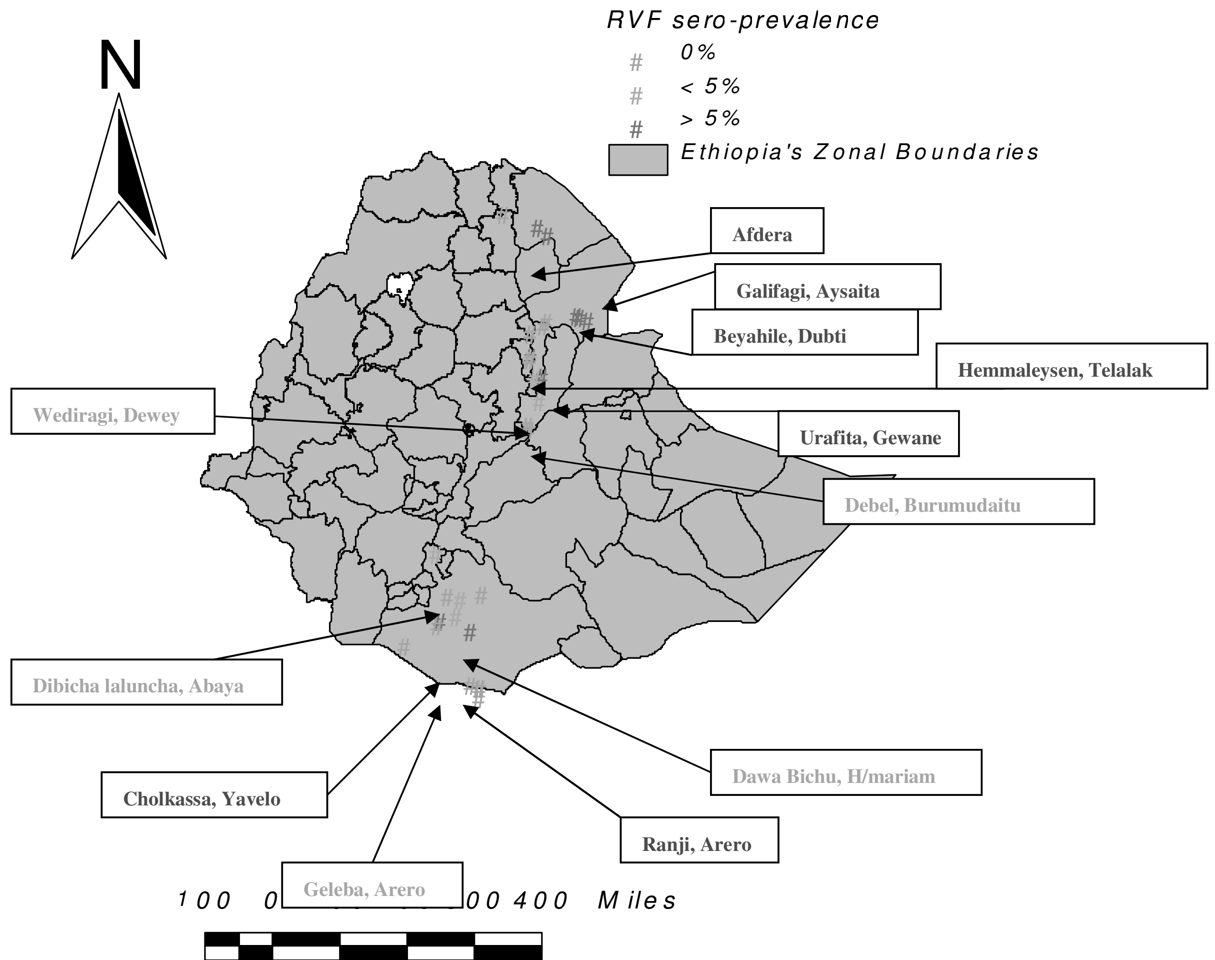


Figure 11: Distribution map of peasant associations of Afar region and Borana zone with Ant-RVF virus IgG sero-positive reactors .

5. DISCUSSIONS

During the cross-sectional survey 900 sheep and goats serum samples from 33 peasant associations located in 17districts were collected in Afar regional state and Borana zone from October 2003 to March 2004. Additional tests were conducted on 246 retrospective sera sorted out of 820 sera collected during 1999 survey from 27 PAs found located in 14 districts (10 Afar and 4 Borana) . Detail information on individual animal and environment related data (flooding, irrigation, problems of biting insects, and DFPWP) and others were included and have been used in the analysis. Recently developed test kit was used to analyze both sera.

Based on the ELISA result of 900 samples of the cross- sectional survey sero-prevalences of 3.22%, 3.98% and 2.02% were found for the total samples, Afar region and Borana zone, respectively. The respective sero-prevalence of the retrospective sera was 3.90%, 2.60% and 5.94%. This shows the prevalence in Borana decreased by more than half during this period; where as in Afar there was an increase by one and half times. This may be due to accumulation of sero-positives in Afar due to lack of access to market, resulted due to the ban, that would have helped in removing the sero-positives animals through selling. Borana has better market access as it had been observed during the fieldwork; in which 400 sheep and goats a day were exported to Kenya from Moyalle livestock market. Other areas of Borana including Teltele and Dire are also with good access to Kenyan markets. Animals were moved to the border of Kenya from areas deeper than 300Kms to Ethiopia such as Yirgachefe. Sheep and goats in Afar are mainly used for milk production for family consumption thus female animals are maintained in the flock for longer periods, while younger male animals are sold. Most of the animals in Afar were nearer to permanent water points and in areas with problems of flooding and biting insects (Annexes 5-7).

A sero-prevalence of 3.22% is higher than that expected in areas with similar ecology that is arid and semiarid areas where there should not be sero-conversion during inter epizootic periods. However, such prevalence was reported in areas with high rainfall and having high mosquito densities; the average being 1-5% (areas sampled were within 5Km of permanent water points such as the Awash and Dawa rivers and ponds (Annexes 3-7) which may favour vector multiplication and might elicit sporadic sero-conversions. This may also be further consolidated by the finding that one of the 29 positives is found in area which is located at a distance greater than 5 km from permanent water. There was no any sero-positive animal in

an area farther than 8 km from permanent water source, though samples were collected in up to 20 km. Of the total 29 positives 62.07% were located within 1 km from permanent water.

After an epizootic it takes 1-3 years the virus activity to die out (OIE, 2000, AGA/FAO, 2002 and Geiger, 2002). Based on vector distribution and prevailing epizootiological factors the prevalence of RVF for IgM ranges from 15-90% which could gradually inevitably be replaced by IgG up to 6 months post epizootic (AGA/FAO, 2002 and Geiger, 2002). From the samples collected during 1999 which in fact was assumed to be a post epizootic period in Afar and Borana was only 3.9%. This does not seem to represent a post epizootic period. The out breaks in Kenya and Somalia in 1997/98 and In Saudi Arabia and Yemen in the year 2000 resulted in the ban on live animals export from east Africa. Therefore, Ethiopia was wrongly included in the ban. In addition if such epidemics had had occurred in pastoral areas, the traditional information systems like that in Afar known as *Daggu* would have been able to disseminate such a large scale catastrophe to the whole community and to responsible government administrative bodies. (When two Afares meet it is accustomed that they must take a few time sit at the road side and exchange information which starts from the history of the new born animals and stop at community issues. This information exchange is locally known as *Daggu.* Because it is supported by the mobility of the community and is able to disseminate a piece of information through out the region within a short period of time.

The limitations of the previous test kit used in the year 2000 were detailed in section 2.2.6. It reported sero-prevalence of anti-RVFV antibodies to be 17.1% in sheep and 11% in goat .This was an exaggerated result which was proved by the retrospective investigation to have 78% false positives.

District level sero-positivity shows high sero-prevalence in Aysaita (11.67%), Dubti (10.37%), and Afdera (6.7%) and of 3.5 % in Gewane, Burumudaitu & Telalak districts of Afar. While sero- prevalence of 5.17 % in Arero, 3.4 % in Yovello and Abaya districts of Borona were diagnosed. The higher sero-prevalence in districts is due to their higher share of the animals living in PAs that have favourable environmental factors such as flooding, irrigation, River Awash and others. (Annex 5-7). Because most districts which were with lower or zero prevalence were devoid of such factors. Such districts include Mille, Awash fentale of Afar and Moyalle and Shakisso of Borona.

Among the sampled PAs Galifagi, Bokaytu of Aysaita; Beyahile Sahil of Dubit and Hemaleysen (10.34%) of Telalak are mostly flooded by Awash river not only due to the rain

in Afar but also due to that from other parts of the country. Galifagi is the collection center of animals from most parts of Zones One and Four that migrate in search of water and grazing. Galifagi, Bokaytu, Beyahile Sahil, Debel and Urafita were PAs found surrounded by spilled over Awash for more than 4 months (Annexes 5-7). Again the flock sizes per house holds in all these PAs were large ranging from 100 to 300. From Borona Chlquassa and Ranji were PAs found to have nurturing environmental situations (annexes 3-4). Most of the PAs with higher sero-prevalence had suitable climate to the breeding of biting insects which might include vectors of RVF virus. The lower sero-prevalence in areas biting insects and water lodgment such as Dukisa Chebiti might be due to the cold weather attributed to its higher altitude 1900m a. s. l.. Areas which support vector multiplication should have warm temperature, (WHO, 1982).

This finding is supported by the different study reports which list the different factors that favour RVF virus vectors. Favorable environmental factors for insects include warm climates, high concentration of domestic animals, irrigations, water pans and ponds, etc. (WHO,1982 ; Davies & Nunn, 1998; Fontenille *et al*., 1998; Kreger,2000 and AGA/FAO, 2002).

There was higher antibody prevalence in sheep than goats in both study areas. The average sero-prevalence was 5.4% and 1.82% in sheep and goats, respectively. This difference was significant $p<0.005$ (Table 19). Similar study results were obtained in many epizootics and inter-epizootic period survey in different countries (WHO, 1982; Davies, 1988 and AGA/FAO, 2002).

Sero-prevalence was higher in the animals living nearer to permanent water and vice versa (Figure 10 and Table 16). This holds true since RVF is vector borne disease and consideration of the average dispersion distance of the *Aedes* mosquito from permanent water, which averages to be 0.15 km as mentioned by AGA/FAO (2002). The dispersion capacity of the vector is mainly affected by the amount of cold cloud cover and wind availability; it can reach up to 500Km. In this survey 58.62% or about 96.55 % of the positive sera were identified in animals living within 0.5 km and 5km, respectively. The prevalence among the various distances from permanent water were with significant differences with $p<0.05$ similar significant differences were observed with $p<0.05$ between different extents of flooding. The reasons are also related to creating favorable conditions for insect breeding (Table 19).

Age specific sero-prevalence and age-specific annual mean sero-conversion rates (Table 11) showed that the level of sero-conversion generally is very little in the population. If there had been a chance of a continuous culling of the sero-positive animals there would have been a higher reduction in both sero-prevalence and sero-conversion rate. This is because, sero-prevalence particularly of IgG, is a result of cumulative experience of the population to the agent (Murphy *et al*., 1999). The relatively high sero-conversion rate but lower sero-prevalence in animals aged one to two years may be indicative of persistent passive immunity. The distribution of these positive animals in to areas found located at distant areas is indicative of the positivity of this susceptible age group not being due to infection. And also since the IgM persists from 6 months (EMPRES/FAO, 2000) up to 9 months (AGA/FAO, 2002) and they might have been protected for up to 6 months there must have been at least a few IgM positive animal specially in this age group but no any IgM positive animal has been detected. But the sharp decline in age group 2-3 yrs supports the former speculation, which is persistent passive immunity. This is also more reflected by the increase of sero-prevalence to the higher ages 4.23% (at age 4yr) to 9.3% at 5yrs and 5.88% at 6Yrs. The absence of sero-positive animals in those animals older than six years might be due to desensitization of the B-cells to respond to signals from the T-cells and even at old age they tend to shift away from IgG production (Thizard, 1982) where detectable amount of antibodies could not be found (Crowther, 1995). The relatively highest sero prevalence of 9.3% at 5yrs and preceded and followed by lower sero prevalence of 5.56% and 4.23%, respectively on ages 6yr and 4yr showed higher sero-conversion indicating the occurrence of slight virus activity during that period. This period Corresponds with the year during which the world wide changes in weather known as EL Ni`no characterized by long lasting heavy rain which resulted in RVF epizooties in other east African countries. This finding is also supported by the results of the test sera of 1999 where sera a sero-prevalence of 3.9% which was not a post epizootic period sero-prevalence. Thus from both findings until the years 2000 there was a slight viral activity in these areas but no epizootics had occurred even during the epizootics that occurred in east Africa in 1997/98. The genetic resistance of the local breeds of Ethiopia might have contributed to the lower prevalence. This is supported by other evidences that states local breeds of Africa to be resistant to RVF and low sero-prevalence are measured even during epizootic periods (WHO, 1982; Fontenille *et al*, 1998; AGA/FAO, 2002 and CIDRAP, 2003).

Most investigations of epidemics in African countries showed that the local or indigenous breeds of African are known to have lower infection rate with shorter vireamic period (AGA/FAO 2002). This when related to the unimproved livestock transport system of the

country which elapses many days from its residence to market places, excludes the risk of exporting vireamic animals.

The estimated positive predictive value of the tes, based on the expected prevalence of the disease in the country that ranges from 1-2% in the lowlands (Asfaw *et al.*, 2002), was 33.36% to 50.28%.That meant the correctness of the test to predict whether sero-positive animals were infected or diseased is 33.36% to 50.28% and. From this the estimated number of animals which had been infected or diseased ranges between 9-15 animals only in the study animals. The same estimation of the age-specific sero-prevalence based on the predictive value was conducted. Of the age groups highest numbers assumed to have had infection or disease were 3 from age groups 2-3years and 3-4. While, the number of animals at age group 1-2 were only 2. Consideration of the nature of the disease with this prevalence excluded the occurrence of new infection in these age groups.

Animal species, distance from permanent water and flooding were the three risk factors associated with sero-positivity to RVF. But flooding and distance from permanent water showed high collinearity in multivariate analysis

6. CONCLUSIONS AND RECOMMENDATIONS

The following conclusions and recommendations are generated from the findings:

1. Sero-positivity to RVF virus IgG antibodies was detected from surveys of both post-epizootic and inter-epizootic periods conducted in Afar and Borana. With Sero-prevalences of the total sera being 3.9% and 3.2% for retrospective and cross-sectional sera, respectively. The respective sero-prevalences of RVF in Afar region and Borana zone were 2.6% and 5.9% as well as 3.98% and 2.02% for retrospective and cross-sectional surveys, respectively.
2. Certain PAs from both study sites were found with higher apparent sero-prevalences ranging from 9.68% to 13.79%. Therefore, attention should be given to these specific sites during the continuous national sero-surveillance, particularly in the establishment of sentinel herds.
3. The age-specific sero-prevalence and annual mean sero-conversion rate showed even younger animals to be sero-positive; but further analysis based on the positive predictive value of the test excluded the probability of any recent virus activity within 4 years in both study sites. This was also supported by the absence of any IgM sero-positive animal even from the highly susceptible age groups. Therefore, export of animals from these sites at this moment doesn't have any risk. However, further control measures have to be implemented to consolidate the current virus free status.
4. There has been species difference in sero-positivity, ovine being with 5.4 % and caprine 1.82%. Thus, further studies should give emphasis to factor(s) of resistance in goats in relation with RVFV.
5. The RVF serological distribution map developed based on laboratory findings is correlated with water roots and water lodging areas such as Awash and Dawa rivers, Lake Afdera and Haro Beke. Therefore, surveillance has to be directed to these sites.
6. The retest result of the 1999 sera using the recent batch of ELISA diagnostic kit showed lower sero-prevalence suggesting a 78% false positivity in the previous kit. Therefore, previously reported results were exaggerated and should be corrected accordingly. From the retest the sero-prevalence of those sera supposed to be of post epizootic period was found to be 3.9%, which is below the expected post-epizootic period's prevalence. Therefore, given such a low positivity rate it is difficult to consider the 1997/98 Ethiopian RVF situation as epizootics.

7. Since interpretations and inferences of results from such serological surveys inevitably rely on the reliability or positive predictive value calculated based on expected prevalence of the disease in the population; the calculated reliability of the test has been found to be from 33.36% to 50.28%. This value indirectly indicated the estimates of sero-prevalence in all the strata of this survey to be with false positives range of 66.64% to 49.72%. The corrected calculation of the sero-prevalence of both surveys dropped to 1.07%-1.62% and 1.30%- 1.96% for the cross-sectional and retrospective surveys, respectively. Therefore conclusions on the actual disease situation in the country need support by other virus isolation studies either from the vertebrate or vector hosts of the disease.

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8. ANNEXES

Annex 1: Sero-prevalence of Rift Valley Fever in PAs of Afar regional state and Borana zone of Oromya regional state.

PAs	*No of Samples*	*No. of Positive*	*Prevalence (%)*
Gali Fagi	29	4	13.79
Bokaytu	31	3	9.68
Beyahile	29	3	10.34
Bekeride`arna	29	0	0
Werenson	29	0	0
Alhiena	29	0	0
Aligenda	29	2	6.9
Kosorawda	29	2	6.9
Debel	29	1	3.44
Tulie	29	1	3.44
Biliforo	29	0	0
Urafita	29	2	6.9
Deho	29	0	0
Duddub	29	0	0
Wediragi	29	1	3.44
Derseda	29	0	0
Hemaleysen	29	3	10.34
Waydelele	29	0	0
Gewis	29	0	0
Buledi	29	1	3.44
Medo migo	29	0	0
Tuqua	29	0	0
Arbelle	29	0	0
Kobo	14	0	0
Cholkassa	15	1	6.67
Bilao	29	0	0
Sarite	29	0	0
Ranji	29	2	6.9
Geleba	29	1	3.44
Dawa Bichu	29	1	3.44
Dukisa Chebiti	14	0	0
Bupo	14	0	0
Dibicha Laluncha	29	1	3.44
Total	**900**	**29**	**3.22**

Annex 2: Questionnaire Format used to collect information related to Rift Valley Fever disease.

Date of visit			Geogr. Coordinates	
Name of surveillance team	(complete)		Heavy rains the last 12 months	(complete)
Name of sampling site	(complete)		High number of mosquitoes in last year	(complete)
District	(complete)		Irrigation carried out	(complete)
Name of owner:			Flooding in last 12 months	
Movement pattern of herd	Resident (tick)	Trade animals (tick)	Transhumant (tick)	
Where animals from elsewhere introduced	Yes (tick)	No (tick)	From where?	(complete)
How many abortions in the herd/flock in the last 12 months	(complete)		How many newborn lambs/kids died in the last 12 months	(complete)
Species in herd	Sheep/goats (tick)	Cattle (tick)	Camels (tick)	Donkeys (tick)

Floding : No_________ Short lasting__________ Long lasting_______________________.

Distance from permanent water point
____.

Others____
____
____
____
____.

Annex 3: A ''Dambo '' depression suitable breeding biotope; Borana Zone.

Photo by Melesse Balcha (March, 2004)

Annex 4: A pond, Haro Beke with its supportive ecology for vector multiplication, both human and animals using it; Cholkassa (PA), Borana. March 2004.

hoto by Melesse Balcha (March, 2004)

Annex 5:A conducive biotope for flood water Aedes species in Gewane and Burumudaitu.

Photo by Melesse Balcha (December, 2003)

Annex 6: The area in Aysaita supported by small scale irrigation from River Awash.

Photo by Melesse Balcha (December, 2003)

Annex 7: River Awash Flooding out its course in Gewane, Lasted for 6 months (2003).

Photo by Hussen Mohammed (a pastoralist; December 2003)

Annex 8: Active blood collection to harvest serum (March, 2004)

9. CURRICULUM VITAE

PERSONAL DATA

Name: Melesse Balcha Ghelan

Date of birth: 28th September 1972 G.C (Butajira, Ethiopia)

Nationality: Ethiopian

Marital status: Married

EDUCATIONAL BACKGROUND

Elementary: Butajira Elementary School, 1979 -1983, Butajira.

Junior and Secondary: Butajira Secondary School, 1984-1988, Butajira.

Higher education: Graduated- from Addis Ababa University (AAU), Faculty of Veterinary Medicine (FVM) with Degree of Doctor of Veterinary Medicine (DVM).

1991: A. A.U, Faculty of Natural Science, Addis Ababa

1992-1995: AAU, FVM, Debre Zeit

1996 (Extern ship programme): Dire Dawa Zonal Veterinary Investigation and Diagnostic Laboratory, Dire Dawa

Since October 2002 Addis Ababa University, Faculty of Veterinary Medicine, as a postgraduate student in Tropical Veterinary Medicine, Veterinary Public Health.

RESEARCH ACTIVITIES:

1. A seminar on: Problems of Veterinary service in Ethiopia (5th Year student seminar, 1997)
2. Gastro Intestinal parasites of the Dromedaries (camel) of Eastern Ethiopia (DVM Thesis, 1998)
3. Problems of backyard poultry production in Dalocha Woreda (SNNP, Silte Zone, 2001/2002).
4. Retrospective and Cross-sectional Serological Investigation of Rift Valley Fever in Small ruminants in Pastoral Areas of Ethiopia (MSc Thesis, 2004)

WORK EXPERIENCE

I have been working in the following areas:

- Since April 1997 Ministry of Agriculture

- April, 1997-June 1999 Sodo Woreda Agriculture office, Sodo, Buee.
- July 1999 – September 2000 Dalocha woreda Agriculture Office, Dalocha.

Posts: - Sodo Woreda Animal Health Team Leader and field veterinarian.

-Dalocha Woreda's Animal Health Team Leader

-Head of the Regulatoty's Department.

TRAINING / SHORT COURSE ATTENDED

Certified for a training on Tsetse and Trypanosomiasis biology, Diagnosis and Control Methods conducted in the National Tsetse and Trypanosomiasis Investigation and Control Center (NTTICC) held from Nov. 29-Dec. 6, 1999 Bedele.

OTHER PROFESSIONAL ACTIVITIES

I have participated in the training of animal health technicians from the eleven Woredas of Gurage Zone on different livestock production problems and diseases in the Zone in 1998 at Wolkite, for a weed and in 1999 at Butajira for one week.

I participated in drafting the Zonal regulations to run a revolving Drug Fund Scheme (RDF), in Gurage Zone, in 1999.

I have trained four field staffs of Action Aid Ethiopia Dalocha programme (NGO) for three months (May to July 2000) as a continuous phase of their Nine months training in Agrafa Agriculture Training center to be certified as an animal health technician each. And I have been working in different developmental activities in partnership with the programme. Since October 2000 for more than a year and half, until I joined the postgraduate programme in Addis Ababa University, I have been serving as a contact person of my office with Action Aid Dalocha programme

ADDITIONAL SKILLS

Computer Skills: Word Processing- Ms-Word

Spread Sheet- Ms-Excel

Driving License: 2nd Level

HOBBIES

I enjoy reading books and papers written on society, philosophy, history and recent scientific achievements, of any stream, as well as research, music and teaching.

CONTACT ADDRESS

Tekalign Balcha Tel. 251-1-637716 or 251-1-632988

REFERENCES

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Graduate studies, Debre Zeit). Tel. 251-1-338917

Dr. Ademe Zerihun (AAU, Faculty of Veterinary Medicine, Debre Zeit. Tel. 251-1-338062 or

251-1-460102 and 09-408137