ADDIS ABABA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

AFRICAN HORSE SICKNESS: STUDY ON SEROPREVALENCE AND DENTIFICATION OF RISK FACTORS IN EQUIDAE AT SELECTED SITES IN ETHIOPIA

A thesis submitted to the School of Graduate Studies of Addis Ababa University in the partial fulfillment of the requirements for the Degree of Master of Science in Tropical Veterinary Epidemiology

BY
KASSA DEMISSIE ABDI

JUNE 2006
DEBRE-ZEIT, ETHIOPIA

TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEXES

LIST OF ABBREVIATIONS

AHS	African Horse Sickness
AHSV	African Horse Sickness Virus
Anon	Anonymous
C-ELISA	Competitive Enzyme Linked Immunosorbent Assay
CFT	Complement Fixation Test
CSA	Central Statistical Authority
CACC	Central Agricultural Census Commission
CI	Confidence Interval
CPE	Cythopathic/Cythopathogenic Effect
CTA	Technical Centre for Agricultural and Rural Co-operation
DNA	Deoxyribonucleic Acid
EARO	Ethiopian Agricultural Research Organization
EEV	Equine Encephalosis Virus
GMEM	Glasgow Modification of Eagel's Minimum Essential Medium
I-ELISA	Indirect Enzyme Linked Immunosorbent Assay
MAbs	Monoclonal Antibodies
MOA	Ministry of Agriculture
NS	Non structural Protein
NVI	National Veterinary Institute
OAU	Organization of African Unity
OCG	Oxalate, Carbolic acid and Glycerin
OD	Optical Density
OIE	Office of International Des Epizooties
OR	Odds Ratio
PAbs	Polyclonal Antibodies
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase PCR
TCID50	Tissue Culture Infective Dose Fifty
VNT	Virus Neutralization Test
VP	Viral Protein

ACKNOWLEDGEMENTS

First and foremost, I would like to express to the Almighty GOD, sustainer and lord of the universe for his innumerable favours and benevolence to me, during the most difficult time of my MSc studies. Special spritual respect and thanks also goes to his mother Saint Mary.

The moral support, encouragement and patience of my wife, Tenagne Molla has no parallel, it was an opportunity for me to witness once again her determination in support of my advancement.

My deepest gratitude goes to the School of Graduate Studies of Addis Ababa University, Faculty of Veterinary Medicine for providing me with the opportunity to pursue my postgraduate studies here in my homeland, for financing the research undertaking, for paying the import tax on the C-ELISA kit, and allowing me to use all facilities.

It is a very good opportunity for me to forward my special thanks and respect to Dr. Kelay Belihu, Associate Dean for research and graduate programmes, for his excellent leadership quality of the postgraduate programmes.

The DONKEY SANCTUARY WORKING WORLD WIDE deserves my heartfelt special appreciation for making available the C-ELISA kit for the detection of African horse sickness virus antibodies serotype 9, with complete bench protocol, without whom the realization of this study would have not been possible. The role played by Dr. ANDREW TRAWFORD and Professor FESEHA GEBREAB in facilitating the purchase of the kit from BDSL in Scotland is an ever-lasting print in my mind.

The allround and enthusiastic support of my advisors in guiding this work and correcting my draft thesis to shape it to its present form is highly acknowledged. For these I am indebted to my academic advisors: Dr. Yilkal Asfaw, Professor Feseha Gebreab and Dr. Berhe Gebreegziabher.

The very vital all rounded technical support of the National Veterinary Institute with free access of all laboratory facilities has enabled me to conduct my work smoothly and according to established time table. The technical support given by w/ro Almaz Sharew, Ato Kinfe Mekuria, Ato Berhanu Beyene, Dr. Fekadu Kebede, Dr. Ahmed Issa and all other staff members of the research and diagnostic department has been immense and very useful and therefore sincerely appreciated.

Last but not least my sincere thanks goes to Drs. Boja Endebu, Fikre Lobago, Gelagay Ayelet, Belay Mulate, Shihun Shimeles, staff members of the Donkey Sanctuary, the Society for the Protection of Animals Abroad (SPANA), Ato Asefa Mekonnen, staff of the Kombolcha Veterinary Regional Laboratory, staff of the Federal Animal Health Department, staff of district veterinary clinics from office of Agriculture and Rural development in the study areas, administrative bodies, friends in the study areas, staff of the National Veterinary Institute, staff of the Faculty of Veterinary Medicine of Addis Ababa University, Equine owners of the study areas have also provided useful inputs to which I am grateful.

ABSTRACT

A study was undertaken to determine the seroprevalence of African horse sickness virus antibodies, isolate and characterize the virus responsible as well as identify potential risk factors in the equine population of selected study areas in Ethiopia. In total 1265 serum samples originating from 824 donkeys, 383 horses and 58 mules were collected from September 2005 to mid of April 2006. Competitive Enzyme Linked Immunosorbent Assay (C-ELISA) configuration was employed to determine the presence of AHSV antibodies. The apparent prevalence of AHSV was found to be 29.7% (95% CI = 26.8-33.0) in donkeys, 10.4% (95% CI = 7.8-14.0) in horses and 10.3% (95% CI =4.8-22.1) in mules. The overall apparent seroprevalence of AHSV was found to be 23% (95% CI=20.8-25.4). There is significant variation amongst the types of equidae in seropositivity ($P<0.05$). Statistically significant ($P<0.05$) difference in seroprevalence was observed in the different study areas, confirming the existence of agro-ecology based variation in the occurrence of African horse sickness. The highest seroprevalence of AHSV was documented in the lowlands followed by midland and highland areas. This has direct correlation with the ecological distribution of the *Culicoides* vectors. As for age dependent variation in seroprevalence no statistical significant difference was found. All age groups as well as male and female populations were equally affected. The risk of acquiring AHS is more than two fold (OR = 2.1) with respect to the types of equidae affected. Moreover, agro-ecology contributes nearly two fold (OR =1.5) for the occurrence of African horse sickness. There is strong association among C-ELISA result of AHSV antibodies, types of equidae and agro-ecology, but age is not part of the interaction. However, sex has weak effect to precipitate the occurrence of African horse sickness. Active disease search was conducted with the aim of virus isolation and identification. After three blind passages were carried out on vero cell lines the sample was subjected to I-ELISA configuration. However, the result was negative. In the presence of the disease in the field with classical pathognomonic signs and postmortem lesions the negative result is probably due to the improper handling of the tissues processed. The indigenous knowledge base of equine owners about African horse sickness in the study areas was assessed through a structured questionnaire format. The survey result indicated that, the indigenous knowledge of owners was found to be unsatisfactory.

Key words: Equidae, AHSV, Seroprevalence, Risk factors, C-ELISA, I-ELISA, Selected areas, Ethiopia, Virus Isolation and Identification, questionnaire.

1. INTRODUCTION

Ethiopia has the largest equine population, probably with the highest density per square kilometer in the world (Alemayehu, 2004) and it has a total of 6.9% of the world's and 42.4% of Africa's equine population. Moreover, it has 65% of all African mules, almost 50% of horses and 30% of donkeys. Horses, mules and donkeys represent a significant share of the working animal population of a number of countries in Africa (Wilson, 1995; Feseha, 1998). According to the Central Statistical Authority of Ethiopia (CSA, 2004) there are about 3.77 million donkeys, 1.45 million horses and 321,339 mules in Ethiopia.

There are 6.1 equines per square kilometer or 1 to 64 equines per hectare. There is also one equine per four people in the agricultural sector and one equine per five people for the total population of Ethiopia (Feseha, 1998).

An important but often overlooked aspect is that, in most cases, donkeys and mules are reared by landless and marginal farmers and are the means of subsistence for millions in the least privileged parts of the world. These beasts of burden receive little care and are subjected to intensive work throughout their lives. Their life expectancy is short, their work output is low and the reproductive performance is poor (Feseha, 1998).

Equids play an important role in the transportation of farm produce, fodder, firewood, agricultural inputs, construction and waste materials. Equine power is used both in a rural and urban transport system which is cheap and viable. It provides the best alternative in places where the road network is insufficiently developed, or the terrain is rugged and mountainous, and in cities where narrow streets prevent easy delivery of merchandise. The importance of equids as working animals needs to be weighed within the prevailing context of their socio-economic value, their demography and distribution, and the implementation of disease control programmes based on strategic system derived from a sound epidemiological knowledge. However, many factors contribute to the poor performance of equids, among which viral diseases characterized by high morbidity and mortality rates are to be mentioned. Hence, it is necessary to examine the present role of these diseases to reappraise the existing control measures with a view to improve them and attain better control strategies (Williams and Masiga, 1998; Feseha, 1998).

African horse sickness (AHS), also known as pernicious fever or typhoid fever, is a highly fatal, infectious disease of horses, mules and donkeys which is caused by African horse sickness virus. It is an endemic disease in equatorial, eastern and southern Africa from which it regularly spreads south and periodically north either along the Nile River or along the west coast of Africa. It appears to be truly an African disease (NVI, 1974; Mellor *et al*., 1998).

African horse sickness virus is a double stranded RNA virus, which causes a noncontagious, but infectious arthropod borne disease of equines and occasionally dogs. Nine distinct internationally recognized serotypes of the virus have so far been identified. All serotypes of African horse sickness virus occur in eastern and southern Africa, this distribution reflects that of zebra, which cycles the virus asymptomatically (Mellor *et al*., 1998). At present, African horse sickness virus (AHSV) is endemic in tropical and sub-tropical areas of Africa south of the Sahara occupying a broad band stretching from Senegal in the west to Ethiopia and Somalia in the east, Kenya and extending as far south as northern part of South Africa (Mellor and Hamblin, 2004).

In Egypt the disease occurred in 1928, 1943, 1953, 1958, and 1971 in which all outbreaks originated in the areas of Aswan and Qena Provinces, and the international boundaries between Egypt and Sudan (Coetzer *et al.*, 1994).

The hosts in order of decreasing severity of AHS are horses, mules, donkeys and zebras. Horses and mules have the highest mortality, donkeys have a lower mortality, and African donkeys have a subclinical infection. The horse is an amplifier of AHSV and source of virus for arthropods. Increased use of irrigation, water leaks, manure, urine, dung pats, tree holes, rotting vegetation, stagnant surface water are ideal larval habitats for the multiplication of *Culicoides* (Mellor *et al*., 1998).

African horse sickness was first recognized in Eritrea around the 16th century. In 1904, French travelers observed the disease in the lowlands of Ethiopia particularly around Baro and Awash valleys. At the end of 1935, there was production of vaccine in Asmara against AHS (A and B types) by the Animal Health Protection Research and Experiment group and the vaccine was found to be effective in mules and donkeys of Tigray and Eritrea. This vaccine was produced using guinea pigs (NVI, 1978). Between 1962-1968, 46 outbreaks of

African horse sickness were declared by OIE of which 27 occurred only in the year 1967. Among the outbreaks, 29 occurred in June, July, August and September of the years. These outbreaks occurred particularly in the Northern part including Tigray and Eritrea. In 1967, 269 sera were collected from horses of Sidamo and 12.8% were positive to AHS virus by CFT (NVI, 1983).

In 1974 virus isolation and serotyping of AHSV was carried out in the National Veterinary Institute of Debre Zeit. In this work, serotype 7 and 9 were isolated on vero cells from pieces of spleen. Isolates were typed with specific sera. Serotype 7 was isolated for the first time and this may necessitate NVI to produce a bivalent vaccine against AHSV in the years to come (NVI, 1978).

Table 1. Isolated serotypes of AHSV from different areas of Ethiopia.

		Host	Technique	
Month	Place		Serotyping	Serology
January	Awassa	Horse	9	-
February	Dire Dawa	Horse	9	-
June	Addis Ababa	Horse	9	-
June	Debre Zeit	Horse	9	-
June	Jimma	Horse	7	-
August	Jimma	Horse	9	-
September	Debre Zeit	Horse	9	-
November	Mekele	Mule	9	-
November	Mekele	Mule	9	-
November	Mekele	Mule	9	-
November	Mekele	Mule	9	-
December	Mekele	Mule	9	-
Summary	Debre Zeit	Horse	9	All serotypes except 2 and 8
	Jimma	Horse	7 and 9	All serotypes except 8

Source: National Veterinary Institute, Debre Zeit (1974)

A report issued by NVI in 1978, indicated that AHS is prevalent in eight out of the previous fourteen provinces of Ethiopia. Moreover, a paper released by the National Veterinary Institute asserts that out of the 9 serological types recognized in the world, type 9 has been isolated from samples received from Awassa, Dire Dawa and Mekele

suggesting that AHS is spread in lowlands and midlands of Ethiopia. In addition to this, some cases of the disease have been encountered in the highlands of the country including Addis Ababa and Arsi. This information may indicate the existence of AHS in almost all agro-ecological zones of Ethiopia (NVI, 1983).

An epidemiological study of AHS was conducted from 1977 to 1981 by the National Veterinary Institute and the study came out with isolation of virus using live animals including intracerebral inoculation of guinea pigs, mice, and vero cell culture (monkey kidney) (NVI, 1983).

The study also involved in an assessment of the seasonal occurrence of the disease and the results of the study indicated that most cases of the disease occurred in the rainy season including June, July, August and September, but also some cases of the disease occurred at the end of the rainy season including November (NVI, 1983).

Two research works were conducted in Wollo administrative region on the pathomorphological and hematological changes in affected equidae due to African horse sickness. The findings of the two research works indicated that AHS occurs every other year and it is very difficult to rear horses in this area. Furthermore, they have confirmed that both cardiac and pulmonary forms of the disease exist in the area (NVI, 1983).

The Virus Neutralization Test (VNT) indicated that two serotypes of AHSV were involved in the outbreak occurred in 2002-2003 in Southern Ethiopia (Awassa, Hossana, Wondogenet, and Hagereselam); Western Ethiopia (Jimma, Bedelle, Nekemte, Horroguduru, and Chaliya) and Central Ethiopia (Debre Zeit, Meki, Zeway, Filtimo, and Bekejo). Serotypes 9 and 6 were isolated from blood, spleen, and lymph nodes collected from 12 sick and dead animals. The identification of serotype 6 represents the first report in Ethiopia (Aschalew *et al., 2005*). The serotypes of AHSV are distinguished by differences in virulence, immunogenicity, and production of different disease patterns. Of the nine serotypes identified, type 9 is predominantly found throughout the African continent, and it is the only serotype previously identified in Ethiopia (Aschalew *et al.,* 2005).

The study conducted by Aschalew and others (2005) at the National Veterinary Institute of Ethiopia has shown the widespread occurrence of AHS across various agro-ecological zones of Ethiopia. In countries such as Ethiopia, where large population of equines are raised, the presence of multiple serotypes of such a devastating virus poses a serious hindrance to the national development was furtherly pointed out by Aschalew and others (2005).

According to the Ministry of Agriculture and Rural development, AHS is reported to occur in most regional states of Ethiopia (personal communication, 2005). Therefore, the present study is initiated to complement the previous findings in some of the study areas and pondered over the following objectives:

- to determine the seroprevalence of African horse sickness virus in different agro-ecological zones of the study areas,
- to undertake AHSV isolation and identification through active disease search in the study areas, and
- to identify the potential risk factors associated with African horse sickness.

2. LITERATURE REVIEW

2. 1. **The Epidemiology of AHS**

2.1.1. The disease AHS

African horse sickness is a peracute, acute, subacute or mild infectious but noncontagious disease of equines caused by an *orbivirus*, of which there are nine serotypes, all transmitted by *Culicoide* midges (Gallowally, 1974; Sewell and Brocklesby, 1990; Coetzer *et al.*, 1994). It is a list A disease according to the OIE classification of diseases. (Mellor *et al.*, 1998).

AHS is endemic in Africa including Ethiopia affecting equidae, which are of paramount importance to the livelihood of millions of farmers (Mellor and Hamblin, 2004). It is quite a serious disease in Ethiopia and has reappeared in Senegal after being unrecorded since 1965 (Hall, 1985).

Among the equidae family, horses are the most susceptible to AHS with a mortality rate of 50-95%, followed by mules with mortality around 50%. In enzootic regions of Africa, donkeys are very resistant to AHS and experience only subclinical infections. In Europeans and Asian countries, however, donkeys are moderately susceptible and have a mortality rate of 10%. Zebras are also markedly resistant with no clinical signs, and may have extended viraemia, up to 40 days (Galloway, 1985; Hunter, 1994; OIE, 2004).

The disease has both a seasonal (late summer/autumn) and cyclical incidence with major epizootics in Southern Africa during warm phase events. But in other areas it may occur regularly if conditions are suitable for midges to be active all the year round. Mortality due to AHS is related to the types of equidae affected and to the strain or serotype of the virus involved (Galloway, 1985; Hunter, 1994; OIE, 2004). Where the disease is enzootic, outbreaks often occur at intervals of 10 to 20 years, and this is considered to be due to the periodic fluctuation/elevation in immunity, the yearly seasonal variation in vector population and periodic redistribution of new or mutated strains of the virus (Hall, 1995; Radostits *et al*., 2000). From time to time, AHS has spread beyond its usual distribution, causing devastating epizootics in many countries, including the Middle East, Pakistan, Afghanistan, India, North Africa, and the Iberian Peninsula, due to importation of infected

zebras into Spain in 1987 (Galloway, 1974; Anon, 1976; Della-Porta, 1985; Knipe and Howley, 2001).

African horse sickness epidemics do not occur in the condition of heavy rain followed by drought. However, the reversed pattern, drought followed by heavy rain favours epidemics. The reasons why this pattern of drought followed by heavy rain is conducive to AHS are unclear though these authors suggested that it might have some thing to do with the congregation of zebra near the remaining waterholes during the drought period where they come into contact with and infect more vectors which then disperse rapidly once rain provides more breeding sites (Mellor and Hamblin, 2004).

2.1.2. Agent

African horse sickness virus is considered enzootic only in sub-saharan Africa, but on several occasions it has caused devastating outbreaks in horses outside Africa. Since world war-II, major epizootics have occurred in the Middle East and Indian sub-continent in 1959-1961 and in North Africa and Southern Spain in 1965-1966 (Fenner *et al*., 1987; Sewell and Brocklesby, 1990).

African horse sickness is caused by a double stranded RNA virus. The virions are 70-80 nm in diameter and have an icosahedral symmetry. They contain 10 double-stranded RNA genome segments encapsulated within a double layered capsid made up of 32 large ring shaped capsomeres, each comprising 7 structural proteins and 4 nonstructural proteins (Joklik, 1988; Seifert, 1996; Anon, 1998; OIE, 2004).

African horse sickness (peste equina africana, peste equine, pestis equorum) virus has similar morphological and biochemical properties with the other members of *orbiviruses* but with distinctive pathological and antigenic properties as well as host ranges. The genome of African horse sickness virus encodes several structural proteins (VP1-7), most of which have been completely sequenced for AHSV serotypes 4, 6 and 9 and four nonstructural proteins (NS1, NS2, NS3, NS3A). Proteins VP2 and VP5 form the outer capsid of the virion, and proteins VP3 and VP7 are the major inner capsid proteins. Proteins VP1, VP4 and VP6 constitute minor inner capsid proteins. Recently, it was indicated that NS3 proteins are the second most variable AHSV proteins, the most variable

being the major outer capsid protein, VP2. This protein, VP2, is also mainly responsible for AHSV serotypes and, together with VP5, takes part in virus neutralization activity. Nine antigenically distinct serotypes of AHSV have been identified by virus neutralization, but no cross-reactions with other known *orbiviruses* have been observed (Hungerford, 1975; Della-Porta, 1985; OIE, 2004).

AHSV serotypes numbering up to 42 are suspected, of which the last was isolated in 1960. Eventhough nine AHS viruses were recognized in South Africa, additional viruses are suspected to occur elsewhere in Africa. Antisera which neutralize homologous virus may cross-neutralize heterologous serotypes. There is cross-neutralization between serotypes 1 and 2, 3 and 7, 5 and 8, and 6 and 9. However, field evidence indicates that no significant intratypic variation occurs (Galloway, 1974; Radostits *et al*., 2000; Seifert, 1996; OIE, 2004). There is no significant serologic or genetic relationship between AHSV and other *orbiviruses* of veterinary importance (Fenner *et al.,* 1999; Murphy *et al*., 1999).

.

The virus is relatively impervious to heat; it does not become inactivated after exposure to temperatures of 55-75^{O}C for 10 minutes and persists for 3 months at 4^{O}C, but at -25^{O}C a marked reduction in virus titre occurs if the virus is not diluted in a stabilizer. Minimal loss of titre occurs in lyophilized vaccine kept at 4^{O}C. Putrid blood remains infective for more than 2 years. The optimal pH for virus survival is 7.0-8.5 (Galloway, 1985; Seifert, 1996). The virus is moderately resistant to external environmental influences such as drying and heating (Radostits *et al*., 2000; Anon, 1998).

A high percentage of sera (79.8%) were positive, confirming the continued prevalence of AHSV antibodies in Nigerian horses and donkeys (Adeyefa and Hamblin, 1995). In the same country 75% of donkeys, 68% of horses, 19% of camels and 9% of dogs were found to have antibodies against AHSV (Baba *et al*., 1992).

AHSV can be inactivated by repeated freezing and thawing, and by treatment with acetic acid (inactivated at a pH of 6.3 or lower), remaining for 2 weeks at 37^{0}C, and being placed for 5 minutes at 70^{0}C (Mellor *et al.,* 1998). In OCG (5g of potassium oxalate, 5g of carbolic acid (phenol), 500ml of water, and 500ml of glycerin), AHSV is stable for more than 20 years at 4^{0}C (Mellor *et al*., 1998). Acetic acid (2%), iodophore disinfectants, and chlorine dioxide disinfectants are all killers of AHSV (Mellor *et al*., 1998).

Serotypes 1-3 and 5-8 are confined mainly to Africa but serotypes 4 and 9 had occurred outside Africa (Mellor *et al*., 1998). The virus is present in the body from the advent of fever to the time of complete recovery (Hall, 1985).

2.1.3. Vector

Species of *Culicoides* have been conclusively identified as the major and biological vectors because AHSV can replicate within them. In the United States, *Culicoides* that can transmit blue tongue virus can most likely transmit AHSV. *Culicoides* are most active at sunset and at about sunrise. Arthropods other than the *Culicoides* (e.g., biting flies and mosquitoes) may spread the virus as mechanical vectors (Mellor *et al.*, 1998, Knowles, 1991). At least two field vectors are involved: *Culicoides imicola* and *Culicoides bolitonos* (Galloway, 1985; Hunter, 1994; OIE, 2004).

The spread of the disease is influenced by climatic conditions which favour the spread of carrier insects (vectors) including warm, moist weather and high rainfall, as well as spread by wind dispersal. It is likely that the virus persists (overwinters) in other unknown species in Africa when the insect is not active. This explains why the disease does not persist in other countries following an outbreak (Mellor *et al*., 1998).

The disease is spread by the passive transfer of very small quantities of blood by biting insects, and spread does not occur between animals in direct contact unless the prerequisite insect vectors are present. Climatic conditions which govern the breeding status and movement of these insects also govern the spread and morbidity rate of horse sickness. These insects have almost worldwide distribution so that spread of the disease could be universal. Many other biting insects have been named as vectors but there is lack of satisfactory evidence in many reports (Radostits *et al*., 2000; Anon, 1998). AHS occurs during rainy warm seasons, which favour propagation of the vectors (Radostits *et al*., 2000; Farouk, 1997).

An entomological survey was conducted with the objective of identifying the types of *Culicoides* species prevailing in Ethiopia. Samples were collected from water meadows near riverine woodland in all months of May to November. The efficiency of catching *Culicoides* was poor, due partly to their smallness and darkness and also because the

weight of their numbers was sometimes overwhelming. *C. grahamii Austen* is a day biting type with peaks shortly after dawn and before dusk; biting intensely during and after showers at any time of the day. It is widely spread around the Rift Valley lakes; particularly prolific at sites of geothermal springs and marshes beside Lake Shala and Lake Abaya in the months of June to August (White, 1997).

C. milnei Austen is a night biting type and frequently abundant in animal shelters, resting by day and biting at night. It is greatly abundant from May to November throughout Shoa, parts of Arsi, Kaffa, Wollo, Gojjam and Gondar areas where main roads permit perennial access (altitudes of 1,550-2,100m.). If it were genetically susceptible to infection with relevant arboviruses, however, *milnei* would obviously rate as an outstanding vector of veterinary disease throughout much of the Ethiopian highlands, where its abundance in association with sheep, cattle and horses far exceeds the numerical densities encountered in the Kenyan highlands (White, 1997).

2.1.4. Host

African horse sickness differentially affects members of the equidae family; horses are the most susceptible, and donkeys and mules show mild symptoms (Galloway, 1974; Anon, 1976; Della-Porta, 1985; Knipe and Howley, 2001). African horse sickness causes diseases in horses, mules and donkeys, with up to 95% mortality among susceptible horse population (Anon, 1976; Robinson, 1987). Dogs fed infected raw horse meat have shown signs similar to those observed in horses. Dogs may also become transient virus carriers (Anon, 1976; Knowles, 1991). The host range of AHSV includes zebra, but clinical disease in this species is unusual. The virus persists in zebra longer than in horses, suggesting that it may be the original reservoir host (Fenner *et al*., 1987; Sewell Brocklesby, 1990).

The virus was isolated from blood of clinically healthy street dogs. Insectborne infection probably never occurs in dogs, because insects are less attracted. Dogs, goats, mice, guinea pigs and rats can be infected experimentally. Elephants demonstrate susceptibility by seroconverting when exposed to infection unlike those of rabbits, camels, sheep and goats (Radostits *et al*., 2000; Farouk, 1997).

In enzootic areas the morbidity rate of susceptible equines varies with the number of insect vectors present. Mules and donkeys do not suffer from high mortality, but the disease is a crippling one in these equines because of gross debility (Radostits *et al*., 2000; Anon, 1998).

After natural infection or vaccination by a homologous strain, immunity is solid but can be overcome by strong challenge by another strain. The development of immunity is slow and may require 3 weeks to be appreciable; titers may continue to rise for 6 months after vaccination. Foals from vaccinated dams appear to derive passive immunity from the colostrum and are immuned until 5-6 months of age (Hall, 1985; Radostits *et al*., 2000; Anon, 1998).

2.1.5. Reservoir host

There were extensive epizootics of AHS in North Africa, the Middle East, and the Indian subcontinent, the disease has always died out there. Possible explanations include the absence of a suitable reservoir mammalian host such as the zebra or lack of persistence of a vector temporarily introduced into these areas (Fenner *et al*., 1987). In tropical countries where there is no cold season it is believed that a reservoir host may not be necessary because the disease is not so strictly seasonal, the virus is being transmitted via the vector from horse to horse continuously (Della-Porta, 1985; Hall, 1985). Nevertheless, *Culicoides* is not considered to be the reservoir which retains the virus between the seasonal appearances of the disease in horses, as the disease can disappear in areas where *Culicoides* abounds. If there is a separate inter-season host reservoir, its density has not yet been established (Hall, 1985). Although clinically affected equidae are the major source of virus during an outbreak, the current view is that in enzootic areas there must be a silent, non-equine reservoir host, which perpetuates the virus between seasons when no insects are present. Dogs, elephants and zebra have all been proposed as reservoir hosts. In some countries the disease has been introduced but has died out in the succeeding winter, presumably because no reservoir hosts were available (Radostits *et al*., 2000; Anon, 1998). In enzootic areas of Africa, there is strong evidence that a non-equine host is a reservoir for survival of the virus between seasonal attacks. (Knowles, 1991; Radostits *et al*., 2000).

There is no confirmed information about a reservoir of infection; but animals recovered from the disease do not remain carriers of the virus. There may, however, be a continuous transmission cycle of AHSV in tropical areas of Africa between *Culicoide* midges and wild or domestic equine species or other wild reservoir hosts (Seifert, 1996; Anon, 1998).

2.1.6. Environment

The ecology of AHS is not properly understood (Radostits *et al*., 2000). *Culicoides* involved in transmission is shown by the fact that, in countries where cold winters occur no new outbreaks have been recorded after 10 days of frost because the insect vectors are killed in the cool conditions. Also, extensive outbreaks are always preceded by heavy rain (Della-Porta, 1985; Hall, 1985).

Consideration must also be given to the wind in carrying the insect vector to new areas. Thus, the disease is carried from one country to another even over long distances. English workers have reported that it has been transmitted from Morocco to Spain and from Senegal to Cape Verde by this means (Hall, 1985; Hunter, 1994). Because of the nature of *Culicoides*, enzootic areas are more likely to be in low-lying, warm, marshy regions (Hall, 1995; Radostits *et al*., 2000).

AHS infection rates of vector *Culicoides* and rates of virogenesis within them are temperature dependent. As temperature increases infection rates also tend to increase. Virogenesis is faster and transmission can occur sooner. However, midge survival rates decrease. Conversely, as temperature is reduced the reverse is true for each of these variables. The likelihood of transmission is therefore a function of the interaction of these two opposing sets of trends (Mellor and Hamblin, 2004).

2. 2. Experimental production

Experimental and natural transmission of AHS to dogs has been reported through ingestion of infected raw horse meat. However, there is only limited and unsubstantiated evidence that dogs can be infected by insect bites (Anon, 1998; OIE, 2004). Artificially, the disease is readily transmitted by the intravenous injection of very small amounts of blood. Transmission can also be effected by subcutaneous injection or oral dosing, but larger amounts of blood are required, particularly with oral dosing (Anon, 1976; Radostits *et al*.,

2000). The disease was transmitted experimentally to a horse by the bites of *Aedes aegypti* mosquitoes which had been fed a virus suspension (Galloway, 1985; Anon, 1998).

2. 3. Economic importance

In a fully susceptible horse population, the effect of African horse sickness can be devastating, because up to 95% mortality can be expected. For instance, the cost of the Newzealand horse industries of an outbreak of AHS would be expected to exceed substantially the cost of an outbreak of equine influenza which has been estimated at nearly 173 million USD. The presence of AHS in a country would cause a major disruption to the export of horses (MAF, 1991).

The serious nature of the disease for equines is compounded by the tremendous problem of eradication. Vaccination reduces the ravages of horse sickness, but even when practiced on a wide scale cannot eradicate the disease because the infection is insectborne, and uncontrolled hosts provide a reservoir of infection (Radostits *et al*., 2000).

2. 4. Human disease

Very rarely, African horse sickness can be zoonotic. The first evidence of this came when laboratory workers, exposed to the virus during vaccine manufacture, developed encephalitis, chorioretinits, and disseminated intravascular coagulation (Anon, 1998; Murphy *et al*., 1999). There is no evidence that humans can become infected with any field strain of viscerotropic AHSV, either through contact with naturally or experimentally infected animals or by virus manipulation in laboratories. However, certain neurotropic vaccine strains that may cause encephalitis and retinitis in humans following transnasal infections have been described (Anon, 1998; OIE, 2004).

2. 5. Pathogenesis

The outcome of infection in horses, including the incubation period and severity of disease, depends largely on the virulence of the virus and susceptibility of the animal. The incubation period varies between 3-10 days (Coetzer *et al.*, 1994; Seifert, 1996).

The factors determining the course and severity of infection are not fully understood (Seifert, 1996). After infection, initial multiplication of virus occurs in the regional lymph nodes and is followed by primary viraemia with subsequent infection of target organs, namely the lungs and lymphoid tissues throughout the body. Virus multiplication at these sites gives rise to a secondary viraema of variable duration; in horses it is generally not higher than 10^5 $TCID_{50}$/ml and lasts 4 to 8 days but does not exceed 21 days, whereas in donkeys and zebras the levels of viraema are lower but they may last as long as 4 weeks. In zebras, viraemia has been reported to occur in the presence of circulating antibodies. The virus is closely associated with the erythrocytes in the blood (Coetzer *et al*., 1994; Hunter, 1994; Seifert, 1996).

In experimentally infected horses, high concentration of virus is found in the spleen, lungs, caecum, pharynx, choroid plexus and most lymph nodes by the second day after inoculation. This precedes the onset of fever or detectable viraemia. By the third day after virus inoculation, it is present in most organs. Even in the “dikkop” or “cardiac” form of AHS, virus levels in the myocardium is no longer than in the blood, indicating that the myocardium is not a primary site of virus replication. High concentration of virus in lymphoid tissues may possibly be responsible for the depletion of lymphocytes which occurs during the course of the disease. However, transmission electron-microscopic studies have not revealed the presence of virus in lymphocytes (Anon, 1976; Coetzer *et al*., 1994).

Effusions into body cavities and oedematous changes of various tissues (particularly of the lungs), as well as serosal and visceral haemorrhages, are often evident in fatal cases of AHS, and indicate endothelial cell damage. Although no significant ultrastructural changes or evidence of viral replication could be detected in endothelial cells in the lungs in one study, the presence of virus and ultrastructural changes in, and separation of, endothelial cells in the lungs were found in another (Pagot, 1992; Coetzer *et al*., 1994).

The development of the various forms of the disease depends on the envelope chemistry of the individual strain of virus concerned and the chemistry dictates the tissue to which the serotype will be directed (Radostits *et al*., 2000; Anon, 1998).

The virus is present in the blood stream from the first day of clinical illness and persists for about 30 days and even up to 90 days. It can be recovered from defibrinated blood by intracerebral inoculation into infant mice (Radostits *et al*., 2000; Anon, 1998).

2. 6. Clinical signs

Small plaque variants of AHSV produce severe clinical reactions, while large plaque variants produce no reactions or only mild ones. Subsequent observations indicated that the rigid distinction between different forms of AHS is not fully justifiable and that most cases of AHS are of the ‘’mixed’’ form. Although more than one serotype may be active during an outbreak, isolation of more than one serotype from a naturally infected animal has never been recorded (Coetzer *et al*., 1994; Anon, 1998).

According to Coetzer *et al*. (1994) and Radostits *et al*. (2000) there are four forms of AHS in horses and these are still useful in categorizing the different effects AHSV may have on equine animals. The four forms are listed below:

1. the peracute, “pulmonary” or “dunkop” form= “thin head”, i.e. subcutaneous swelling of the head is absent;
2. the acute or “mixed” form;
3. the subacute, oedematous, “cardiac” or “dikkop” form= “thick head” or swollen head and
4. the horse sickness fever (abortive) form.

2.6.1. “Dunkop” form

This form of the disease occurs most commonly when AHSV infects fully susceptible horses, notably foals that have lost their passively acquired maternal immunity; and when equines are infected with virulent strains of virus. It is also the usual form in dogs (Coetzer *et al*., 1994; OIE, 2004).

This form of the disease has a short incubation period (3-5 days), is characterized by very marked severe dyspnoea, and progressive respiratory involvement. An acute febrile reaction, lasting 1-2 days and reaching a maximum of approximately 40-41^{O}C may be the only sign. This is followed by various degrees of respiratory distress. The respiratory rate may increase to 60 or even to 75 breaths/minute. The animal may be observed to stand with its fore legs spread apart, its head extended and its nostrils fully dilated. Profuse sweating is common and spasmodic coughing may be observed terminally, with frothy serofibrinous fluid exuding from the nostrils. Death usually occurs with in a few hours after the first clinical signs are observed, the animal having literally drowned in its own serous fluid. Recovery from this form is very rare, occurring in less than 5% of cases (Radostits *et al*., 2000; Hunter, 1996; OIE, 2004).

2.6.2. "Mixed" form

This form represents a mixture of the pulmonary and cardiac forms. Although seldom diagnosed clinically, it is the most common form and is seen at postmortem examination in most fatal cases of AHS in horses and mules. The incubation period varies from 5-7 days, and the disease may manifest itself in the following ways (Galloway, 1974; Hunter, 1996; OIE, 2004):

- Initial pulmonary signs of a relatively mild degree are followed by marked oedemaotous swellings of the head and neck, with death resulting from heart failure,
- Oedematous swelling, typical of the subacute form, is followed by sudden onset of dyspnoea and other clinical signs typical of the peracute pulmonary form.

The mortality rate in the mixed form is greater than 80% and death usually follows within 3-6 days after the onset of febrile reaction (OIE, 2004).

2.6.3. "Dikkop" form

The incubation period of this form varies from about 7-14 days, and the onset of clinical disease is marked by a febrile reaction (39-41^{O}C) that lasts for 3-6 days. Shortly before the decline of fever, characteristic oedematous swellings may appear. These initially involve the temporal or supraorbital fossae and the eyelids, and later extend to the lips, cheeks, tongue, intermandibular space and laryngeal region. Subcutaneous oedema sometimes

extends a variable distance down the neck towards the chest and, in severe cases, may involve the chest and shoulders, but generally not the lower limbs. Terminally, petechial haemorrhages may be observed in the conjunctivae and under the ventral surface of the tongue. The animal finally becomes restless and may show signs of colic before death from cardiac failure. Difficulty in swallowing due to paralysis of the oesophagus is also seen. The mortality rate is about 50% and death usually occurs within 4-8 days after the onset of febrile reaction. In recovering cases, swelling gradually subsides within a period of 3-8 days. This clinical form of AHS is usually associated with infection by virus strains of low virulence or is encountered in vaccinated animals infected by heterologous virus strains, or may be a function of biological variation in the infected animal (Coetzer *et al*., 1994; Hunter, 1996; OIE, 2004).

2.6.4. Horse sickness fever (abortive form)

Horse sickness fever is the mildest form and is frequently overlooked in natural outbreaks. The incubation period varies from 5-14 days, and is followed by a febrile reaction (39-40^{O}C) of the remittent type, with morning remissions and afternoon exacerbations, lasting for 5-8 days. Apart from the febrile reaction, other clinical signs are rare. The conjunctivae may be slightly congested, the pulse rate may be increased, and a certain degree of anorexia and depression may be present. This form of the disease is usually observed in partially immuned animals or in resistant species, such as the donkey and zebra (Sewell and Brocklesby, 1990; Hunter, 1996; OIE, 2004).

2.7. Pathology

The pathological features are most prominent in the pulmonary and cardiac forms. The most striking features of the pulmonary form are severe oedema of the lungs and hydrothorax. Several liters of pale yellow fluid which may coagulate on exposure to air are found in the thoracic cavity. Epicardial and endocardial haemorrhages of the endocardium may be evident. Congestion of the mucosa of the stomach and patchy congestion and petechiation of the serosa and sometimes of the mucosa of the intestine appear. The liver may be slightly enlarged and congested; there is usually some degree of ascites (Radostits *et al*., 2000; Seifert, 1996).

The most characteristic signs of the cardiac form are the distinctly yellowish gelatinous oedema of the subcutaneous and intramuscular connective tissues of the head and neck, which in severe cases extends to the back, shoulders and chest. The eyelids, supraorbital fossae, lips, cheeks, tongue and intermandibular space are commonly involved. The tongue may be severely swollen, cyanotic and have mucosal petechiae on its ventral surface. Lesions in the heart are more severe than described, for the pulmonary form and accompanied by severe hydropericardium. Similarly, the lesions in the gastrointestinal tract are usually more severe than in the pulmonary form (Seifert, 1996; Anon, 1998).

There are few published reports on the histopathology of AHS. However, exudative pneumonia, congestion of alveolar capillaries, arterioles and venules as well as perivasculitis are evident. Degeneration and necrosis of myocytes, oedema of the myocardium with infiltration of mononuclear cells, plasma cells, siderocytes and polymorphonuclear leukocytes as well as lysis of necrotic myocytes appear (Coetzer *et al*., 1994; Seifert, 1996).

2. 8. Diagnosis

Wherever AHS is endemic the epidemiology, clinical signs and macroscopic lesions are sufficiently specific to allow its diagnosis. The chronic form is more difficult to diagnose (OIE, 2004).

2.8.1. Identification of the agent

Several techniques are already available for AHSV identification ranging from the rapid Enzyme Linked Immunosorbent Assay (ELISA), using either polyclonal antibodies (PAbs) or monoclonal antibodies (MAbs), to the polymerase chain reaction (PCR) test, including a new reverse-transcription (RT) PCR for discrimination of the nine AHSV serotypes, or cell culture and inoculation of newborn mice. If possible more than one test should be performed to diagnose an outbreak of AHS, especially the index case. The initial test can be a quick test such as ELISA or PCR, followed by virus isolation in tissue culture. Virus neutralization (VN) for serotype identification should be performed as early in the outbreak as possible so that the correct vaccine can be selected. Subsequently, the ELISA may be very useful in laboratory diagnosis (OIE, 2004).

At present, there are no international standards for viruses or diagnostic reagents, and there is no standard methodology for the determination of AHSV. However, a panel of viruses has been evaluated, and comparative studies between different ELISAs for AHSV antigen determination have been carried out in different laboratories. The results have demonstrated a high level of correlation for antigen detection using the indirect sandwich ELISAs for antigen studies (Seifert, 1996; OIE, 2004). A very important aspect of the diagnosis is the selection of samples and their transportation to the laboratory (OIE, 2004).

2.8.1.1. Samples for virus isolation

Unclotted whole blood collected during the early febrile stage of the disease from sick animals, as well as small pieces (2-4g) of spleen, lung and lymph nodes from animals that have recently died, are the samples of choice for diagnosis. Samples should be kept at $4^{O}C$ during transportation and storage (OIE, 2004).

2.8.1.2. Cell culture

According to OIE (2004) direct isolation of AHSV on baby hamster kidney (BHK-21), monkey stable (MS) and African green monkey kidney (Vero) cell lines have been used successfully. Blood samples collected in heparin can be used undiluted. After 60 minutes of adsorption, the cell cultures are washed and maintenance medium is added. Alternatively and more usually, the blood is washed, lysed and diluted 1/10. This procedure removes unwanted antibody, which could neutralize free virus, and promotes release of virus associated with the red blood cell membranes. When tissue samples, such as spleen, lung etc., are used, a 10% tissue suspension is prepared in phosphate buffered saline (PBS) or cell culture medium, containing antibiotics. A cytopathic effect (CPE) may appear between 2 and 8 days post infection. Three blind passages should be performed before considering the samples to be negative.

2.8 1.3. Newborn mice inoculation

This method of isolation of AHSV involves the intracerebral inoculation of two families of 1-3 day-old mice. In positive cases, animals develop nervous signs between 3 and 15 days post-inoculation. The brains from sick animals must be collected, homogenised and re-inoculated intracerebrally into at least six 1-3 day-old mice. This second passage should present a shortened incubation period (2-5 days) and 100% infectivity (Coetzer *et al*., 1994; OIE, 2004).

2.8.1.4. Polymerase chain reaction (PCR)

A number of PCR-based assays for the specific detection of AHSV RNA have been developed. Primers correspond to the 5' end (nucleotides 1-21) and 3' end (nucleotides 1160-1179) of RNA segment 8 have been developed (Coetzer *et al*., 1994; OIE 2004).

Extraction of nucleic acids from spleen samples is carried out as follows: 1g of tissue sample is homogenize in 1ml of denaturing solution (4M guanidium thiocyanate, 25mM sodium citrate, 0.1M 2-mercaptoethanol, 0.5% sarcosyl). After centrifugation, 1µg of yeast RNA, 0.1ml of 2M sodium acetate pH 4, 1ml of phenol and 0.2ml of chloroform/isoamyl alcohol mixture (49/1) are added to the supernatant. The suspension is vigorously shaken and cooled on ice for 15 minutes. After centrifugation, the RNA present in the acqueous phase is phenol extracted, ethanol precipitated and resuspended in sterile water. The methods for cDNA synthesis and PCR amplification are performed using, in all cases, 37^0C as renaturing temperature. The sequences of the PCR primers used are 5'-GTT –AAA –ATT –CGG –TTA –GGA –TG –3', which corresponds to the messenger RNA polarity and 5'-GTA-AGT-GTA-TTC-GGT-ATT-G-3', which is complementary to the messenger RNA polarity. The PCR procedure itself involves 40 cycles (94 ^{0}C for 1 minute, 55^0C for 1.5 minutes, 72^0C for 2.5 minutes and 70^0C for 7 minutes) and then the PCR tubes are kept at 4^0C. Analysis of the PCR products is carried out by electrophoresis in 1.2% (w/v) agarose gels containing ethidium bromide. AHS positive samples will resolve in an 1179 base-pair band (OIE, 2004).

A new RT-PCR for discrimination of the nine AHSV serotypes has been described. Nine pairs of primers were designed for each specific serotype. The results obtained show a

perfect agreement between the RT-PCR and the VNT. Typing of the nine AHSV serotypes has also been carried out with probes developed from a set of cloned full-length VP2 genes and can be an alternative to amplification of genome segment (OIE, 2004).

2.8.1.5. New approaches

Genomic probes can also be developed and applied for *in-situ* hybridization in tissues. Immunohistochemical staining methods have also been used successfully to determine the localization of AHS antigen within various tissues. The advantages of these new approaches are that, they have the potential to be rapid, sensitive and versatile, and they may supplement or replace some of the older conventional methods. Furthermore, they can be applied to specimens from clinical cases that do not contain live virus (Coetzer *et al*., 1994).

2.8.2. Serological test

There is an international reference sera developed to standardize the ELISA, which is an OIE prescribed test. In addition, a panel of reference antisera has been evaluated and comparative studies between different ELISAs using MAbs and PAbs, and involving several laboratories have been carried out. The results have demonstrated a high level of correlation using indirect or competitive ELISAs for antibody detection. The competitive ELISA (C-ELISA) can also be used for testing wild life, as species-specific anti-globulin is not required with this method (OIE, 2004). VNT is the method of choice for serotyping (OIE, 2004; Mellor and Hamblin, 2004).

The indirect sandwich ELISA is an extremely useful method for the rapid identification of AHSV antigen in solid tissues taken from animals that have died following an acute infection (Mellor and Hamblin, 2004; OIE, 2004). Sandwich ELISA's that detect viral antigen in mammalian and insect tissue homogenates as well as in cell culture supernatant fluid have been reported to be used in the diagnosis of AHS (Coetzer *et al*., 1994).

2. 9. Differential diagnosis

The clinical signs and lesions reported for AHS can be confused with those caused by closely related *Orbivirus*, equine encephalosis virus (EEV). Fortunately, rapid, sensitive and specific ELISA's are available to enable the detection of the antigen and antibody of both AHSV and EEV. The haemorrhages and oedema reported in cases of purpura haemorrhagica and equine viral artritis may be similar to those seen in the pulmonary form of AHS, although with AHS the oedema tends to be less extensive and the haemorrhages are less numerous and widespread. The early stages of babesiosis (*B. equi* and *B.caballi*) can be confused with AHS, particularly when the parasites are difficult to demonstrate in blood smears (Mellor and Hamblin, 2004).

2. 10. Treatment

Apart from supportive treatment, there is no specific therapy for AHS. Affected animals should be carefully nursed, well fed and given rest as the slightest exertion may result in death. During the recovery process they should be rested for at least 4 weeks before being returned to light work. As babesiosis may be a complication of AHS, blood smears as well as the body temperature (to detect a secondary febrile reaction) should be taken regularly and, if found positive, animals should be appropriately treated. Careful massaging of the oesophagus in those cases in which it is paralyzed may result in a gradual improvement of the condition (Hall, 1985; Coetzer *et al*., 1994; Hunter, 1994).

2. 11. Prevention and control

Polyvalent and, to a limited extent, monovalent vaccines are used for immunization. A polyvalent, formalin-inactivated spleen tissue (viscerotropic) vaccine gives immunity for 1 year. A second vaccine is a mouse-brain attenuated live virus (neurotropic) vaccine that has been passaged in mice by intracerebral inoculation, which is also polyvalent. Annual vaccination is advocated to obtain true polyvalent immunity. It has been observed that the use of polyvalent vaccine rarely results in polyvalent immunity after the 3rd or 4th annual vaccination. Prophylactic immunization against AHS is a very efficient method of preventing serious losses (Coetzer *et al*., 1994).

2.11.1. Endemic Areas

2.11.1.1. Vaccination

Annual vaccination in late winter or early summer (September to November) which is sometime before the peak of AHS season with a live polyvalent attenuated vaccine is mandatory and allows immunized animals to respond adequately to the vaccine before possibly being challenged by natural exposure. Vaccinated animals should not be exercised for three weeks following vaccination. Foals should be vaccinated at six months of age and there after annually (Galloway, 1974; Della-Porta, 1985; Pagot, 1992; Hunter, 1994; Coetzer *et al*., 1994; Seifert, 1996).

Horses that have received three or more courses of immunization are usually well protected against the disease. Generally immunization has no, or only limited side effects. A slight temperature response may ensue between 5 and 13 days after inoculation as a result of low level virus replication in immunized animals. Cases of fatal encephalitis, characterized by blindness and neurological disorders, in fully susceptible horses and donkeys 6 to 8 weeks after initial vaccination with neurotropic mouse strains of virus has occasionally been reported (Coetzer *et al*., 1994; Seifert, 1996).

Cross-immunity between serotypes may be enhanced by repeated inoculation of the same virus. There is evidence that animals recovered from infection with a field virus require a broader cross-immunity to the other serotypes than that which is obtained from immunization. Animals should be vaccinated twice in their first and second years of life and thereafter only once annually to avoid unresponsiveness (Coetzer *et al*., 1994). Through application of recombinant DNA technology, subunit vaccines are expected to become available for AHSV within the foreseable future (Seifert, 1996).

2.11.1.2. Vector Control

Infection of susceptible horses can be prevented to a large degree by stabling them some hours before sunset and letting them out a few hours after sunrise as *Culicoides s*pecies are nocturnal and are not inclined to enter buildings (Coetzer *et al.,* 1994; Seifert, 1996).

2.11.2. Non-endemic Areas

Following an outbreak of AHS in a country that has been free of it, much more stringent control measures must be taken which involve quarantine, slaughtering of viraemic animals, vaccination, stabling and controlling *Culicoides (*Coetzer *et al*, 1994).

3. MATERIALS AND METHODS

3.1. Description and physiography of the study areas

The study was conducted in selected sites of Addis Ababa city administration and its surroundings as well as Amhara and Oromia regional states of Ethiopia. In total 4 zonal administrations, 13 districts and 19 peasant associations were included in the study. Specific study sites encompassed Addis Ketema from Addis Ababa region; Dessie Zuria, Kalu and Kombolcha of South wollo; Debrebrihan and Basona Werana of North shoa from Amhara region; Adama, Dugda Bora, Akaki, Ada'a Liben, Berehna Aleltu and Boset of East shoa from Oromia region. These study areas are classified into three agro-ecological zones based on temperature and length of plant growing period (LGP) (EARO, 1998). Keith Powell's (2005) adopted classification that categorizes areas with elevations of 1500 meters above sea level (m.a.s.l.) or less was endorsed as lowland, between 1500-2500 m.a.s.l. as midland and over 2500 m.a.s.l. as highland.

The altitudinal range of the study areas is from 1413 (Boset) to 2812 (Addis Ababa) meters above sea level. The rainfall in the study areas is bimodal in disribution and falls in the range of 900 to 1000 mm per annum. There are long and short rainy seasons extending from June to September and February to March, respectively. The minimum, maximum and average air temperature of the study areas are 16^0C, 27.5^0C and 21.75^0C respectively. The mean relative humidity of the study areas is in the range of 35-68 %. Study areas such as Ada'a Liben, Adama, Boset and Dugda Borra are near to watering bodies such as Awash river, Koka dam, irrigation canals, Zeway lake and different lakes of Ada'a. Ada'a is near but others are within the belt of Rift Valley (see figure 1).

3.2. Geo-reference (GPS database)

Table 2. Measured Geo-reference database of the study areas.

No.	Study sites	Elevation (m.a.s.l.)	Latitude (N)	Longitude (E)
1	D/B kebele 8	2678	09^0 01’ 232’’	038^0 48’ 177’’
2	D/B kebele 4	2641	09^0 01’ 232’’	038^0 48’ 177’’
3	Tulamba	2694	09^0 01’ 232’’	038^0 48’ 177’’
4	Chollie	2501	09^0 01’ 232’’	038^0 48’ 177’’
5	S.chollie	2495	09^0 01’ 232’’	038^0 48’ 177’’
6	Legebollo	2465	09^0 01’ 232’’	038^0 48 ’177’’
7	Addis Ababa	2812	09^0 01’ 232’’	038^0 48’ 177’’
8	Akaki	2025	09^0 01’ 232’’	038^0 48’ 177’’
9	Sidamawash	2064	09^0 01’ 232’’	038^0 48’ 177’’
10	Gogiecha	1956	09^0 01’ 232’’	038^0 48’ 177’’
11	Ada’a	1856	09^0 01’ 232’’	038^0 48’ 177’’
12	Gerado	2321	11^0 09’ 810’’	039^0 61’ 740’’
13	Kombolcha	1818	11^0 07’ 090’’	039^0 73’ 851’’
14	Kalu	1837	11^0 08’ 475’’	039^0 73’ 741’’
15	Adama	1566	09^0 01’ 232’’	038^0 48’ 177’’
16	Boset	1413	09^0 01’ 232’’	038^0 48’ 177’’
17	Godino	1922	08^0 45’ 681’’	039^0 00’ 143’’
18	Guaworko	2025	08^0 51’ 245’’	039^0 01’ 329’’
19	Gende gorba	1963	08^0 51’ 856’’	039^0 00’ 574’’
20	Meki	1600	$08^0$1 2’ 887’’	038^0 52’ 488’’
21	Alemtena	1672	08^0 09’ 472’’	038^0 05’ 248’’

D/B = Debrebrihan

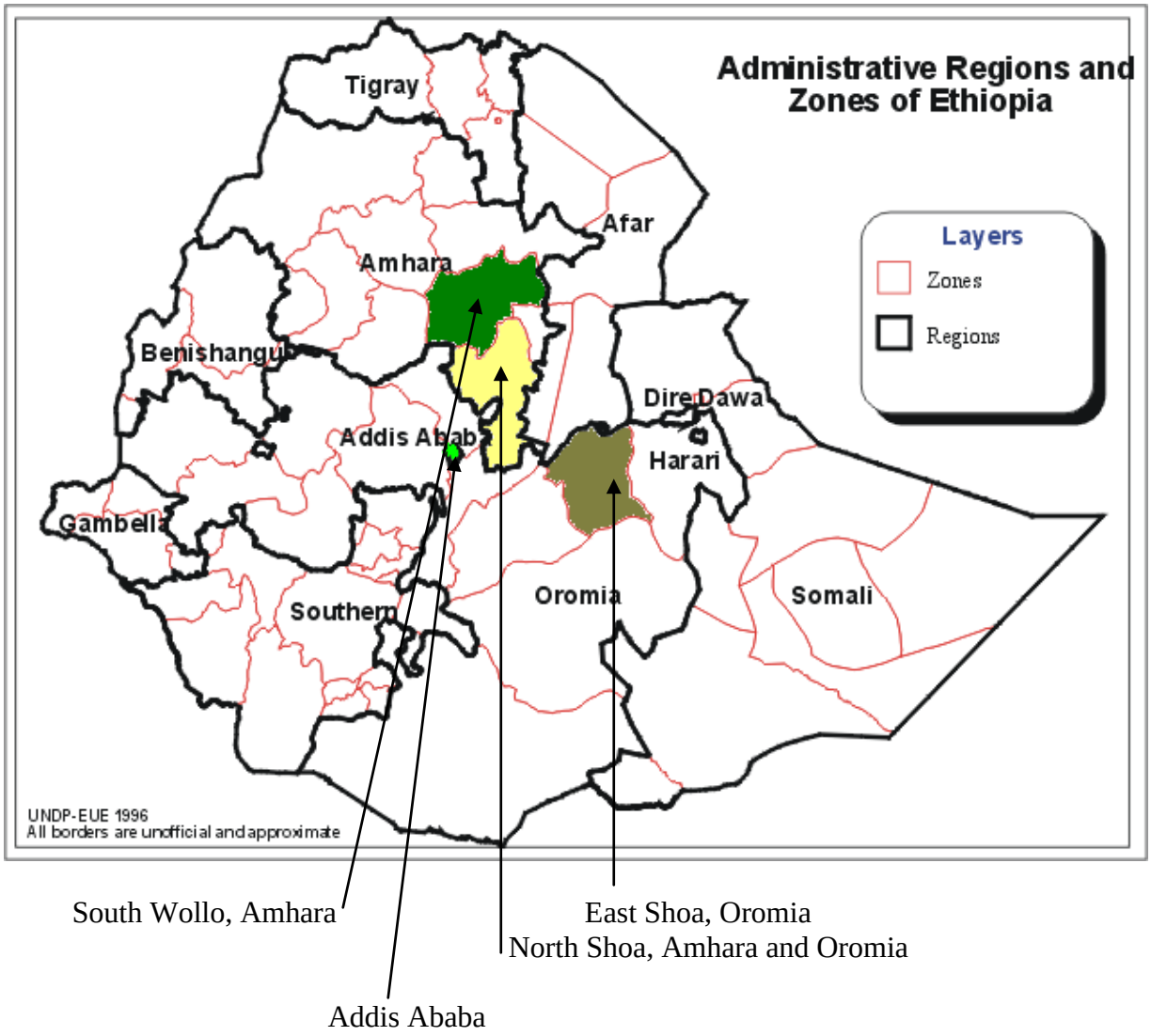

Figure 1. Map of the study areas

3.3. Target Population

The target population of the study areas were 16,480 donkeys, 4,791 horses and 58 mules. Equidae above six months of age, both sexes and with no previous history of vaccination against African horse sickness were sampled. The age category of sampled equidae is shown by table 3.

Table 3. Number of sampled equidae in different age category.

Category	Age range (years)	Sampled equidae			
		Donkey	Horse	Mule	Total
1	1-7	364	112	25	501
2	8-14	455	239	32	726
3	15-20	5	32	1	38
Total	-	824	383	58	1265

3.4. Sampling strategy

Purposive sampling method was employed up to district level. Stratification of equines into donkeys, horses and mules was made at peasant association level. Equines were taken as the primary units of the stratum and the stratum was determined to be one (equines). Discussion was made with equine owners of different peasant associations to know the equine resource base of their respective areas. The number of equines to be sampled from different study areas was determined by the method of proportional allocation based on 5%, 8% and 100% sampling fractions for donkeys, horses and mules, respectively. Random sampling was made by employing a lottery system to select the primary units. Since owners were not willing their mules to be sampled, their sampling was determined to be 100% (see table 4).

3.5. Sample size determination

The sample size was determined according to Thrusfield (1995) simple random sampling method for an infinite population with 95% confidence level, 5% desired absolute precision and 50% expected prevalence, since there was no previous information on the prevalence of AHSV antibodies in the study areas. The sample size was calculated by making use of the following formula after Thrusfield (1995).

$$n = \frac{1.96^2 \, p_{exp} \, (1-p_{exp})}{d^2}$$

Where,
n = required sample size

p_{exp} = expected prevalence

d = desired absolute precision

1.96 = z-value for the 95% confidence level

In total 1265 serum sample comprising 824 donkeys, 383 horses and 58 mules were collected.

3.6. Study design

A cross-sectional observational epidemiological study was conducted using competitive Enzyme Linked Immunosorbent Assay (C-ELISA) configuration. Data on the potential risk factors associated with the occurrence of African horse sickness were collected during sampling through recording and questionnaire administration. Active disease search was carried out in those areas with a history of AHS outbreak and manifestation of typical clinical signs by sick equidae. Field sera and data collection was undertaken from September 2005 to mid of April 2006.

3.7. Study Methodologies

3.7.1. Blood Collection and Assay Methodology

3.7.1.1. Seroprevalence survey

Equine serum collected from different study areas are shown by table 4.

Table 4. Serum sample collected from equines of different study areas.

Study areas	Number of equines			Number sampled		
	Donkeys	Horses	Mules	Donkeys	Horses	Mules
Gerado	1900	1000	43	95	80	43
Kombolcha	-	500	-	-	40	-
Kalu	-	700	-	-	56	-
Debrebrihan 8	780	613	-	39	49	-
Debrebrihan 4	800	675	-	40	54	-
Tulamba	840	-	-	42	-	-
Fagi	800	-	-	40	-	-
Senkolliechollie	1020	163	-	51	13	-
Legebollo	820	138	-	41	11	-
Sidamawash	1200	-	1	60	-	1
Gogiecha	1120	-	-	56	-	-
Godino	2280	-	1	114	-	1
Ada'a town	580	438	13	29	35	13
Abeno one	140	-	-	7	-	-
Alemtena	740	13	-	37	1	-
Soridolliesa	900	-	-	45	-	-
Adama town	-	413	-	-	33	-
Treesengota	1560	138	-	78	11	-
Addis ketema	1000	-	-	50	-	-
Total	16,480	4,791	58	824	383	58

Whole blood of 10ml was collected by vein puncture using sterile venoject needles and plain vacutainer tubes including needle holder under aseptic precautions. Each sample was labelled with identification number. The blood was allowed to clot over night at room temperature. The recovered serum was decanted into another vacutainer tube and labelled with similar identity. The samples were kept at -20^0C until evaluated with C-ELISA. Area of sampling, age, sex, type of equidae (donkey, horse, mule), date of collection, agro-ecology, management practices, and vacutainer ID number were recorded at the time of sampling. The test was done in 2-4 months time after serum collection. The kit was AHSV serotype 9 specific. Sensitivity and specificity of the test kit was not given in the bench protocol. Standardization and validation of the kit was conducted before the test sera are subjected to C-ELISA test procedures. Then similar results were obtained with the interpretation given in the test protocol except the conjugate. The information given in the protocol to prepare chromogen/substrate complex was also insufficient. Through a request forwarded to get clarification on minor issues to the manufacturer, problems of some parameters and documentations were pointed out. Finally the overall test procedure was evaluated and confirmed to run safely. The C-ELISA test was conducted at NVI immunology laboratory, Ethiopia according to the Standard Operating Procedure (SOP) developed by the joint venture of Biological Diagnostic Supplied Limited (BDSL) Company and the Institute of Animal Health (IAH) Pirbright, Scotland, United Kingdom. The SOP procedure is described as follows:

AHSV antigen was thawed and mixed by vortexing to ensure homogeneity. It was diluted at 1:150 in PBS. 50µl of the reconstituted antigen was coated to each microwell of the ELISA plates (Nunc Maxisorb) and sealed with plate sealer so as to be incubated for 1hour at 37^0C on an orbital shaker. The stock antigen was returned to the freezer (-20^0C).

Plates were washed 4 times by flooding and emptying the wells with PBS and blot dried on an absorbent towel. Blocking buffer of 40µl was added to all the test wells and to each of the negative control wells. 50µl of blocking buffer was added to each of the guinea-pig control wells. Moreover, 100µl of blocking buffer was added to the conjugate control wells. Finally 50µl of blocking buffer was added for the positive control of wells G up to A and 80µl in well H of the first column. Test samples of 10µl were added (in duplicate) to

their respective wells and 10µl of the negative control sera was added to the negative control wells after it was reconstituted with 1.0ml bidistilled water.

After it was reconstituted with 1.0ml bidistilled water, positive control of 20µl was added to well H in the first column and mixed by careful pipetting up and down a number of times. A 1:5 dilution of 100µl of the positive control was obtained in well H. A two-fold serial dilution was carried out up the remaining wells of this column by taking 50µl from well H and dispensing into well G, to give a 1:10 dilution. After mixing and dispensing, 50µl was taken from well G and dispensed into well F, to give a 1:20 dilution. This procedure was continued until a 1: 640 dilution in row A was obtained. The last 50µl of the positive control from well A was discarded so that all wells in column one were found to have 50µl in total.

The immuned guinea-pig antiserum (monoclonal antibody) was thawed and gently mixed to ensure homogeneity. It was reconstituted with 1.0ml bidistilled water. The guinea-pig antiserum was diluted to 1:100 in blocking buffer and 50µl of this was added to each well except the duplicate conjugate control wells. The ELISA plates were covered with plate sealer and incubated for 1hour at 37^0C on an orbital shaker. The stock guinea-pig antiserum was returned to the freezer (-20^0C).

After washing 4 times the rabbit anti-guinea-pig conjugate was diluted to 1:1000 in blocking buffer and 50µl of this was added to all wells. The plates were sealed and incubated at 37^0C for 1hour in an orbital shaker. The other microwells were filled with test serum samples, which were duplicated as indicated by figure 2.

	1	2	3	4	5	6	7	8	9	10	11	12
A		-Ve cont.	8	8								
B		-Ve cont.	7	7								
C		Conj.Cont.	6	6								
D		Conj. Cont.	5	5	Etc	Etc						
E		Mab. Cont.	4	4	Etc	Etc						
F		Mab. Cont.	3	3	11	11						
G		Mab. Cont.	2	2	10	10						
H	+Ve cont.	Mab. Cont.	1	1	9	9						

Figure 2. Plate layout for spot test

One plate can accommodate a maximum of 40 samples.

After the plates were washed 4 times, the chromogen (OPD = Orthophenylene diamine Dihydrochloride) solution was prepared at a rate of 0.4mg/ml in sterile bidistilled water. Then a substrate (hydrogen peroxide) was added to the OPD solution. 50µl of the OPD solution was added to the wells of each micro plate, sealed and left in dark place for 10minutes at an ambient temperature. The reaction was stopped by the addition of 50µl of 1M H_2SO_4 to all wells including the wells acting as the blank. Finally the reaction was read spectrophotometrically at 492 nm filters (wavelength) and interpreted according to the interpretation given in the SOP (see annex 1).

3.7.1.2. Virus isolation and identification

Whole blood was collected (and kept at -20^{0}C until processed) in heparinized vacutainer tubes by venoject sterile needles, with needle holder under aseptic precautions from the jugular vein of horses and a mule manifesting the cardiac form of African horse sickness characterized by supraorbital oedema and febrile reaction (40^{0}C). An equal volume of blood and bidistilled water were mixed together. After three times washing of the RBCs with bidistilled water centrifugation was carried out at 1500 rpm for 15 minutes so that the RBCs are haemolyzed to let the virus free. The supernatant was inoculated onto vero cells originated from African green monkey kidney (Pan African Veterinary Vaccine Center/FAO origin) at the National Veterinary Institute in Debre Zeit, Ethiopia. Vero cells were grown in GMEM at an initial concentration of 380,000 cells/ml, which were supplemented with 10% calf serum, antibiotics (Penicillin and Streptomycin) and mycostatin. The cells were prepared 2-3 days prior to use. After the cells reached 100% confluency 0.2ml of the inoculum was seeded onto early confluent monolayers of vero cells in tissue culture flasks. The cultures were incubated at 37^{o}C for 30 minutes. After 30 minutes of adsorption time, the inoculum was washed with PBS and refilled with growth medium containing antibiotics (Penicillin and Streptomycin). After 4 days of post inoculation maintenance medium containing 5% calf serum is added and incubated at 37^{o}C for 7 days. The development of cytopathic effect was daily followed through an inverted microscope.

A cytopathic effect (CPE) was expected to appear between 2 and 8 days post inoculation. Three blind passages were performed to recover AHSV. AHSV serotype specific serum titration by 10 fold serial dilution was supposed to be carried out to determine $TCID_{50}$/ml of solution. The 50% end-point titre of the serum was supposed to be calculated by the Spearman-Karber method and expressed as the negative log_{10}. Then virus neutralization test with the objective of serotyping by serotype specific serum was planned to be conducted by taking 25µl from either of them in order to seed on confluent monolayer of vero cells. However, serotype specific serum was not available at NVI and the procedure was discontinued. Then the sample was recommended to be tested by I-ELISA protocol by making use of the monoclonal antibody developed for AHSV serotype 9 of the C-ELISA configuration. This procedure was carried out to rule out the possible existence of African horse sickness serotype 9.

3.7.1.2.1. Indirect-ELISA

The control antigen was thawed and diluted in PBS at a rate of 1:150. Sixteen microwells of polystyrene microplate were precoated with 50µl of the reconstituted antigen as a control. The supernatant of the suspected nine samples was coated in nine microwells at different rates of 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128 dilutions in the rows of A3-A11 and in columns of A-H. Totally 8*9=72 microwells were filled with test samples. Then 50µl from each dilution was added to the nine different rows of microwells until 72 microwells were filled. Both control antigen and the samples were incubated for 1hour in an orbital shaker. After washing four times with PBS and drying, 50µl of monoclonal antibody diluted at a rate of 1:100 with blocking buffer was added to the control and test microwells and incubated for 1hour in an orbital shaker. After washing four times and drying, 50µl of the conjugate diluted at a rate of 1:1000 with blocking buffer was added to the controls as well as to the test microwells and incubated for 1hour in an orbital shaker. Chromogen/substrate complex was prepared, of which 50µl was added to all of the controls and test microwells. After 10 minutes 50µl of 1M H_2S0_4 was added to each of the controls and test microwells in order to stop the reaction and facilitate the reading. Then reading was made spectrophotometrically at 492nm filter (wavelength). The test is said to be positive if there is colour development and negative if there is no colour development.

3.7.2. Questionnaire survey

A structured questionnaire format was prepared to interview individual owners of donkeys, horses and mules. The format was used to assess the potential risk factors such as management practices (stabling in enclosed, semi-opened and fenced but with no cover of roof type of housing during the night), presence of equine biting insects and availability of water bodies near the study areas (see annex 4) and the knowledge base of equine owners about AHS. The format was initially being pretested so as to incorporate relevant points in the final questionnaire format. The interviewees were initially being pretested for their knowledge of AHS and attention was paid not to miss key informants. A total of 57 equine owners, three from each of the 19 study areas were interviewed and the result was summarized.

3.8. Data Management

Data recorded during sampling, laboratory findings and questionnaire survey were entered and stored in separate MS-access database and MS-Excel spread sheet. The data were thoroughly screened for errors and proper coding before subjected to statistical analysis.

3.9. Data Analysis

The data were imported from the Microsoft Excel spread sheet and analyzed using Intercooled Stata 7.0 software to establish association (χ^2 test), to measure the strength of associations between serological test results and the identified potential risk factors (logistic regression). Win Episcope 2.0 software was used to calculate confidence intervals for seroprevalence of AHSV antibodies. Descriptive statistics was used to calculate seroprevalence of AHSV antibodies.

4. RESULTS

4.1. Seroprevalence survey

The C-ELISA result of AHSV antibodies in equidae of different study areas is shown by Table 5.

Table 5. C-ELISA result of AHSV in different study areas.

Study areas	C-ELISA result		Seroprevalence (%)	95% CI
	Total tested	Positive		
Abeno One	7	6	85.7	63.3-116.0
Ada'a	54	16	29.6	19.6-44.7
Adama town	42	10	23.8	13.9-40.9
Addis Ketema	50	7	14	7.0-27.8
Senkolliechollie	64	11	17.2	12.0-34.5
Debrebrihan 8 and 4	264	20	7.6	5.0-11.5
Sidamawash	61	24	39.4	28.8-53.7
Gerado	218	0	0	0
Godino	55	25	45.5	34.0-60.7
Guaworko	74	32	43.2	33.3-56.1
Kalu	56	17	30.4	20.4-45.1
Legebollo	52	21	40.4	29.0-56.2
Kombolcha	40	0	0	0
Kombollie	38	19	50	36.4-68.7
Gogiecha	56	25	44.6	33.3-59.8
Alemtena	45	22	48.9	36.3-65.9
Boset	89	36	40.4	31.4-52.0
Total	1265	291	23	20.8-25.4

(Pearson χ^2 (16) = 248.2619; P = 0.000)

The variation in seroprevalence of AHSV in different areas was found to be significant ($P<0.05$).

Of the total 1265 sampled equidae a mean seropositivity of 23% was obtained. Seroprevalence increases as one goes from highland and midland to lowland areas. Seroprevalence was found to be high areas near permanent water bodies such as lakes, rivers, irrigation canals and water reservoirs. The seroprevalence of African horse sickness virus in different types of equidae is presented in Table 6 and Figure 3.

Table 6. C-ELISA result of AHSV in different types of equidae.

Types of equidae	C-ELISA result		Seroprevalence (%)	
	Total tested	Positive		95% CI
Donkeys	824	245	29.7	26.8 - 33.0
Horses	383	40	10.4	7.8 - 14.0
Mules	58	6	10.3	4.8 - 22.1
Total	1265	291	23	20.8 - 25.4

(Pearson χ^2 (2) = 60.4253; P = 0.000).

In this study a significant variation of seropositivity was observed among different types of equidae (P<0.05). Figure 3 shows the highest seropositivity result in donkeys, horses and mules showed almost similar seropositivity.

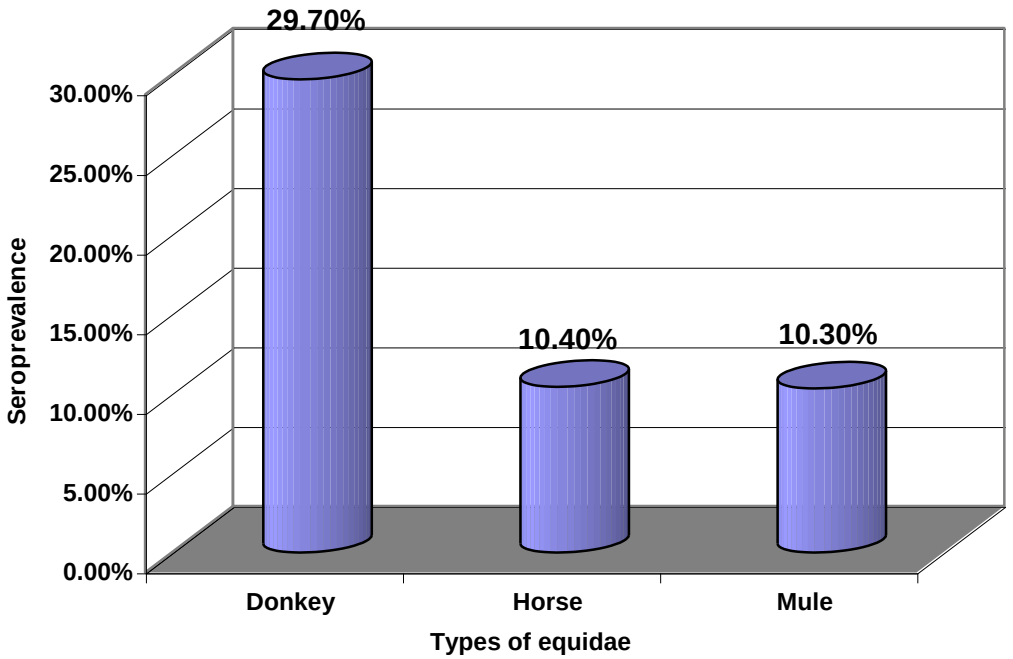

Figure 3. C-ELISA result of AHSV in different types of equidae.

Table 7. C-ELISA result of AHSV in different age categories of equidae.

	C-ELISA Result		Seroprevalence	
Age category (years)	Total tested	Positive	(%)	95% CI
1 (1 - 7)	501	125	25	21.4 - 29.0
2 (8 -14)	726	161	22.2	19.4 - 25.4
3 (15 - 20)	38	5	13.2	5.8 - 29.8
Total	1265	291	23	20.8 - 25.4

(Pearson χ^2 (2) = 3.4320; P= 0.180)
There was no significant variation in seroprevalence among the different age groups of equidae affected by African horse sickness virus (P>0.05).

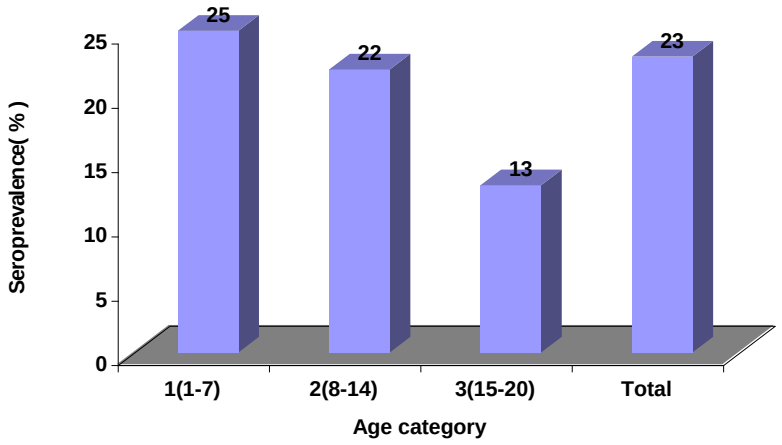

Figure 4. C-ELISA result of AHSV in different age categories of equidae.

Table 8. C-ELISA result of AHSV in the two sexes of equidae.

	C-ELISA Result		Seroprevalence	
Sex	Total tested	Positive	(%)	95% CI
Female	447	129	28.9	24.9 - 33.4
Male	818	162	19.8	17.3 - 22.7
Total	974	291	29.9	20.8 - 25.4

(Pearsons χ^2 (1) = 13.3796; P = 0.000)
The seroprevalence in the two sexes of equidae was found to be statistically significant (P<0.05).

Table 9.C-ELISA result of AHSV in different types of equidae and sexes.

Types of		Sex			Seroprevalence (%)	
equidae	Result	Female	Male	Total	Female	Male
Donkey	Negative	282	297	579	69	71
	Positive	124	121	245	31	29
	Total	406	418	824	-	-
Horse	Negative	14	329	343	74	90
	Positive	5	35	40	26	10
	Total	19	364	383	-	-
Mule	Negative	22	30	52	100	83
	Positive	-	6	6	-	17
	Total	22	36	58	-	-

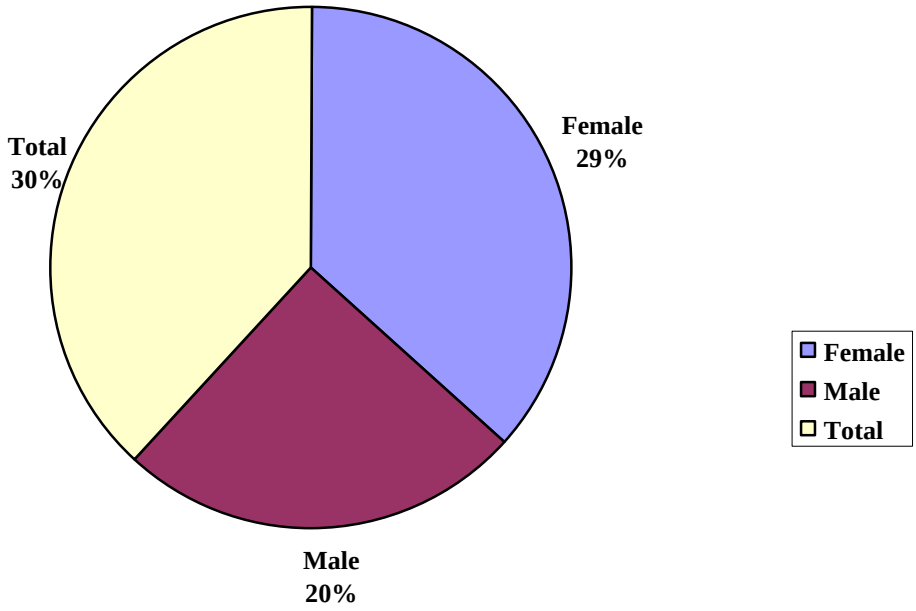

Figure 5. C-ELISA result of AHSV in the two sexes of equidae.

Table 10. C-ELISA result of AHSV in different agro-ecological zones of the study areas.

	C-ELISA Result		Seroprevalence	
Agro-ecology	Total tested	Positive	(%)	95% CI
Highland	378	38	10	7.4 - 13.6
Lowland	212	92	43	37.2 - 50.6
Midland	675	161	24	20.8 - 27.3
Total	1265	291	23	20.8 - 25.4

(Pearson χ^2 (2) = 85.8429; P=0.000)

There is significant variation in seroprevalence of AHSV antibodies in different agro-ecological zones of the study areas (P<0.05). The highest seropositivity was obtained in the lowland followed by midland and highland.

Table 11. C-ELISA result of AHSV in different types of equidae in different agro-ecological zones.

Types of equidae	Result	Agro-ecology			
		Lowland	Midland	Highland	Total
Donkey	Negative	86	265	228	579
	Positive	81	130	34	245
Horse	Negative	34	197	112	343
	Positive	11	25	4	40
Mule	Negative	-	52	-	52
	Positive	-	6	-	6
Total		212	675	378	1265

The seroprevalence of AHSV in donkeys is 13% in the highlands, 33% in the midlands and 49% in the lowlands. Seropositivity values of 3% in the highlands, 11% in the midlands and 24% in the lowlands were obtained in horses. Since mules were sampled from only midlands a seroprevalence of 10.3% is obtained.

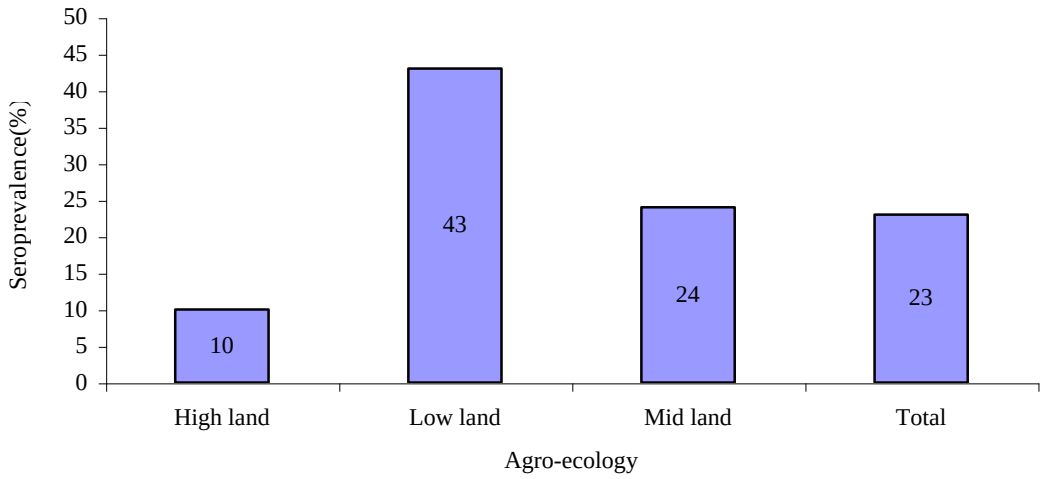

Figure 6. C-ELISA result of AHSV in different agro-ecological zones of the study areas.

Table 12. Multivariate logistic regression estimates for assumed risk factors of AHSV in different types of equidae.

C-ELISA Result	Odds Ratio	Std. Err.	P	95%CI
Types of equidae	2.1	0. 22	0. 000	1.73 - 2.60
Agro-ecology	1.5	0. 13	0. 000	1.27 - 1.77
Age	1.0	0. 02	0. 115	0.99 - 1.06
Sex	0.9	0. 15	0. 937	0.74 - 1.32

The risk of acquiring African horse sickness is more than two fold with respect to the types of equidae affected. Agro-ecology contributes nearly two fold for the occurrence of African horse sickness. There is association among C-ELISA result, type of equidae and agro-ecology. Age is not part of the interaction. Sex is less likely to be part of the interaction.

4.2. Virus Isolation and Identification

Virus isolation and identification was carried out on blood collected from clinical cases of AHS. Three blind passages were undertaken on vero cell lines. Then serotyping could have been carried out. However, due to lack of specific serum in order to run the test the procedure was discontinued. So the samples were tested with indirect ELISA by making use of the monoclonal antibodies developed for C-ELISA kit of AHSV serotype 9. The result was negative.

4.3. Questionnaire Survey

All of the fifty seven owners interviewed complied to the minimum requirement of equine possession i.e. owning at least either a donkey, horse or mule. 26% (15/57) of the respondents replied of having encountered the disease once or more in their life time. While 74% (42/57) of the respondents proved to know nothing regarding the disease. Their experience of other equine health problems such as lymphangitis (Bichie), rabies, anuria, impaction, gastrointestinal parasitism, cough (furro), lameness, colic and car accidents was considerable.

Respondents reported horses to be more affected, followed by mules. The interviewees were not able to describe the age groups of equines affected by the disease. However, they said that both sexes are equally affected. In addition owners with the indigenous

knowledge of the disease said that the disease is quite common in the lowlands and midlands, and that it is seen occasionally in the highlands. None of the respondents were able to explain the mode of AHS transmission, except few, who said that it is through contact with equines with wounds and bites by *Tabanus*.

All of the owners replied that, there are equine biting insects in their localities called by local names such as " kerchassa, ewir zinb'' implying *Tabanus*. These flies are abundant in the months of September to November as well as from mid April to June. Respondents were however, unaware of *Culicoides* and their role as vectors.

As to the maintenance and shelter of equines, owners replied that stabling their equines to be a common practice during the night. The type of stables however, vary from place to place. Equine owners of South Wollo stable their equines in houses made of intact walls and complete roof cover. On the other hand equines of North Shoa are stabled in houses without walls but with well-constructed roof. Equines of Akaki, Ada'a, Adama and Dugdaborra areas are stabled in round fenced compound without roof, traditionally called "Beret". While some equines in Adama area are stabled in a fence made of wood and plastic covered roof.

Environment geared enquiry led to the establishment of the following scenario: The study areas in South Wollo zone are near to rivers such as Borkena in Kombolcha. In North Shoa zone the existence of rivers such as Beriesa was reported. Equine owners from Senkolliechollie and Legebollo responded that, their localities are a bit far from the rivers. However, daily watering of equines in rivers is a routine activity. Owners of Akaki area reported the existence of marshy areas near their localities. Similarly interviewees from Ada'a replied that, their localites are surrounded by small lakes and rivers. Equine owners of the Rift Valley areas described that, there are irrigation canals, marshy areas, water reservoirs, water wells, large lakes and rivers near their localities.

In view of the importance of animal movement in the epidemiology of AHS, attempt was made to probe into the degree and pattern of equine migration in the study area. In the response obtained the informants said generally the movement of equines is from highlands and midlands to the lowlands. Cart horses are being purchased from the midlands and highlands.

5. DISCUSSION

5.1. Seroprevalence Survey

This study has once again confirmed the existence of agro-ecology based variation in the occurrence of AHS. The difference in seroprevalence of the various study areas is statistically significant ($P<0.05$). Seroprevalence rates of 0 at Gerardo, 0 at Kombolcha, 30.4% at Kalu, 7.6% in Debrebrihan, 17.2% at Senkolliechollie and 40.4% at Legebollo were obtained in this study. The altitude of these study areas ranges from 1818 (Kombolcha) to 2694 (Debrebrihan) meters above sea level with mean annual rainfall ranging between 900-1000 mm with bimodal pattern; a long rainy season extends from June to September and a short rainy season from February to May. The average monthly minimum air temperature is in between 2.4^0C in November to 8.5^0C in August. The average monthly maximum air temperature ranges from 18^0C to 24^0C in June. The mean relative humidity of these study areas is 68%. Frost usually occurs in the months of October to January. The length of growing period (LGP) of plants in these study areas ranges from 121-180 days.

Seroprevalence rates of 14% in Addis Ababa, 39.4% at Sidamawash, 44.6% at Gogiecha, 45.5% at Godino and 29.6% in Ada'a were the findings of this study. The altitudinal range of these areas is between 1820 (Ada'a) to 2812 (Addis Ababa) meters above sea level. The mean annual rainfall of these study areas is also in the range of 900-1000 mm with similar bimodal pattern of the study areas mentioned previously. The average monthly air temperature is in between 18-24^0C. The mean relative humidity of these study areas is similar with the one mentioned above. The length of plant growing period also ranges from 121-180 days.

Seroprevalence rates of 23.8% in Adama, 40.4% at Boset, 48.9% at Alemtena, 85.7% at Abeno one and 50% at Kombollie were the findings of this study in these study areas. The altitude of these study areas ranges from 1413 (Boset) to 1672 (Alemtena) meters above sea level. The mean annual rainfall of these areas is in the range of 900-1000 mm with bimodal distribution pattern mentioned above. The minimum and maximum temperature ranges from 30-32^0C. The daily temperature is said to be 10^0C. The relative humidity of

these areas is between 35-40%. The length of plant growing period ranges from 61-120 days.

According to the seroprevalence study of AHSV conducted by Keith (2005) in different agro-ecological zones of Amhara and Tigray regions seropositivity values of 48% in donkeys and 70.6% in mules were obtained. The study further described that 44% donkeys and 71.7% mules of Amhara region as well as 50% donkeys and 62.5% mules of Tigray region were found to be seropositive of AHSV. In Keith's study the main areas of sampling were lowlands and midlands (endemic areas) as well as areas near to permanent water bodies such as lake tana and the likes.

The seroprevalence findings in donkeys in this study is in agreement with that of Keith (2005) except minor variations. The reasons for variations are the sample size, study design and agro-ecology. The sample size of mules in the present study is not representative to compare with the findings of others.

The findings in seroprevalence of AHSV in this study in donkeys of different study areas were 86% at Abeno one, 38% in Ada'a, 14% in Addis Ababa, 44% in Boset, 16% at Senkolliechollie, 12% in Debrebirhan, 40% at Sidamawash, 46% at Godino, 48% at Guaworko, 39% at Legebollo, 51% at Kombollie, 45% at Gogiecha and 49% at Alemtena. Moreover, the seroprevalence obtained in this study in horses of different study areas were 9% in Ada'a, 27% in Adama, 18% at Boset, 23% at Senkolliechollie, 1% in Debrebrihan, 45% at Legebollo and 18% at Kalu. 46% of the mules sampled from Ada'a were found to be seropositive in this study.

In this study 29.7% of donkeys, 10.4% of horses and 10.3% of mules were found to be seropositive. Seropositivty increases as one goes from highland and midland to lowland areas. Hence higher seropositivity was indicated in the lowland followed by midland and highland areas. Study areas in the lowlands are near permanent water bodies such as lakes, rivers, irrigation canals, watering points and water reservoirs that support insect breeding and multiplication. The annual report of NVI (1974), Hall (1995), and Radostits and others (2000) also suggested that endemic areas are more likely to be in low-lying, warm and marshy regions that creat favourable environment for multiplication of *Culicoides* and mechanical vectors.

These days and in every day life the movement pattern of equidae, other livestock and people is from the highlands and midlands to the lowlands, because of natural resource scarcities. Some of the highland areas are known to be free from AHS. So in the naïve population that moves from the highlands, there is maximum mortality at the time of first exposure to the disease. Horses are not normally kept in the lowlands due to failure of physiological adaptation mechanisms; only donkeys and mules that are adapted to semi-arid conditions are let to the lowlands for transportation and packing purposes.

The seroprevalence of African horse sickness virus antibodies in different types of equidae was determined ($P<0.05$). In this study seroprevalence was higher in donkeys (29.7%) and almost similar in horses (10.4%) and mules (10.3%) eventhough the sample size of mules is not representative like the case encountered by Keith (2005) in Amhara and Tigray regions. According to Robinson (1987) and OIE (2004) AHS causes disease in horses, mules and donkeys, with up to 95% mortality in susceptible horse population. Robinson further described that mules have got high morbidity but low mortality. Moreover, Robinson explained that donkeys are the least sensitive showing only a mild febrile response without mortality. Sewell and Brocklesby (1990), Hunter (1996) and OIE (2005) described that donkeys are affected by horse sickness fever, the mildest form, which is frequently overlooked in natural outbreaks. Furthermore, the cohort study conducted among the donkey and mule population of Amhara and Tigray regions (Keith, 2005) confirmed that African horse sickness was not a significant cause of mortality in donkeys, but it was a significant cause of mortality in mules. In this study the sample size of mules is not representative because owners were not willing for their mules to be sampled. According to Galloway (1974), Hunter (1994) and OIE (2004) among equidae, horses are the most susceptible to AHS with a mortality rate of 50-95%, followed by mules with mortality around 50%. They further pointed out that, in enzootic regions of Africa including Ethiopia donkeys are very resistant to AHS and experience only subclinical infections. Moreover, they described that, in Europeans and Asian countries however, donkeys are moderately susceptible and have a mortality rate of 10%. From this fact of life the author can infer that, there is 90-95% chance of recovery in donkeys from infection due to AHS unlike horses and mules. Contrary to this, exposed horses and mules often die of AHS viral infection. Field experiences of the author during active disease search and serosurvey indicated that, getting cases of seropositive recovered horses and mules after being exposed to the natural challenge is rare in the study areas. The higher seroprevalence

observed in donkeys in this study is in agreement with what had been said by the previous authors.

In this study the seroprevalence of AHSV in different age groups of equidae was assessed.. In the findings there was no significant variation in seropositivity among the different age groups of equidae (P>0.05). Seroprevalence in the ranges of 20-45% in age category one (1-7 years), 19-38% in age category 2 (8-14 years) and 19-50% in age category 3 (15-20) were the findings of this study in donkeys. By the same talken seroprevalence in the ranges of 5-19% in age category 1 (3-7 years), 6-15% in age category 2 (8-14 years) and 6-24% in age category 3 (15-20 years) were obtained in this study in horses. Similarly seropositivity values of 14% in 3 years of age, 17% in 6 years of age, 20% in 8 years of age, 17% in 10 years of age, 33% in 14 and 16 years of age were documented in this study in mules. This finding is supported by Keith (2005) in that, all foals that have lost their maternal antibody by six months of age would be protected by vaccination. Keith further described that different age groups of equidae that are above six months of age had equally seroconverted and protected after they were being vaccinated. From the findings of this study and Keith's survey results the author can infer that all age groups of equidae seem likely to be equally affected by AHS provided that the equines were not previously exposed and recovered as well as vaccinated.

In the present study the seroprevalence of AHSV was determined in the two sex groups of equidae. A Seroprevalence of 31% in female donkeys and 29% in male donkeys; 26% in female horses and 10% in male horses as well as 17% in male mules were indicated in this study. In the result statistically significant difference of seropositivity was obtained in the two sexes (P<0.05). However, the author did not come across previous work/s done pertaining to seroprevalence and sex of equidae in the present study areas to compare with the finding of this study. From field experience the author can infer that both sex groups are equally likely to be affected by AHS.

In this study the seroprevalence of AHSV was assessed in different agro-ecological zones of the study areas. In the findings statistically significant difference of seropositivity of AHSV was obtained in the three agro-ecological zones of the study areas. Seroprevalence rates of 13% in highland donkeys, 3% in highland horses; 49% in lowland donkeys, 24% in lowland horses; 33% in midland donkeys, 11% in midland horses as well as 10% in

midland mules. Mules were sampled only from the midlands. According to NVI (1983) the distribution of the disease seems to have positive correlation with the ecology of its vectors. Furthermore, the disease is considered to be endemic to the lowlands and midlands of Ethiopia. However, some cases of the disease are known to appear in surveyed highlands of Ethiopia. According to Anon (1998) and Radostits *et al*. (2000) the breeding status and movement of vectors is governed by climatic conditions. They further described that *Culicoides* have almost worldwide distribution so that spread of AHS is universal. Mellor *et al*. (1998) described that increased use of irrigation, water leaks, manure, urine, dung pats, tree holes, rotting vegetation, stagnant surface water are ideal larval habitats for the multiplication of *Culicoides*. Galloway (1974), Anon (1976), Della-Porta (1985) and Knipe and Howley (2001) explained that, from time to time AHS has spread beyond its usual distribution, causing devastating epidemics in many countries including Ethiopia. The report issued by NVI (1983) documented that AHS is prevalent in eight out of the previous fourteen provinces of Ethiopia. Keith (2005) explained that, it is environment rather than species or husbandry that is relevant to sero-conversion. Therefore, from the findings of this study and contextual comparison of the present findings with previous survey results of other authors in different parts of Ethiopia, it is likely to infer that African horse sickness exists in all agro-ecological zones of the present study areas.

In this study the association of seropositivity with different potential risk factors that facilitate the precipitation of AHS as well as the production of antibodies against AHSV was assessed. In the result the risk of acquiring African horse sickness is more than two fold (OR=2.1) with respect to the type of equidae affected. Agro-ecology contributes nearly two fold (OR=1.5) for the occurrence of African horse sickness. The strength of association among seropositivity and putative risk factors indicated that types of equidae and agro-ecology contribute for important arrays of interaction to facilitate the occurrence of AHS. Ages of the animals in question are not part of the interaction (OR=1). On the other hand sex of equidae has got insignificant effect to favour the occurrence of AHS (OR=0.9). Hence all age groups and both sexes of equidae are equally affected. However, the author was unable to get research work/s done pertaining to the degree of association among seroprevalence rates and assumed risk factors in the present study areas to compare with the findings of this study.

To put in a net shell this epidemiological study is the first of its kind in some of the study areas and the second of its kind in areas where the National Veterinary Institute undertook its last epidemiological study in the year 1978 (28 years ago) pertaining to the epidemiological status of AHS in some of the present study areas.

5.2. Virus Isolation and Identification

Virus isolation and identification was carried out on whole blood collected during active disease search from clinical cases of horses and a mule. However, the test result is negative. In the presence of the disease in the field with classical pathognomonic signs and history of postmortem lesions, the negative result is probably due to the improper handling of the tissues processed.

5.3. Questionnaire Survey

Knowledge of equine owners on African horse sickness was assessed. The majority of the owners were found to have no experience of the disease. However, those owners whose livelihoods depend on equines (cart horse taxi owners) were able to recognize the disease. They described horses to be more affected than mules and donkeys. Mules were the second most susceptible and donkeys were seen rarely with the disease.

The majority of owners did not know the age of their equines, because of lack of know-how on age determination. It was determined that both sexes of equines were equally affected by AHS. The disease was quite frequently seen in the lowlands and midlands and. rarely seen in the highlands. Like in all other livestock diseases the respondents were unable to describe the mode of transmission of AHS.

Equine biting flies are very common mainly in the lowlands followed by midlands. It is not uncommon to find equine biting flies in the highlands too. Equine biting flies are called by different names in different regions: Kerchassa in Oromia, Ewir zinb in Amhara which all mean *Tabanus*. In the assessment made to know the knowledge base of equine owners about *Culicoides* vectors, none of the respondents were be able to know these vectors and the role they play as well. Rather, they are totally unaware of them. It was conclusively described that flies are abundant immediately after the rainy season, because the climate is

conducive for their multiplication. All equine owners stabled their equines in different types of houses. Those stables with intact walls and complete cover of roof are insect proof. Stables with open walls but with complete cover of roof and those fenced ones without roof can equally predispose equines for insect bite.

Almost all the study areas are located close to permanent water bodies such as lakes, rivers, watering points, water reservoirs and irrigation canals which support insect breeding and multiplication. The movement pattern of equines from the highlands and midlands to the lowlands is a predisposing factor to AHS.

6. CONCLUSIONS AND RECOMMENDATIONS

This epidemiological study is the first of its kind in the present study areas since the 1981 comprehensive epidemiological study undertaken by NVI. The significant variation in seropositivity of African horse sickness virus, serotype nine, obtained in donkeys, horses and mules in the present study areas of this study indicated that the three equine types have got differences in the degree of their response to AHSV. Moreover, the disease is equally likely to affect all age groups of equidae; hence there is no significant variation in seropositivity among the different age categories of equines. Moreover, both sexes are equally affected by African horse sickness.

In this study the highest seroprevalence was observed in the lowland followed by midland and highland areas. Furthermore, types of equidae and agro-ecology contributed more than two and nearly two folds for the occurrence of AHS, in the given order. Age was not part of the association and sex has got weak effect to precipitate the disease. Generally AHSV exists in all agro-ecological zones and affects all types of equidae in the study areas.

The result of virus isolation and identification on vero cell lines was negative which may be due to improper handling of the specimen during processing. The questionnaire survey result indicated that the knowledge base of equine owners about AHS is unsatisfactory. They are conclusively unaware of *Culicoides* vectors and the mode of transmission of AHS.

Based on the above concluding remarks the following points are recommended:

- There is a need of deeper understanding of the epidemiology of African horse sickness in the present study areas through an integrated approach of serotyping and identification of the *Culicoides* complex as well as other potential vectors.
- Appropriate equine enclosure system to avoid insect bite by stabling them some hours before sunset and letting them out a few hours after sunrise, as *Culicoides* species are nocturnal in nature and are not inclined to enter equine stables, should be adopted.
- There is a need to classify the present study areas into endemic and epidemic zones in relation to AHS. There should be a need of serotyping in out break cases. Based on the epidemiological situation of the areas and representativeness of the study,

annual vaccination programme in endemic areas by making use of the vaccine that incorporates the local serotype/s should be practiced. There should be vaccination strategy that indicates the degree of coverage and protective value of the vaccine. This is because there have been repeated outbreaks of AHS now and then in the present study areas that might probably be related to insufficient vaccination coverage and lack of feed back from field veterinary personnels. Since donkeys are affected by the disease subclinically, they are potential source of infection for horses and mules through insect bite. So all types of equines should be vaccinated against AHS.

- ❖ The mechanism of AHSV survival between interepidemics should be studied in detail and the precise reservoir host be properly identified.
- ❖ The seasonal occurrence, feeding behaviour, vector ecology and veterinary importance of *Culicoides* complex should be studied through an in depth approach and be well documented in order to institute economically feasible and practically applied AHS control interventions.
- ❖ Immediate outbreak reporting system to the relevant bodies should be in place.
- ❖ The epidemiological status of the disease should be studied in domestic as well as wild animal species.
- ❖ The present knowledge base of the disease among equine workers is not satisfactory. So there should be awareness creaction among the people through an organized extension package.

7. REFERENCES

Adeyefa, C. A., and Hamblin, C. (1995): Continuing prevalence of African horse sickness in Nigeria. *Rev. Elev. Med. Vet. Pays Trop*. **48** (1), 31-33.

Alemayehu, L. (2004): Case studies on reproductive activity of equines in relation to environmental factors in central Ethiopia. Humboldt University of Berlin: Books on Demand, GmbH, PhD thesis, p. 1.

Anon (1976): Handbook on Animal Diseases in the Tropics. 3rded. London: British Veterinary Association, pp. 1-3.

Anon (1998): Foreign Animal Diseases, United States Animal Health Association. Virginia: Carter Printing Company, pp.41-49.

Aschalew, Z., Teshale, S., Keith, P., Feseha, G., Bojia, E. (2005): Isolation and identification of circulating serotypes of African horse sickness virus in Ethiopia. In: USA, Veterinary Solutions LLC. *Intern. J. of Appl. Res. Vet. Med*, **3** (1), 40-42.

Baba, S.S., Akinyele, H.A., and Olaleye, O.D. (1992): Prevalence of complement-fixing antibody to the African horse sickness virus in domestic animals in Nigeria. *Beitr. Trop. Landwirtsch Veterinarmed.* **30** (4), 471-477.

CACC (2003): Statistical report on livestock and farm implements. Ethiopian Agricultural Sample Enumeration, 2001/2,Addis Ababa.

CACC (2003): Statistical report on livestock and farm implements. Ethiopian Agricultural sample enumeration, 2001/2, Oromia Region.

CACC (2003): Statistical report on livestock and farm implements. Ethiopian Agricultural sample enumeration, 2001/2. Amhara Region.

Coetzer, J.A.W., Thomson, G.R., and Tustin, R.C. (1994): Infectious Diseases of Livestock with Special Reference to South Africa. Vol.1, Cape Town: Oxford University Press, pp.460-472.

CSA (2004): Federal Democratic Republic of Ethiopia. Central Statistical Authority (CSA), Agricultural Sample Survey 2003/2004, Volume II, Report on Livestock and Livestock Characteristics (Private Peasant Holdings) Statistical Bulletin 302, Addis Ababa, June 2004.

EARO (1998): Animal Science Research Strategy and Short Term Plan. Executive Summary, August 1998, Addis Ababa, p.9.

Farouk, M. (1997): African horse sickness. In: The Merck Veterinary Manual (1997), 8th ed. Whitehouse station: Merck & Co. Inc., pp. 496-498.

Fenner, F., Bachmann, P.A., Gibbs, E.P.J., Murphy, F. A., Studdert, M. J., White, D. O. (1987): Veterinary Virology. United Kingdom Edition, London: Academic Press, Inc., pp.587-590.

Fenner, F.J., Gibbs, E.P.J., Murphy, F.A., Rott, R., Studdert, M. J., White, D.O. (1993): Veterinary Virology. 2nd ed., California: Academic Press, Inc., pp.547-549.

Feseha, G. (1998): Proceeding on Equine Infectious Diseases, 8th International conference on Equine Infectious Diseases. Dubai, April, 1998 pp. 318-319.

Galloway, J.H. (1974): Farm Animal Health and Disease Control. Philadelphia: Lea & Febiger, pp. 215-219.

Hall, H.T.B. (1985): Diseases and Parasites of Livestock in the Tropics. Intermediate Tropical Ag. Series, 2nd ed. Singapore: Print House, pp. 57-61.

Hanson, R.P. and Hanson, M.G. (1983): Animal Disease Control, Regional Programs, 1st ed. Ames, Iowa, Australia: the Iowa State University Press, p. 12.

Hungerford, T.G. (1975): Diseases of Livestock. 8th ed. University of Sydney, Auckland, Australia: McGraw-Hill Book Company, pp. 833-834.

Hunter, A. (1994): The Tropical Agriculturalist, Animal Health Vol.2, Specific Diseases. London: The Macmillan/ CTA Press Ltd. pp. 111-113.

Hunter, A. (1996): The Tropical Agriculturalist, Animal Heath Vol.1, General Principles. London: Macmillan/ CTA Education Ltd. pp. 49, 71.

Joklik, W.K. (1988): Virology 3rd ed. USA: Prentice-Hall International Editions, p. 32.

Keith, R.P. (2005): Survey into the factors affecting the health and welfare of donkeys and mules in Amhara and Tigray regions of northern Ethiopia. Report on the Health and Welfare of Donkeys and Mules to the Joint Venture of Addis Ababa University Faculty of Veterinary Medicine, Debre Zeit, Ethiopia and the Donkey Sanctuary, Sidmouth, Devon, England, UK, pp. 68-81.

Knipe, D. M. and Howley, P.M. (2001): Fields Virology, Vol.2, 4th ed. Philadelphia: Lippincott Williams & Wilkins, p. 1861.

Knowles, R. C. (1991): African horse sickness. In: Colahan, P. T., Mayhew, I. G., Merritt, A. M., Moore, J. N. (1991): Equine Medicine and Surgery, Vol.1, 4thed. California: American Veterinary Publications, Inc. pp. 378-380.

Mellor, P.S. and Hamblin, C. (2004): African horse sickness. http: // www. edpsciences. Org.

Mellor, P.S.; Baylis, M.; Hamblin, C.; Calisher, C. H.; Mertens, P.P.C. (ed) (1998): African horse sickness. Vol. 8, http: // www. aphis. usda. gov/ us/ep/ fad.

Ministry of Agriculture and Fisheries (1991): Surveillance. Exotic Disease Issue, MAF, Newzealand, 18 (3), 3-4.

Murphy, F.A., Gibbs, E.P.J., Horzinek, M.C., Studdert, M.J. (1999): Veterinary Virology, 3rd ed. California: Academic Press, pp. 400-402.

NVI (1974): National Veterinary Research Institute. *Rev. Elev. Med. Vet. Paystrop*., **36**(2), 117-129.

NVI (1983): The haematological changes of African horse sickness. Annual report, Debre Zeit, pp.1-11.

NVI (1983): The pathomorphological changes of African horse sickness. Annual report, Debre Zeit, pp.1-2.

OIE (2004): African horse sickness. In: Manual of Diagnostic Tests and Vaccine for Terrestrial Animals, 5th ed., Paris: Office International des Epizooties, pp. 1-21.

Pagot, J. (1992): Animal Production in the Tropics and Subtropics, 1st ed. The Netherlands: The Macmillan Press, pp. 233, 266.

Palya, V., Prandota, J., Rweyemamu, M.M., Sylla, D. (1994): Quality control testing of rinderpest cell culture vaccine. FAO Animal Production and Health Paper, Standard Operating Procedure (SOP), Pan African Veterinary Vaccine Centre (PANVAC), Addis Ababa, pp.1-78.

Personal communication (2005): Dr. Zelalem Tadesse, Ministry of Agriculture, Epidemiology Unit, Addis Ababa.

Proceeding of an International Seminar on Virus Diseases of Veterinary Importance in South-East Asia, held at Australian National Animal Health Laboratory, Geelong, Australia, 27-30 August 1984, Academic Press, pp.89-97. In: Della-Porta, A.J. (1985): Veterinary Viral Diseases, Their Significance in South-East Asia and the Western Pacific.

Radostits, O.M., Gay, C.C., Blood, D. C., Hinchcliff K.W. (2000): Veterinary Medicine, A textbook of the Diseases of Cattle, Sheep, Pigs, Goats, and Horses, 9th ed. London: W.B. Saunders Company Ltd. pp. 1038-1040.

Robinson, N. E. (1987): Current Therapy in Equine Medicine-2. Philadelphia: W.B. Saunders, pp. 582-583.

Seifert, H.S.H. (1996): Tropical Animal Health, 2nd ed. Dordrecht: CTA-Kluwer Academic Publishers, pp. 234-240.

Sewell, M.M.H., Brocklesby, D.W. (1990): Handbook on Animal Diseases in the Tropics, 4th ed. London: Bailliere Tindall, pp. 257-259.

Stata Corp. (2001): Stata statistical software, Release 7.0. Lakeway Drive, College station, Texas.

Thrusfield, M. (1995): Veterinary Epidemiology, 2nd ed. Edinburgh: Blackwell, p. 339.

Thrusfield, M., Ortega, C., Blas, I. DE., Noordhuizen, J.P., Frankena, K. (2001): Win Episcope 2.0. Improved epidemiological software for Veterinary Medicine. *Veterinary record*, **148**:567-572.

White, G. B. (1977): Man-biting species of *Chrysops* Meigen, *Culicoides* Latreille and *Simulium* Latreille in Ethiopia, with discussion of their vector potentialities. Department of Entomolgy, British Museum (Natural History), Transactions of the Royal Society of Tropical Medicine and Hygien, London, **71**(2), pp.164 and 171.

Williams, A.O. and Masiga, W.N. (1988): Viral Diseases of Animals in Africa. Lagos: OAU/STRC Scientific Publication, p. 174.

Wilson, R.T. (1995): The Tropical Agriculturalist. Livestock Production Systems, Distribution and Importance of Livestock in the Tropics, 1st ed. London: CTA-Macmillan, p. 5.

8. ANNEXES

Annex 1. Expression of results of spot test

The principle of the test is the interruption of the reaction between AHSV antigen and an immuned anti-AHSV guinea-pig serum by a test serum sample. AHSV antibodies in the test serum sample will compete with those in the immuned guinea-pig antiserum resulting in a reduction in the level of expected colour (following the addition of enzyme labelled anti guinea-pig antibody and substrate).

The optical density (OD) values should be converted to percentage inhibition (PI) values by using the following formula after the mean of the 4 guinea-pig control is determined:

$$\text{Percentage inhibition (PI)} = 100 - \frac{\text{OD of each test or control value}}{\text{Mean OD of Guinea-Pig Controls}} \times 100$$

The data expressed as OD values and PI values are used to determine whether or not the test has been performed within acceptable limits of variability and therefore, whether or not the test sera data may be accepted for any given microplate. The ranges which are acceptable for each of the controls are listed below:

	OD value range
Guinea-Pig Antibody control	>0.4 but <1.4
	PI value range
Conjugate control	>95
Guinea-Pig control	-20 to +20
Positive control serum	>50
Negative serum	-25 to +25

First level of acceptance:

Both intermediate OD values (disregard the highest and the lowest) for the guinea-pig control must be within the lower and upper limits. If not the plate must be rejected.

Second level of acceptance:
At least three of the quadruplicate wells for the guniea-pig control (PI) values must be within the limits set above. If more than one is incorrect the plate must be rejected.

Both of the duplicate Conjugate and Negative control (PI) values should be within the value range. Although failure to fall within the acceptable limits does not provide grounds for rejecting the plate, this provides a warning that background levels are increasing and fresh reagents should be used.

The Positive control, which has been titrated up column 1 of the ELISA plate, should record a 50% end-point of PI, that falls somewhere in the dilution range of 1:120 to 1:480. In some circumstances, the 50% end-point may fall outside of this range (>1:480), however a test can still be accepted provided the negative controls are negative and the test sera are also negative.

Acceptance of individual test sera data
The diagnostic threshold is set at 50% inhibition (50% PI). This is a reduction of 50% of the OD value shown in the guinea-pig control. Any PI value greater than or equal to 50% is considered positive.

To accept individual test sera results, both of the replicate PI values must fall either Above (*i.e.* positive) or below (*i.e.* negative) the 50% threshold. Test sera should be re-tested if their replicate PI values lie either side of 50% PI.

If both values are acceptable, the mean PI value for the two replicates is then calculated by dividing the sum of both values by 2. If the resulting value is greater than 50%, the sample is considered seropositive for AHS. In some circumstances, you may wish to repeat such samples on a titration test to determine the level of positivity, by calculating the 50% end-point.

Annex 2. C-ELISA result of AHSV in equidae of each study area.

Study areas	Result	Sampled equidae			
		Donkey	Horse	Mules	Total
Abenogebriel	Negative	1	-	-	1
	Positive	6	-	-	6
Ada'a	Negative	18	32	7	57
	Positive	11	3	6	20
Adama	Negative	-	24	-	24
	Positive	-	9	-	9
Addis Ababa	Negative	43	-	-	43
	Positive	7	-	-	7
Boset	Negative	44	9	-	53
	Positive	34	2	-	36
Chollie	Negative	43	10	-	53
	Positive	8	3	-	11
Debrebirhan	Negative	142	102	-	244
	Positive	19	1	-	20
Gellan	Negative	36	-	1	37
	Positive	24	-	-	24
Gerado	Negative	95	80	43	218
	Positive	-	-	-	-
Godino	Negative	29	-	1	30
	Positive	25	-	-	25
Guaworko	Negative	31	-	-	31
	Positive	29	-	-	29
Kijeri	Negative	25	6	-	31
	Positive	16	5	-	21
Kombolcha	Negative	-	79	-	79
	Positive	-	17	-	17
Kombollie	Negative	18	1	-	19
	Positive	19	-	-	19
Mendella	Negative	31	-	-	31
	Positive	25	-	-	25
Tejitu	Negative	23	-	-	23
	Positive	22	-	-	22
Total		824	383	58	1265

Annex 3. C-ELISA result of AHSV in different ages of equidae.

Age (yrs)	Types of equidae								
	Donkey			Horse			Mules		
	Nega.	Post.	%	Neg.	Post.	%	Nega.	Post.	%
1	20	5	20	1	-	-	2	-	-
2	34	11	24	7	-	-	1	-	-
3	31	14	45	15	3	17	6	1	14
4	49	11	18	21	1	5	1	-	-
5	29	8	22	11	2	15	3	-	-
6	49	35	42	22	5	19	5	1	17
7	43	25	37	21	3	13	5	-	-
8	93	22	19	23	3	12	4	1	20
9	45	24	35	15	1	6	2	-	-
10	52	25	32	33	-	-	5	1	17
11	25	11	31	8	1	11	2	-	-
12	36	22	38	32	1	3	1	-	-
13	13	6	32	11	2	15	3	-	-
14	22	8	27	24	3	11	2	1	33
15	17	4	19	17	1	6	3	-	-
16	8	7	47	30	5	14	2	1	33
17	5	3	38	7	1	13	1	-	-
18	2	2	50	22	1	4	1	-	-
19	1	-	-	7	2	22	-	-	-
20	5	2	29	16	5	24	3	-	-

Annex 4. Questionnaire format to assess the indigenous knowledge of equine owners

1.Owner's address: Name _____

Region_____Zone_____District_____

Peasant association_____

2. Equine owning status: Donkey____Horse_____Mule_____

3. Do you know the disease of equidae characterized by supraorbital oedema and dullness?

A / yes B / no

4. Which type of equines is more affected? A / Donkeys B / Horses C / Mules

5. Which age groups of equines are more affected? A / young age B / Middle age

C / Old age D / Difficult to define

6. Which sex of equines is more affected? A / Female B / Male

7. Can you tell the agro-ecology in which the disease is frequently seen?

8. Can you tell the mode of its transmission?

9. Are there equine biting insects in your locality? A / yes B / no

10.Can you mention their local names?

11. Which months of the year that flies are abundant?

12. Do you stable your equine/s during the night? A / yes B / no

13. What is the type of your stable in use? A / intact wall with cover of roof B / semi-intact wall with cover of roof C/ round fence but no cover of roof (Beret)

14. Are there water bodies near to your locality? A / yes, there are lakes, rivers etc. B / there are no water bodies

15. Is there equine movement? If yes, from where to where?

9. CURRICULUM VITAE

1. Personal data

Name:	Kassa Demissie Abdi
Date of birth:	April 12, 1964
Nationality:	Ethiopian
Marital status:	Married
Children:	None
Religion:	Orthodox Christian
Position:	Field veterinarian, Team leader, District Livestock Project Coordinator
Membership:	Ethiopian Veterinary Association
Office contact:	E-mail: kassa_demissie@yahoo.com Mobile 0911 99 32 42

2. Educational background

September 1972 – July 19976 E.C	Elementary and Junior education at Deneba Junior Secondary School
September 1977 – July 1980 E.C	High school education at Hailemariam Mamo Senior Comprehensive Secondary School, Achievement: ESLCE Certificate
September 1981 – July 1981 E.C	Addis Ababa University, Science Faculty
September 1982 – July 1987 E.C	Addis Ababa University, Faculty of Veterinary Medicine Achievement: Degree of Veterinary Medicine (DVM)

3. Work experience

19 May 1988 – 2 March 1992 E.C	Field veterinarian at Metema District Off. Of Agriculture
25 March 1990 -24 February 1992 E.C	Regulatory and protection acting team leader at Metema District Office of Agriculture
3 March 1992 – 1 September 1997 E.C	Field veterinarian at Chilga district Office of Agriculture
21 March 1992 – 1 September 1997 E .C	District coordinator of Integrated Livestock Development Project
1 September 1995 – 1 September 1997E.C	Team leader of livestock, Veterinary and fisheries resource Development
1 September 1995 – 1 September 1997E.C	District coordinator of National Livestock Development Project And UK Overseas bee keeping Development Project

4. Research Papers

Camel-Cephalopsis: The Epidemiology and Biodaynamics in dromedary of Eastern Ethiopia, DVM thesis (1987), FVM, AAU.

Feed resource inventory at Chilga district, ILDP booklet number one, Gondar.

African horse sickness: Seroprevalence and Identification of risk factors in Equidae at selected sites in Ethiopia, MSc.thesis (2006), FVM, AAU.

5. Additional Training:

1. Continuing Professional Development Training course organized by the Donkey Sanctuary Working Worldwide and Donkey Health and Welfare Project of Ethiopia (from 19 to 23 March, 2001).

2. Applied sampling methods, socio-economic and agricultural research data analysis and interpretation using SPSS and MSTATC at ILDP head quarter office (9-13 March 2002).

3. Forage and seed production, and inventory held at ILDP head quarter office, Gondar (15-20 April 2002).

4. Participatory Rural Appraisal organized by ILDP head quarter office and the Ethiopian Management Institute, held at gorgora port, Gondar (23 Dec.2002 to 1 Jan 2003).

5. Educational tour to National Dairy Development Board of India (NDDB), Anand , Gujirat state, organized by ILDP and AEDC (1 July – 26 August 2003).

5. Reference Persons

1. Professor Feseha Gebreab: Addis Ababa University Faculty of Veterinary Medicine, Consultant of the Donkey Sanctuary Working World Wide, Ethiopia, P.o.Box 34, Tel. 0114 33 85 33, 0114 33 03 04, 0114 33 93 26.
2. Dr. Kelay Belihu, Addis Ababa University Faculty of Veterinary Medicine, Associate Dean for research and graduate programmes, P.O. Box 34, Tel. 0114 33 85 33.
3. Dr. Yilkal Asfaw, Addis Ababa University Faculty of Veterinary Medicine, P.O.Box 34, Tel. 0114 33 85 33.

10. SIGNED DECLARATION SHEET

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

Name Kassa Demissie Abdi

Signature _____

Date of submission _____

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

Dr. Yilkal Asfaw _____

Prof. Feseha Gebreab _____

Dr. Berhe Gebreegziabher _____