SEROPREVALENCE OF AFRICAN HORSE SICKNESS VIRUS IN HORSES IN KADUNA STATE, NIGERIA

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FEBRUARY, 2013

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By

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MSc/VET-MED/17481/07-08

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

IN PARTIAL FULFILLMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN EQUINE MEDICINE

DEPARTMENT OF VETERINARY MEDICINE,

FACULTY OF VETERINARY MEDICINE,

AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

FEBRUARY, 2013

DECLARATION

I declare that the work in the thesis entitled "**SEROPREVALENCE OF AFRICAN HORSE SICKNESS VIRUS IN HORSES IN KADUNA STATE, NIGERIA"** has been performed by me in the Department of Veterinary Surgery and Medicine under the supervision of Prof. I. A. Abdulkadir (Chairman) Dr S.N.A Saidu (Member) and Dr David Shamaki (Member).

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work was previously presented for another degree or diploma at any University.

Wayuta Philip MSHELIA		
(Name of student)	**Signature**	**Date**

CERTIFICATION

This thesis entitled "Seroprevalence of African Horse Sickness Virus in Horses in Kaduna State, Nigeria" by Wayuta Mshelia Philip, meets the regulation governing the award of the degree of Master of Science of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my Wife, Blessing Philip-Mshelia.

"Her price is far above rubies"

ACKNOWLEDGEMENT

I give God the glory for the gift of life and for keeping me through those years of academic turmoil. I also want to appreciate God for giving me the opportunity to present the fruits of my intellectual labour. My appreciation goes to my cheerful wife who possesses an innate sense of balance and discernment, who personally assisted me in making this work a success. To my Parents, Mr. and Mrs. Wayuta Miduku Mshelia who taught me virtues that have remained indelible to my life, I am grateful.

My heartfelt appreciation goes to my supervisors Professor I. A. Abdulkadir, Dr S.N.A Saidu and Dr D. Shamaki for meticulously supervising this work.

My profound gratitude goes to my Head of Department, Professor A.K.B Sackey for his concern and encouragement. I am also indebted to Dr F. Fasina, Animal Production Section, University of Pretoria and Dr D.D Lazarus, National Veterinary Research Institute (NVRI), Vom, Nigeria for their valuable support- a particular kindness.

My profound gratitude goes to a vibrant group of students I cherish so much Richard Edeh, Sunday Agbonika, Danjuma Isiyaku and Ibrahim Josiah whom despite all odds, assisted me during this work.

I appreciate my friends and colleagues, my late friend and brother, Dr Samdi Musa, my family members and their wives, also my in- laws-thanks for being there for me.

ABSTRACT

A study was conducted in the seven Local Government Areas of Kaduna state, Nigeria to determine the prevalence and the various horse management and environmental factors associated with AHS viral infection in horses in Kaduna state using I-ELISA test. A total of 183 horses in 50 stables were used in the study. Out of the 110 of the non vaccinated horses sampled 102 (92.7%) were seropositive. There was a significant difference ($P< 0.05$) in serum titre value of the horses tested with regards to breeds, sex, use of horses, and vaccination status. While there was no significant ($P>0.05$) difference between the different ages of horses studied. The high titre levels as seen in this study could indicate that the horses might have long standing infection since I-ELISA detects Ig G. The high titre value could also mean that AHS may be endemic in the study area. The endemicity could be as a result of the presence of the insect vectors due to the favourable conditions that make these vectors to survive optimally as shown in the study. The husbandry practices as practiced by the stables seemed to be adequate, though disease prevention and control practices were found to be inadequate as to prevent the presence of the vectors, let alone control them. The stable managers and grooms had close working relations with their consulting veterinarians as many of the stables reported vaccinating their horses. Six of the consulting veterinarians to these stables reported experiencing outbreaks of AHS. The study has showed that the AHS virus seems to be circulating among horses in the study area as non-vaccinated horses were seropositive with titres as high as 243 even in the presence of a seemingly adequate husbandry and management practices.

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ABREVIATIONS

AHS	-	African Horse Sickness
AHSV	-	African Horse Sickness Test
VNT	-	Virus Neutralization Test
SNT	-	Serum Neutralization Test
RIM	-	Resource Inventory and Management Limited, UK
FAO	-	Food and Agricultural Organization
BTV	-	Blue Tongue Virus
RNA	-	Ribonucleic Acid
RT-PCR	-	Real Time Polymarase Chain Reaction
VP7	-	Viral Protein Seven
I-ELISA	-	Indirect – Enzyme – Linked Immunosorbent Assay
pH	-	Hydrogen Potency
OCG	-	Sodium Oxalate, Carborlic acid and Glycerine
EEV	-	Equine Encephalitis Virus
EHDV	-	Epizootic hemorrhagic disease virus
PHSV	-	Peruvian Horse Sickness Virus
TCID	-	Tissue Culture Infective Dose
OIE	-	Office International Des Epizootics
FCT	-	Federal Capital Territory
LGA	-	Local Government Area
NVRI	-	National Veterinary Research Institute
USA	-	United States of America
IgG	-	Immunoglobulin G
VN	-	Viral Neutralization
PI	-	Post Infection
PP	-	Cut-off Point
CF	-	Complement Fixation

CHAPTER ONE

INTRODUCTION

1.1 Background

The population of horses in Nigeria is estimated at 200,000 (RIM, 1992). Garba, (2006) reported different breeds of horses in Kaduna state namely, Argentine Polo Ponies, South Africans and the Local breeds which originated from Arabian breeds. Dent and Hendricks (2001) reported that the Nigerian breed of horse is a member of the group of horses often known collectively as West African Dongola or West African Barb. Horses of this classification are often called "degenerate Barbs" and are known to have various admixtures of Barb blood. It is possible the Nigerian Horse has genetic connections to the pony mousseye of Cameroon (Dent and Hendricks, 2001).

Horses play very important roles in Nigeria. Horses were used for ceremonial processions in towns. They are also used for pleasure riding, polo, racing, durbar and traditional ceremonies, and are also kept by mounted police and army for defense and security operations (RIM, 1992). A few horses are still kept to run sugar-mills in the Zaria area (RIM, 1992).

African Horse Sickness has been reported to be a source of major loss to the equine industry in Nigeria, especially in the Northern states where the disease has a significant negative impact on the health and welfare of horses (Mshelia *et al.,* 2010). Fasina *et al* (2008) reported that illegal importation with no regards for import control and quarantine favoured the introduction and spread of the AHS virus in Nigeria. Field investigations have revealed the presence of biting midges at horse stables and warm and damp environment that favour the development of the midges are present in

various part of the country where outbreaks have been reported. The presence of AHS serotype 2 with wide geographic distribution in Kaduna, Kano, Katsina, Ibadan, Yola and Lagos has severe socioeconomic implications.

1.2 African Horse Sickness

Coetzer and Guthrie, (2004) reported that African horse sickness (AHS) is a devastating insect-borne viral disease of equids that occurs throughout sub-Sahara Africa. It is non-contagious and the virus is spread by hematophagous *culicoides* insects. The disease has periodically occurred in North Africa, the Mediterranean Basin and the Middle East (Mellor and Hambling, 2004). Regular outbreaks of AHS have occurred in Southern Africa since its introduction to the region following European settlement of the Cape of Good Hope several centuries ago (Mellor and Hambling, 2004). It has been shown to economically cripple the region because it compromised the lucrative export sales of horses to the British army in India throughout the latter part of the 19th century, and it was a substantial obstacle to the British army during the wars against the Zulus and in the South African war of 1899-1902 (Gilfoyle, 2006). Similarly AHS was reported to have a severe negative socioeconomic effect within the equestrian community in Nigeria. (Mshelia *et al*., 2010 and Fasina *et al.,* 2008).

Information on the epizootiology of AHS in Nigeria is very scanty (Baba *et al*., 1993). Sporadic cases of the disease have been reported. (Kemp *et al.,* 1971; Abegunde, 1975; Parker *et al.,* 1977). Equine deaths, presumably due to AHS, have been reported in different parts of Northern Nigeria since 1931 (Kemp *et al.,* 1971; Abegunde, 1975; Best *et al.,* 1975).

1.3 Statement of the Problem

Epidemiological surveys among equine populations and virus isolation in Nigeria have shown that the virus is active in Nigeria (Howell, 1963; Kemp *et al.,* 1971; Parker *et al.,* 1977; Nawathe *et al.,*1981; Fasina *et al.,*2008). Despite the presence of the vertebrate and invertebrate hosts and other ecological factors that favour the establishment of AHS in Nigeria, the extent of the virus activity in equine population has not been adequately investigated (Baba *et al*.,1993). Currently, the true field situation of AHSV in West Africa does not seem to be known either. (Fasina *et al.,* 2008).

1.4 Justification

The official equine population figure in Nigeria was given as 200,000 (RIM, 1992 and FAO, 2003).

Horses have social and economic value in Nigeria as they are used for polo, racing, traditional ceremonies and other festivals. In the early days Zaria was reported to be a national and international centre of attraction for durbar, polo and racing activities. A few horses are still kept to run the sugar-mills in the Zaria area (RIM, 1992).

Multiple health problems like AHS, ulcerative lymphangitis and equine piroplasmosis have been reported to have adverse effects in the equine cycle in Nigeria, especially in the Northern states where such diseases exerts a significant impact on the use of horses in the states (Mshelia *et al.,* 2010).With the seeming paucity of information on AHS among the local breeds in Nigeria, there is a need to determine the prevalence of the disease in both imported and indigenous horses. This will help in maintaining a healthy

equine population, considering the increasing interest in polo, racing and ceremonial activities involving horses in Nigeria.

1.5 Aim

To determine the distribution of AHS virus in Kaduna State

1.6 Objectives

- To determine the prevalence of AHS antibodies in horses according to breed, age, sex, use, vaccination status and location in Kaduna State.
- To determine the various horse management and environmental factors associated with AHS viral infection in horses in Kaduna state.

CHAPTER TWO

LITERATURE REVIEW

Guthrie, (2007) and Guthrie and Quan, (2009) have defined African horse sickness (AHS) as a non-contagious, insect-transmitted disease of equids caused by AHSV. Mules are less susceptible than horses while African donkeys and zebras rarely show clinical signs of the disease (Quan *et al.,* 2008).

Mellor and Hamblin, (2004) reported that the effects of the disease, particularly in susceptible populations of horses can be devastating and mortality rates for this species of animals may exceed 90% in non-endemic areas. As a consequence of its severity and because it is able to expand rapidly out of its endemic areas, AHS has been accorded the OIE (Office International des Epizooties) List A status (OIE, 2012).

The disease is characterized by clinical signs, which develop as a result of damage to the circulatory and respiratory systems giving rise to serous effusion and haemorrhage in various organs and tissues. The extent and severity of the clinico-pathological findings have been used to classify the disease into four forms. In ascending order of severity, these are horse sickness fever (mild form) which usually affects only mules, donkeys and partially immune horses, the subacute or cardiac form (dikkop), the cardio-pulmonary or mixed form and the peracute or pulmonary form (dunkop). All forms of the disease can occur in any one outbreak. However, in susceptible populations of horses the mixed and pulmonary forms tend to predominate. Mortality rates in these animals can be very high (Mellor and Hamblin, 2004). The clinical signs occurring in naturally and experimentally infected horses have been described in detail by various

workers (Theiler, 1921; Maurer and McCully,1963; Awad *et al*.,1981; Newsholme *et al.,* 1983; Lubroth,1988; Coetzer and Erasmus, 1994).

2.1 History

African horse sickness is an ancient disease of horses and the first report of the disease was in Yemen in 1327 (Moule, 1896 as quoted by Mellor and Hamblin, 2004).

The first observation on AHS was by Father Monclaro in 1569 (Theal, 1899). However, the virus most certainly originated in Africa and was first recognized there subsequent to the introduction of horses during the exploration of Central and East Africa, (Mellor and Hamblin, 2004). In South Africa the virus has probably been present from distant times though the disease was not recognised until some 60 years after the first introduction of horses in 1657 (Henning, 1956). The first major outbreak of the disease in South Africa was in 1719 when over 1,700 horses died (Henning, 1956). Subsequently, outbreaks of the disease between 1854— 1855 where over 70 000 horses were reported to have died in Southern Africa (Coetzer and Erasmus,1994; Barnard, 1998). The frequency, extent and severity of outbreaks in South Africa have declined significantly over the last century, coinciding with major decrease in the horse and zebra populations and the introduction of vaccines. (Mellor and Hamblin, 2004).

The disease was first reported in Nigeria in 1971 by Kemp and his colleagues (Kemp, *et al*., 1971). The disease was reported in an epizootic proportion among the imported horses (Kwanashie and Abdullahi, 1991; Oladosu *et al*., 1993). Recently Fasina *et al*., (2008) reported outbreaks of AHS due to AHSV serotype 2 in Ibadan and Lagos

between December 2006 and January, 2007.This seemed to be the first report of serotype 2 in the northern hemisphere (Fasina et al.,2008).

2.2 Aetiology

African horse sickness (AHS) is a viral disease of equidae, caused by an arbovirus that is closely related to bluetongue virus (BTV). (Mellor and Hamblin, 2004).

The virion is an unenveloped particle of about 70 nm in diameter and is made up of a two- layered icosahedral capsid, which is composed of 32 capsomeres (Coetzer and Erasmus, 1994). The genome comprises of 10 double stranded RNA segments, each of which encodes at least one polypeptide (Grubman and Lewis, 1992). The genome is enclosed within the core particle, which comprises of two major proteins, VP3 and VP7 that are highly conserved among the nine AHSV serotypes, and three minor proteins, VP 1, VP4 and VP6 (Oellerman *et al.,*1970; Bremer *et al.*,1990; Roy *et al.,* 1991; Grimes *et al*., 1995). Together they make up the group specific epitopes (Grimes *et al*., 1995; Hewat, *et al.,* 1992, Prasad *et al.,* 1992). The core particle is surrounded by the outer capsid which is composed of two proteins VP2 and VP5. The VP2 is the protein that is responsible for antigenic variation (Burrage *et al.,* 1993; Vreede and Huismans, 1994; Martinez-Torrecuadrada and Casal 1995). At least three non-structural proteins have been identified in infected cells (NS 1, 2 and 3/3a) (Huismans and Els, 1979; Devaney, *et al.,* 1988; Laviada *et al.,* 1995).

To date, nine antigenically distinct serotypes have been identified; serotype 9 was identified in 1960 (Howell, 1962). There is cross relation among the serotypes, typically between AHSV types 1 and 2; 3 and 7; 5 and 8; and 6 and 9, although there is no

evidence from the field that there is any intratypic variation (Coetzer, and Erasmus, 1994). Of the nine serotypes, types 1 to 8 are typically found only in restricted areas of sub-Saharan Africa while type 9 is more widespread and has been responsible for virtually all epidemics outside Africa. The only exception being the 1987-1990 Spanish-Portuguese outbreaks which were due to AHSV-4 (Coetzer, and Erasmus,1994).

The physio-chemical characteristics of AHSV are fairly typical for the genus *Orbivirus.* (Mirchamsy and Hazrati, 1973; Coetzer and Erasmus, 1994;) The virus is acid sensitive, readily inactivated at pH values 6.0 but remains generally stable at more alkaline pH values (7.0-8.5) (Mirchamsy and Hazrati,1973; Coetzer and Erasmus, 1994). It is resistant to lipid solvents and relatively heat resistant. (Mirchamsy and Hazrati,1973; Coetzer and Erasmus, 1994). Infectivity is remarkably stable at 4 °C, particularly in the presence of stabilisers such as serum and sodium oxalate, carbolic acid and glycerine (OCG). Minimal loss of titre occurs with viruses that have been lyophilised or frozen at -70 °C with Parker Davis Medium though the viruses are fairly labile between -20°C and -30 °C (Mirchamsy and Hazrati,1973;Coetzer and Erasmus, 1994).

2.3 Transmission

The virus (AHSV) is transmitted by biting midges of the genus *Culicoides,* which become infected after ingesting a blood meal from an infected and viraemic host (Mellor and Hamblin, 2004). Sporadic outbreaks of AHS have been reported to have devastating effects in North Africa, Europe, Middle East and India (Howell, 1960; Rafyi,1961; Diaz and Panos,1967; Verwoerd *et al.,* 1979; Stanley, 1981; Spence *et al.,*1984; Rodriguez *et al*.,1992).

2.4 Re-emergence of African Horse Sickness and other Orbiviruses

Arthropod-transmitted viruses are important causes of disease in humans and animals, and it is proposed that climate change increase the distribution and severity of arboviral diseases. Arbiviruses are the cause of important and apparently emerging arboviral diseases of livestock, including bluetongue virus (BTV), African horse sickness virus (AHSV), equine encephalitis virus (EEV), and epizootic hemorrhagic disease virus (EHDV) that are all transmitted by haematophagous *Culicoides*. The global and seasonal distribution of individual viruses coincide with that of their specific biological vectors and appropriate climatic conditions. (MacLachlan and Guthrie, 2010). Bluetongue virus (BTV) and African horse sickness virus (AHSV) are the most economically important members of this genus, while several others like, Equine encephalitis virus (EEV), Epizootic hemorrhagic disease virus (EHDV), Palyam virus, and Peruvian horse sickness virus (PHSV) are potentially important, either regionally or globally (Howell *et al.,* 2004; Coetzer and Guthrie, 2004; Verwoerd and Erasmus, 2004; MacLachlan and Osburn, 2004; MacLachlan and Guthrie, 2010).

Although there have been somewhat less dramatic recent alterations in the distribution of EHDV, AHSV, and EEV, it is not yet clear what the future holds in terms of these diseases, nor of other potentially important but poorly characterized Arbiviruses such as Peruvian horse sickness virus (MacLachlan and Guthrie, 2010).

2.5 Host Range

The zebra have long been considered as the natural vertebrate host and reservoir of AHSV, and are believed to play a vital role in the persistence of the virus in Africa. (Mellor and Hamblin, 2004). This species rarely exhibits clinical signs of infection. All other equid species and crossbreeds are also susceptible to the infection. Because of the high mortality rate frequently recorded in horses and occasionally mules these species should be regarded as accidental or indicator hosts. (Mellor and Hamblin, 2004).

It has been shown that dogs are susceptible to experimental infection with AHSV and may die from the effects of the virus. (Van Rensburg *et al.,* 1981). Infection also occurs readily following consumption of infected horsemeat by dogs (Van Rensburg *et al.,* 1981).

Infection in camels is rare and inapparent and no details are available on the level and duration of viraemia, if any, in them (Awad *et al.,* 1981). It is likely that, like the dog, this species has no significant role in the epidemiology of AHS (Awad *et al.,* 1981).

2.6 Pathogenesis

On entry into the vertebrate host following deposition of the virus by insect vector, initial multiplication of AHSV occurs in the regional lymph nodes. This is followed by dissemination of the virus throughout the body via the blood stream (primary viraemia) and subsequent infection of target organs and cells, namely the lungs, spleen and other lymphoid organs, and certain endothelial cells like squamous cells of the posterior portion of the cornea anterior surface of the iris (Coetzer and Erasmus, 1994). Virus

multiplication goes back to lymphoid tissue and then to blood which gives rise to secondary viraemia. (Mellor,1994). Under natural conditions the incubation period to the commencement of secondary viraemia is less than nine days although experimentally it has been shown to vary between 2 and 21 days, (Mellor, 1994). In donkeys and zebra the levels of viraemia are lower (< 103.0 TCID50ImL) and viraemia may extend for up to four weeks (Coetzer and Erasmus, 1994; Hamblin *et al.,* 1998).

In experimentally infected horses, high concentrations of AHSV rapidly accumulate in the spleen, lungs, caecum, pharynx, choroid plexus and most lymph nodes. (Theiler, 1921; Henning,1956). Subsequently the virus is found in most organs, due to their blood content. (Theiler, 1921; Henning, 1956). In the blood, the virus is associated with the cellular fraction (especially buffy coat) while very little is present in the plasma (Theiler, 1921; Henning, 1956; Laegreid, 1996*)*. This may be similar to the situation that occurs with the related blue tongue virus of ruminants where the virus is sequestered in the cell membrane of infected red blood cells and is thereby protected from the effects of humoral antibody. (Theiler, 1921; Coetzer and Erasmus, 1994). In the process viraemia, a situation that seems not to occur with AHSV in horses although viraemia in the presence of circulating antibody has been reported in zebra (Theiler, 1921; Coetzer and Erasmus , 1994,). In AHS the onset of viraemia always corresponds with the appearance of fever (Laegieid *et al.,* 1993).

Wohlsein *et al.* (1998) have shown that in experimentally infected horses with the per-acute form of the disease, the antigen is found primarily in the cardio-vascular and lymphatic systems, and to a lesser extent throughout the body. In animals with horse sickness fever the antigen is concentrated in the spleen and in lesser amounts elsewhere.

2.7 Clinical Signs

African horse sickness can cause four forms of the disease: horse sickness fever (mild form), cardiac form (subacute or dikkop), mixed form and the pulmonary form (dunkop). (Erasmus, 1976). Horse sickness fever is invariably mild, usually involving only mild to moderate fever and oedema of the supraorbital fossae and there is usually no mortality (Howell, 1963). It usually occurs following infection with the less virulent strains of the virus or as a result of immunization. It is the common form of disease exhibited by the African donkey and zebra (Howell, 1963).

The cardiac or sub-acute (dikkop) form of the disease is characterised by fever that may last for several weeks. The main clinical finding here is subcutaneous oedema, particularly of the head, neck, chest and the supraorbital fossae. Oedema is not usually seen in the lower limbs. Gravitated oedema has been seen in pulmonary and cardiac forms.So oedema may be seen in both dunkop and dikkop forms. The conjunctivae may be congested and that petechial haemorrhages may be seen in the eyes while ecchymotic haemorrhages may be seen on the ventral surface of the tongue. Colic is often a feature and mortality rates may exceed 50% (Coetzer and Erasmus, 1994).

The mixed form is often the most common form and is characterized by a combination of the cardiac and pulmonary forms of the disease. The mortality rate is approximately 70% and death usually occurs within 3 to 6 days after the onset of fever (Coetzer and Erasmus, 1994).

The pulmonary form (dunkop) has been reported by Coetzer and Erasmus, (1994) and Mellor, (1994). This form is peracute and may develop so rapidly that an animal can die

without previous indication of illness. Usually there will be marked depression and fever (39-41 °C) followed by onset of respiratory distress and severe dyspnoea. Coughing spasms may occur. The head and neck are extended and severe sweating develops. There may be periods of recumbency at terminal stage frothy fluid may be discharged from the nares. Anorexia is not a feature. The prognosis for horses suffering from this form of the disease is extremely poor and mortality rates commonly exceed *95%.* The pulmonary form is also the form most usually seen in dogs (Coetzer and Erasmus, 1994).

2.8 Pathology

2.8.1 Gross pathology

These vary in accordance with the different forms of the disease. In the pulmonary from the most conspicuous lesions are interlobular oedema of the lungs and hydrothorax. The sub-pleural and interlobular tissues are infiltrated with a yellowish gelatinous exudate and froth. Ascites and hydrothorax may occur while the mucosa of the stomach may be hyperaemic and oedematous (Mellor and Hamblin 2004).

In the cardiac form, the most prominent lesions are gelatinous exudate in the subcutaneous, sub-fascial and intramuscular tissues and lymph nodes. Hydropericardium is seen and haemorrhages are found on the epicardial and endocardial surfaces. Petechial haemorrhages and cyanosis also occur on the serosal surfaces of the caecum and colon. In the mixed form of AHS, lesions common to both the pulmonary and cardiac forms of the disease occur (Mellor and Hamblin 2004).

2.8.2 Histopathology

Maurer and McCully, (1963), Mirchamsy and Hazrati, 1973 Hess, (1988), have documented the histopathological changes commonly seen in the disease. They reported increased permeability of the capillary walls and consequent impairment of blood circulation. The lung is said to exhibit strong serous infiltration of the interlobular tissues with distension of the alveoli and capillary congestion. Cellular infiltration can be seen in the cortex of the kidneys while the spleen is heavily congested. Congestion may also be seen in the intestinal and gastric mucosae, and cloudy swelling due to fibrin in the myocardial and skeletal muscles.

2.9 Epidemiology

2.9.1 Distribution of AHSV

At present the 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, and extending as far south as the Northern border of South Africa (Howell, 1963; Mellor and Boorman, 1995). Sailleau *et al.* (2000) reported that the Sahara desert, however, seems to provide an effective geographical barrier which has prevented the virus from establishing itself permanently in Northern Africa or beyond. The virus may also be endemic outside Africa, as in Yemen in the Arabian Peninsula. Its current status has not yet been reported.

2.9.2 AHSV in South Africa

Barnard, (1998) reported that in recent historic times AHS has been endemic across virtually the whole of Southern Africa and that outbreaks of the disease in horses have been recorded since 19th Century and into the first few decades of the 20th Century. However, the decline in the annual number of AHS outbreaks over this period, particularly in the southern areas of South Africa, seems to have coincided with the elimination of the AHSV, through hunting of the large free-ranging populations of zebra considered to be the reservoir host for AHSV. In the northeast of South Africa seroconversion to AHSV occurs in zebra in every month of the year and virtually all adult animals have specific antibodies to all the nine serotypes of the virus (Mellor and Hamblin, 2004). At present populations of zebra in South Africa comprises of isolated herd of less than 100 individuals. Each of the herd is presumably insufficient to maintain AHSV throughout the year as the virus only occurs occasionally in these areas (Barnard, 1998). As restocking of zebra is reported to be taking place in many parts of South Africa it may well be that at some ill-defined population density, conditions for the re-establishment of permanent foci of AHSV across the country may be generated (Barnard, 1998).

2.9.3 AHSV outside sub-Saharan Africa

Until relatively recently AHSV was believed to be confined to sub-Saharan Africa except for occasional incursions into North Africa or the Arabian Peninsula (Rafyi 1961; Mirchamsy and Hazrati, 1973). However, between 1959 and 1961 AHSV serotype 9 spread out of Africa to Saudi Arabia, Syria, Lebanon, Jordan, Iraq, Turkey,

Cyprus, Iran, Afghanistan, Pakistan and India (Howell, 1960; Rafyi, 1961; Howell, 1963; Gohre *et al.,* 1965; Mirchamsy and Hazrati, 1973;). Nevertheless, by the end of 1961, in the face of a massive vaccination campaign and the death of over 300, 000 horses, the disease in Asia was regarded to have diminished (Anwar and Qureshi, 1972). The inability of the virus to persist in these regions was probably due to a combination of factors including vaccination, vector control campaigns as against the disease and adverse climatic conditions that reduced or prevented adult vector activity during the winter periods (Mellor, 1993). It was also the case that the mortality rate from AHS was so high that much of the area was virtually depopulated of susceptible equids. (Mellor and Hamblin, 2004).

In 1965, AHSV-9 again spread beyond its sub-Saharan endemic zones and appeared first in Morocco, then spread into Algeria and Tunisia before crossing the Straits of Gibraltar into Cadiz Province, Spain, in October 1966 (Diaz Montilla and Panos Marti, 1967; Mornet *et al.,* 1967; Diaz Montilla and Panos Marti, 1968; Laaberki, 1969). Diaz Montilla and Panos Marti, (1968) reported that the virus was eliminated from Spain within three weeks, following the application of a vigorous vaccination and slaughter policy. However, the disease persisted in North Africa for almost two years The appearance of AHSV in North Africa was thought to be due to the movement of nomads and their animals, particularly donkeys, across the Sahara from West Africa where AHSV-9 is endemic (Maurice and Provost, 1976; Pilo-Moron, et al., 1969). Before 1966, AHSV was reported only from sub-Saharan Africa for over 20 years. (Lubroth, 1988). However, in July 1987 an outbreak of the disease due to serotype 4 of the virus was reported in the Provinces of Madrid, Toledo and Avila in central Spain

(Lubroth, 1988). The outbreak was apparently caused by the importation of a number of sub-clinically infected zebra from Namibia into a Safari park at Aldea del Fresno, some 45 km Southwest of Madrid (Lubroth, 1988). This park subsequently became the site of the first 27 cases of AHS in Spanish horses in 1987. The epidemic continued for 3 to 4 months in central Spain and then apparently ended during October (Diaz Yubero, 1987). The 1987 AHS outbreak in Spain caused some alarm for fear of its extending into the whole of Europe. Consequently, the apparent end of the epidemic in October 1987 was in line with expectations and was assumed to be the end of the matter. However, this was not the case as more severe outbreaks occurred in Spain during 1988, 1989 and 1990, in Portugal in 1989 and in Morocco in 1989, 1990 and 1991 (Mellor, 1993). All these outbreaks were due to AHSV-4, a serotype that had never previously been seen outside Southern Africa (Mellor, 1993). During the course of the outbreaks no evidence of any serotype was documented within 2000 km of Spain or northern Morocco. So it seemed certain that the virus had persisted in the area for at least five years overwintering. At the time this situation was regarded to be unprecedented and led to much speculation on how and why the epidemiological situation had changed (Mellor and Hamblin, 2004).

It is now known that AHSV is able to overwinter in southern Spain and Portugal, and in Morocco as an efficient vector species of Culicoides is present in these regions (MeIlor *et al*., 1994; Bouayoune, *et al.,* 1998; Capela, *et al.,* 2003;). Further the climate is sufficiently mild for the adults of this vector to be active throughout the year (MeIlor *et al*., 1994; Bouayoune, *et al.,* 1998; Capela, *et al.,* 2003).

2.10 AHS and Serological Survey in Nigeria

2.10.1 AHSV in Nigeria.

African horse sickness was mentioned in the annual reports of the Northern Nigerian Veterinary Division in the early 1950's (Kemp, *et al*., 1971). It was not until the late 1960's and early 1970's that the disease attracted the attention of veterinarians (Kemp, *et al*., 1971; Alhaji *et al*., 1974; Kemp, 1974; Abegunde, 1975; Parker *et al*., 1977). The disease was first officially reported in Nigeria in 1971 by Kemp and his colleagues (Kemp, *et al*., 1971). The disease has been reported in almost epizootic proportion among the imported stock of horses (Abdulkadir, 1989; Kwanashie and Abdullahi, 1991; Oladosu *et al*., 1993). The seeming non-vaccination of these horses in Nigeria resulted in outbreaks of the disease in these horses. The same reason accounted for the high morbidity and mortality rates in horses in Kano state in 1982 (Kwanashie and Abdullahi, 1991; Oladosu, *et al*., 1993). Adeyefa and Hamblin, (1995) also reported the existence of AHS in Nigeria. Clinical cases of AHS have been reported after vaccination with polyvalent live attenuated vaccines (Mshelia *et al.,* 2008). Fasina *et al*., (2008) reported outbreaks of AHSV serotype 2 in Ibadan and Lagos between December 2006 and January, 2007.This seemed to be the first report of serotype 2 in the northern hemisphere.

Adeyefa and Hamblin, (1995) carried out a serological survey to demonstrate the continuing presence of AHSV in Nigeria by collecting 254 horses and 58 donkeys sera from 10 widely separated regions throughout Nigeria using a competitive enzyme-linked immunosorbent assay (ELISA). The animals sampled included imported horses,

indigenous and locally cross-bred (local) horses and African donkeys. Those animals were shown to have a prevalence of 79.8%. Similarly Baba *et al.* (1993) carried out a serological survey, using haemaglutination inhibition test with serum from 81 horses from 6 states of Nigeria and recorded a prevalence rate of 95.1%.

2.11 Diagnosis of African Horse Sickness

Clinical signs and lesions in association with previous epidemiological information may be sufficient for clinical diagnosis. However, because most of the clinical signs and macroscopic lesions are not pathognomic, AHS must be confirmed by isolation and identification of the virus. Susceptible horses usually die following an acute infection. The virus may also be readily recovered and identified from tissues such as the spleen, lung, lymph nodes and salivary glands of suspected animals after death (Erasmus 1972).

2.11.1 Antigen identification

African Horse Sickness Virus (AHSV) can be identified directly in whole blood and other tissues using molecular probes and RT-PCR using group specific primers for segment 3 of the virus and VP7 (Venter *et al.,* 1992; Zientara *et al.,* 1993 Sakamoto *et al.,* 1994; Moulay *et al.,* 1995). The indirect sandwich ELISA is also useful for the rapid identification of AHSV antigen in solid tissues taken from animals that have died following an acute infection (Du Plessis *et al.,* 1990, Hamblin *et al* 1991, Laviada *et al.,* 1992). In addition, isolated viruses can be identified by group specific tests such as complement fixation and direct and indirect fluorescence antibody test (McIntosh, 1956; Davies and Lund, 1974).

Until recently serotyping AHSV has relied largely upon virus neutralisation tests (VNT) using type specific antisera. This test depends upon the presence of live replicating virus (McIntosh, 1958; Howell, 1962; Hazrati and Ozawa, 1965; Verwoerd *et al.,* 1979). They tend to be onerous to perform and may take five or more days before results are obtained. The recent development of a type-specific RT-PCR by Sailleau *et al*. (2000) has now provided a method of confirming the identity of AHSV in tissue samples within 24 hours. A significant advantage of this method is that it can be used to type very low levels of AHSV antigen in samples that do not contain live virus, and the results are available within a few hours (Mellor and Hamblin, 2004).

2.11.2 Antibody identification

Group specific antibody against AHSV can be detected using several diagnostic assays that are directed primarily toward the VP7. The assays include complement fixation, agar gel immunodiffusion , immunofluorescence and ELISA (Davies and Lund, 1974; Anon 1987; Hamblin *et al*., 1991b, and Laviada *et al.,* 1992). An indirect ELISA based on the detection of antibody against segment 10 (NS3) has also been described for AHSV serotype 4 (Laviada *et al.,* 1995). This assay can be used to distinguish between naturally infected animals and those vaccinated with purified, inactivated vaccine. Serotype specific antibody can be detected using serum neutralisation tests (SNT) (House *et al.,* 1990; Baylis *et al.,* 1999). The SNT is not often used for primary diagnosis but may have value in epidemiological surveillance and transmission studies. In endemic regions, where multiple AHSV serotypes are likely to be present, it is essential that paired sera collected at a minimum of two weeks interval be assayed serologically to confirm a diagnosis (Mellor and Hamblin, 2004).

2.12 Differential Diagnosis

The clinical signs and lesions reported for AHS can be confused with those caused by a closely related *Orbivirus,* equine encephalitis virus (EEV). Many aspects of the epidemiology of the diseases caused by these two viruses are also similar (Venter *et al.,* 2002). They have a similar geographical distribution and vertebrate host range and the same vector *Culicoides* species (Coetzer and Erasmus 1994 and Venter *et al.,* 2002). As a result both can occur simultaneously in the same locations and even in the same animal. Fortunately, rapid, sensitive and specific ELISA tests are available to enable the detection of the antigens and antibodies of both AHSV and EEV (Hamblin *et al.*, 1991a and Crafford *et al.,* 2003).

Several other diseases may also be confused with one or other of the forms of AHS. The haemorrhages and oedema reported in cases of purpura haemorrhagica and equine viral arteritis may be similar to those seen in the pulmonary form (dunkop/acute form) of AHS, although the oedema in AHS tends to be less extensive and the haemorrhages are less numerous and widespread (Coetzer and Erasmus, 1994). The early stages of babesiosis *(Theleria equi* and *B. caballi)* can be confused with AHS, particularly when the parasites are difficult to demonstrate in blood smears (Coetzer and Erasmus, 1994).

2.13 Prevention and Control

There is no specific treatment for animals suffering from AHS apart from rest and good husbandry (Radostits *et al.,*2003). Complicating and secondary bacterial infections should be treated appropriately during the recovery period. African horse sickness virus is non-contagious and can only be spread via the bites of infected vector species of

Culicoides. Control may therefore be effected by introducing animal movement restrictions to prevent infected animals initiating new foci of infection (Mellor and Hamblin 2004).

2.13.1 Husbandry modification

This measure is aimed at denying or reducing vector access to susceptible animals. Most vector species of *Culicoides* including *C. imicola* are exophilic. So stabling susceptible equids during times of maximum vector activity (crepuscular period) and during the night will significantly reduce biting rates and hence the likelihood of infection (Meiswinkel *et al.,* 2000). In addition, prevention of portals of access to such housing such as windows and doors are screened with material of fine mesh such as sand-fly netting or with coarser material impregnated with insecticide (e.g. a synthetic pyrethroid) will further reduce biting rates (Braverman, 1989).

2.13.2 Vector control

It is rarely possible to completely eliminate populations of the vector, *Culicoides* spp. (Mellor and Hamblin, 2004). The main aim is to reduce the number of potential infective bites that susceptible horses may receive, to the levels where maintenance of an epidemic becomes unsustainable (Anderson and May, 1991). Vector control can be tackled in a number of ways but it is important to remember that a combination of approaches is likely to yield the best results (Mellor and Hamblin, 2004).

2.13.3 Vaccination

2.13.3.1 Live attenuated vaccines

This commercially available polyvalent AHS vaccine (live cell cultured-attenuated) is produced and widely used in South Africa (Botha *et al.,* 1996; OIE, 2000). The vaccine formulation consists of seven of the nine serotypes divided into two vials (combination 1: types 1, 3 and 4; combination 2: types 2, 6, 7 and 8) that must be administered 28 days apart. Serotypes 5 and 9 are not included because of putative cross protection, respectively , afforded by immunization with serotypes 8 and 6 (Van Dijk, 1998). That indicates that the presence of serotype 8 and 6 in the second vial protects against outbreaks of serotype 5 and 9.

2.13.3.2 Inactivated vaccines

Inactivated vaccines have the advantage that they do not contain a live and potentially dangerous agent (Laegreid 1996). During the 1987 to 1991 AHSV epidemic in Spain, Portugal and Morocco, one of these, a formalin-inactivated vaccine "Equipest®" was developed commercially (Dudourget *et al.,* 1992). The initial clinical trials were carried out in France, and were followed by two field trials in Spain and Morocco. Although this vaccine proved to be reasonably efficacious, it was withdrawn shortly after the eradication of AHSV from Europe and is no longer available. This is because they may be expensive to produce and multiple inoculations may be required to elicit and maintain high levels of protective immunity. It may also be difficult to ensure complete vaccine inactivation (Laegreid, 1996).

2.13.3.3 Sub-unit vaccines

Sub-unit vaccines against AHS have not so far been developed commercially although a considerable amount of research has been undertaken on the subject dealing mainly with the related BTV (Roy *et al.,* 1992, Roy *et al.,* 1993, Roy *et al.,* 1994).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Kaduna state ($10^{o}29'N$, $7^{o}25'E$) is bordered by Katsina and Kano states to the North, Nasarawa state and FCT to the south, Niger and Zamfara to the West and Plateau and Bauchi state to the East (Fig 1).

The state has a total annual rainfall varying between 1,000 mm and 1,500mm and a rainy season which is between 150 and 200 days. The northern part of the state is semi-arid, while further south, as the rainfall level increases, the climate becomes sub-humid. The extreme south of the state is marked by a series of rocky hills which are responsible for an island of high rainfall. The people are mostly involved in farming during the raining and dry season (RIM, 1992).

The survey was carried out in seven out of the 23 Local Government Areas (LGA's) of the state namely Zaria, Sabon Gari, Igabi, Kaduna North, Lere, Ikara, and Makarfi (Fig 1.). The 7 LGAs were randomly selected among the 10 LGAs that have the highest population of horses as reported by RIM (1992). Furthermore, the stables within the selected LGAs were similarly selected randomly.

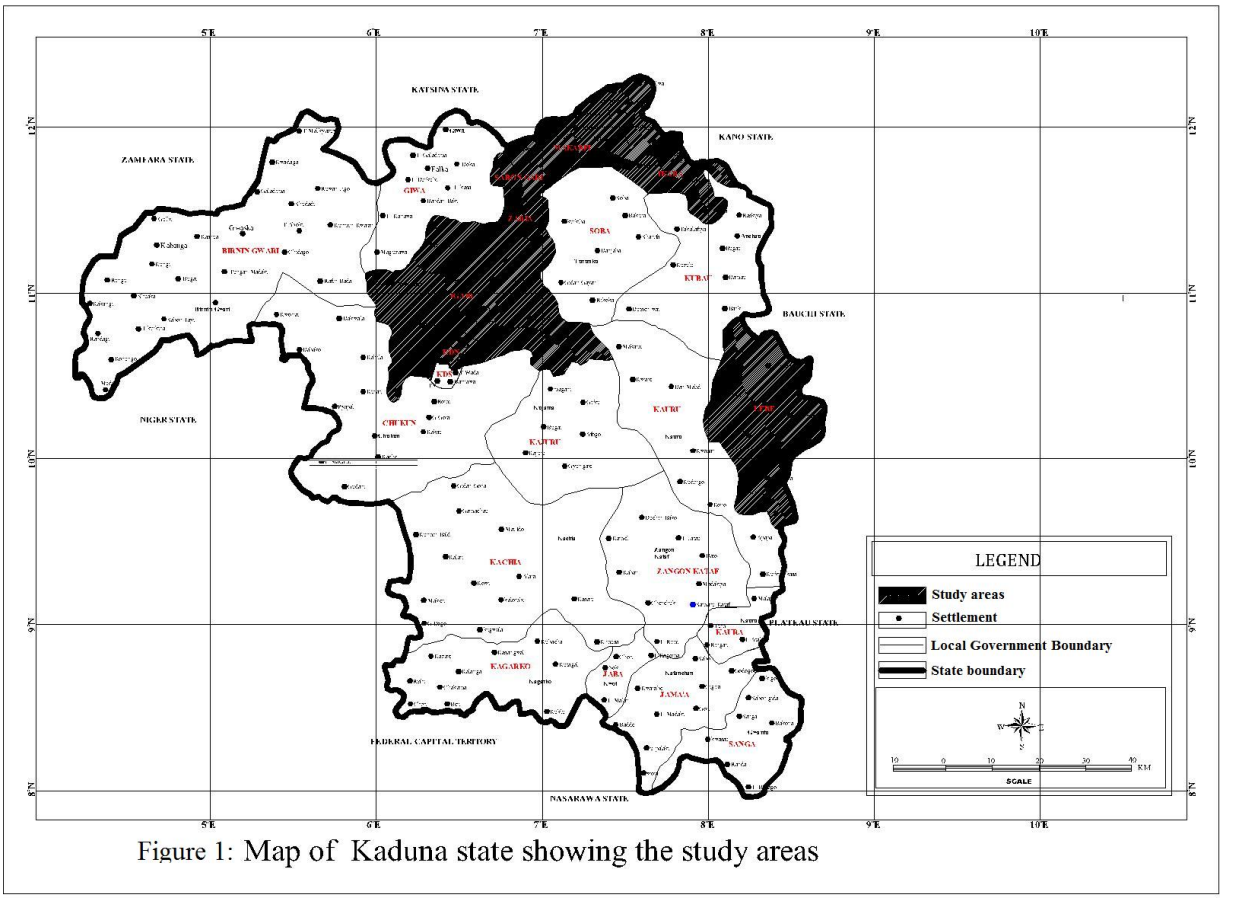


Figure 1: Map of Kaduna state showing the study areas

Source of Map: RIM

3.2 Horses

The population of horses in Kaduna state is estimated at 2,986 (RIM, 1992). Garba, (2006) reported different breeds of horses in Kaduna state namely as Argentine polo Pony, South African and the Local breeds which are of Arabian decent.

Horses in Kaduna state are kept for ceremonial purposes, pleasure ride, defense (mounted police and military troops), polo and racing (RIM, 1992; Edwards, 1993; Garba, 2006). These horses are unevenly distributed in different parts of the State.

3.3 Study Design

Random sampling was carried out in the study area as described by Trochim, (2004). Seven LGAs were selected because the LGAs was where the highest number of horses could be found (RIM, 1992). The horses in the seven LGAs are considered to be a representative of the entire population selected based on availability of horses in the twenty three LGAs. The stables and horses were selected randomly in their various locations in the LGAs studied

3.4 Sample Size

From the estimated population of horses given by RIM (1992) it indicates that there is an average of 130 horses per LGA. Therefore, the seven selected LGAs for this study have an average of 909 horses.

Conceivably, 10% of the total population of the 7 LGAs is statistically significant (Selvin, 1991) which is about 91 samples. For this study, a total of 183 horses were sampled with a minimum of 25 horses per LGA.

3.5 Sampling Procedure

3.5.1 Identification of horses

All horses sampled were identified with a combination of indices like serial number, Local Government Area, and sample serial number. The first digit represents stable number, the Local Government represented with the initial of the LGA, and a serial number represented as a subscript. In this regard, Kaduna North was represented by Kn and Igabi by Ig. Thus, horses and blood samples from them had numbers ranging from $1Kn/S_1$ to S_{35}. A permanent marker pen was used to inscribe numbers on masking tape placed on the sample bottles.

3.5.2 Blood sampling for serum

Blood samples were collected from horses between 7:00 am and 10: 00 am. The time was considered appropriate to get adequate assistance from grooms to assist in restraining the horses for blood collection. The horses were physically restrained by holding unto the halter and the jugular furrow disinfected with methylated spirit. Approximately, 10 ml of blood was collected aseptically from the external jugular vein using a 19 gauge needle and a 10 ml syringe. The blood samples were then transferred into plain vacutainer tubes and allowed to clot for two hours at room temperature before transporting them to the Physiology Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The clotted blood samples were then spinned for 10 minutes at 1580g to separate the sera which were then transferred into cryovials encased by a cryoware. They were then labeled as for their corresponding bottles and placed in

ice box and transported to the National Veterinary Research Institute (NVRI), Vom and stored at -20^{o}C till used.

3.5.3 Serological investigation

The indirect ELISA was performed according to the standard procedure prescribed by the OIE, (2008). The kits were obtained from African Horse Sickness Reference Laboratory, Ondersteport Veterinary Institute, South Africa (Plate I).



Plate I: Equine sera stored in Nalgene cryovials contained in cryoware suspended in a rackunder an ultra low volume temperature refrigerator (-80) at National Veterinary Research Institute, Vom.

3.6 Questionnaire Preparation and Administration

A structured questionnaire was prepared and administered in person to stable managers and grooms (Appendices I and II). In a group interview, the questionnaires were piloted and later in the field and adjusted based on the findings on the field. Purposive sampling was used to select the key informants.

Data on demographic variables which included horse breed, sex, age, stable size, purpose of keeping horses, vaccination history, and level of interaction of the horses with other animals were collected. The ability of the managers and grooms to recognize signs, prevention and control methods of AHS were similarly.

Another structured questionnaire was administered to practicing veterinarians in the study areas to determine the occurrence of AHS and measures employed to control it. Also information was generated on whether they notified the appropriate authorities in times of outbreaks and means of diagnosis in times of outbreaks. Information on the types of vaccines currently in use and the morbidity and mortality rates due to AHS were generated.

3.7 Data Analysis

The Data generated from this work was analyzed using graph pad and Statistical Analysis for Social Sciences (SAS). Prevalence and mean standard error of antibodies were calculated. Chi-square was used to measure the degree of association among factors such as age, sex, breed, use of horses and vaccination status.

CHAPTER FOUR

RESULT

4.1 Frequency of Animals Sampled

A total of 183 horses were sampled from the 7 LGAs studied. The number of horses sampled per LGA is given in Table 4.1. Among the horses studied 107 were indigenous while 76 were exotic. No exotic horses were sampled in Lere, Ikara and Makarfi (Table 4. 2). Ninety (90) out of all the horses sampled were males while ninety three (93) were females (Table 4. 3). Two racing horses, 100 polo horses and 81 pleasure horses were sampled with an equal distribution of polo horses in Zaria, Sabon Gari, Igabi and Kaduna North (Table 4.4).

None of the horses sampled from Lere, Ikara and Makarfi LGAs had vaccination history while a total of 73 horses from all sampled were reported to have been vaccinated (Table 4.5). Eighty two (82) horses out of 183 fell within the range of 6-10 years (Table 4.6).

Table 4.1: Frequency of Animals Sampled per Local Government Area of Kaduna State.

Variables	Local government Area							Total
	Zaria	S/Gari	Igabi	K/North	Lere	Ikara	Makarfi	
Horses	40	37	35	37	10	12	12	183

Table 4.2: Frequency of Animals Sampled based on Types per Local Government Area of Kaduna State

Variables	Local government Area							Total
	Zaria	S/Gari	Igabi	K/North	Lere	Ikara	Makarfi	
Exotic	19	19	20	18	0	0	0	76
Indigenous	21	18	15	19	10	12	12	107
Total	40	37	35	37	10	12	12	183

Key: S/Gari = Sabon Gari; K/North = Kaduna North

Table 4.3: Frequency of Animals Sampled based on Sex from seven Local Government Areas of Kaduna State

Variables	Local Government Area							Total
	Zaria	S/Gari	Igabi	K/North	Lere	Ikara	Makarfi	
Male	22	22	18	21	1	1	5	90
Female	18	16	17	15	9	10	8	93
Total	40	38	35	36	10	11	13	183

Key: S/Gari = Sabon Gari; K/North = Kaduna North

Table 4. 4: Frequency of animals sampled based on purpose from the Seven Local Government Areas of Kaduna State.

Variables	Local Government Area							Total
	Zaria	S/Gari	Igabi	K/North	Lere	Ikara	Makarfi	
Racing	2	0	0	0	0	0	0	2
Polo	25	25	25	25	0	0	0	100
Pleasure	13	12	10	12	10	12	12	81
Total	40	37	35	37	10	12	12	183

Key: S/Gari = Sabon Gari; K/North = Kaduna North

Table 4.5: Frequency of animals sampled based on vaccination status per Local Government Area of Kaduna State

Variables	Local Government Area							Total
	Zaria	S/Gari	Igabi	K/North	Lere	Ikara	Makarfi	
Vaccinated	19	17	19	18	0	0	0	73
Non-vaccinated	21	20	16	19	10	12	12	110
Total	40	37	35	37	10	12	12	183

Key: S/Gari = Sabon Gari; K/North = Kaduna North

Table 4.6: Frequency of animals sampled based on age

Age range	Zaria	S/Gari	Igabi	K/North	Lere	Ikara	Makarfi	Total
1mth – 5yr	3	5	3	3	5	4	4	27
6-10yrs	15	5	7	15	12	12	16	82
11-15yrs	10	9	8	12	10	10	9	68
16-20yrs	2	1	0	2	1	0	0	6

Key: Mth-Month

4.2 Seroprevalence

A total of 110 non-vaccinated horses were serologically tested for AHS virus using I-ELISA. Out of these 102 were seropositive with a prevalence of 92.7%. The range of seropositivity in horses in the various LGAs was 83.3-100%. (Table 4.7). The sera titre range obtained in the study was 9- 243 (Appendix III). The overall mean titre for the indigenous and exotic horses was 80.35 SEM 5.28 and 97.51SEM 5.75 respectively. Those for pleasure, racing and polo were 70.74 SEM 4.97, 41.50 SEM 25.50 and 102.95 SEM 5.45 respectively (Appendix IV). Similarly the mean serum titre by sex was 80.82 SEM 49.04, and 100.09 SEM 53.60 for male and female horses respectively while the corresponding figures per age groups are 77.72 SEM 9.83, 95.07 SEM 50.3, 88.51 SEM 6.57 and 92.50 SEM 23.42 for horses in the age ranges of 1 month to 5 years, 6 to 10 years, 11 to 15 years and 16 to 20 years respectively (Appendix V and VI). Similarly, the seroprevalence per type of use of the horses studied given in Table 4.8.While corresponding figures for the various LGAs are given in appendices VII to XIII. Similarly the data obtained in the cause of the study are given in appendix XIV. Polo horses had overall seroprevalence of 95%, while racing and pleasure horses had 100% and 92.6% seroprevalence respectively. (Table 4.8; Fig II) Further the Seroprevalence per age of horses 15 to 20 years had the highest seroprevalence followed by those between the age of 5 and 10 years and least by those between the ages of 1 month to 5years.

Table 4.7: Seroprevalence of African Horse Sickness Virus in the seven (7) local government areas of Kaduna state.

	Local Government Area							Total
	Zaria	Sabon Gari	Igabi	Kaduna North	Lere	Ikara	Makarfi	
Total number tested	21	20	16	19	10	12	12	110
Number positive	20	18	14	19	10	11	10	102
Prevalence (%)	95.2%	90.0%	87.5%	100%	100%	91.7%	83.3%	92.7%

Table 4.8: Seroprevalence per use of Horses in Seven Local Government Areas of Kaduna State

Variables	Use of Horses		
	Polo	Racing	Pleasure
No. Tested	100	2	81
No. Positive	95	2	75
Prevalence	95%	100%	92.6%

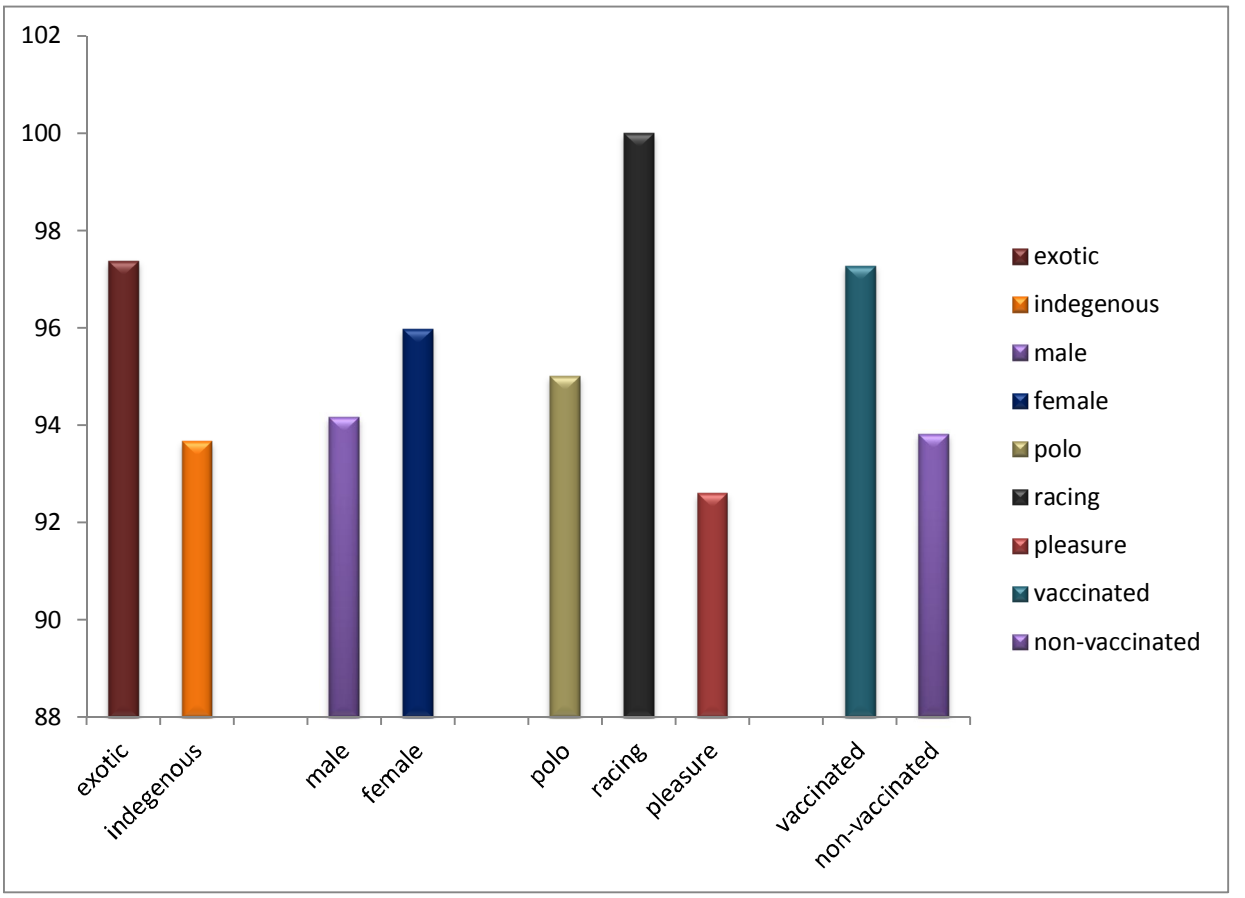


Figure II. Seroprevalence of AHSV based on various variables

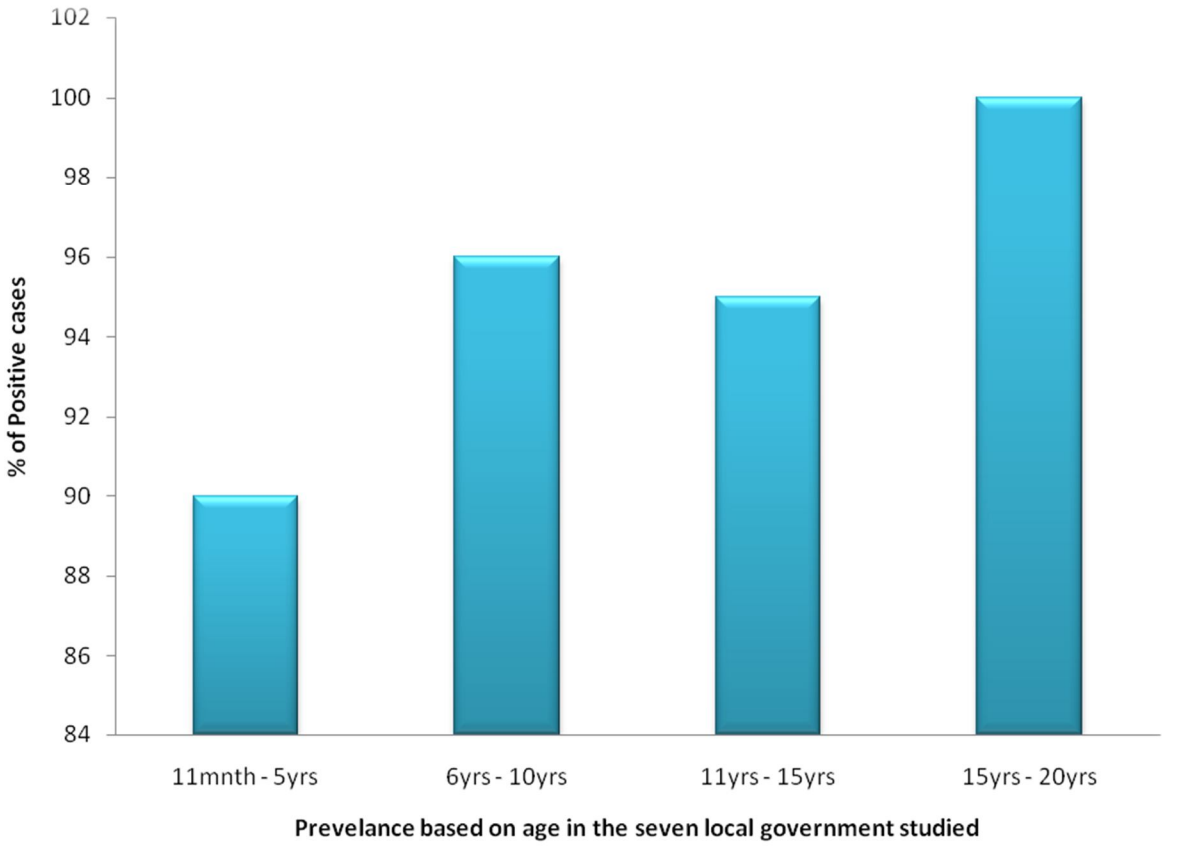


Figure III. Seroprevalence of AHSV based on age in all the LGAs studied.

4.3 Stable/Farm Management Practices

4.3.1 Horse holdings and purpose per stable

The responses from the interviewed stable managers/grooms revealed that the number of horses on each facility was small scale ownership of between 1 and10 horses (50%) while 11 to 20 horses per facility were owned by 20% of the facilities studied (Table 4.9 and Figure IV). The use or purpose of keeping animals, 25 facilities (50%) kept horses for polo while 50% of the facilities kept horses for various reasons which include, racing, draught and pleasure (Table 4.10 and Figure V).

Among the respondents in the facilities interviewed 20 reported having exotic horses while 30 had indigenous horses (Table 4.11). Further 25 of each of the respondents reported being either mobile (travelled wide for tournaments or festivities) or sedentary (used mainly in their immediate environment).

Table 4.9: Number of horses in the facility in the seven local governments studied

No of Horses in the facility	No. of Facilities	Frequency (%)
1 – 10	25	50
11 – 20	10	20
21 – 30	5	10
31 – 40	9	18
41 – and above	1	2
Total	**50**	

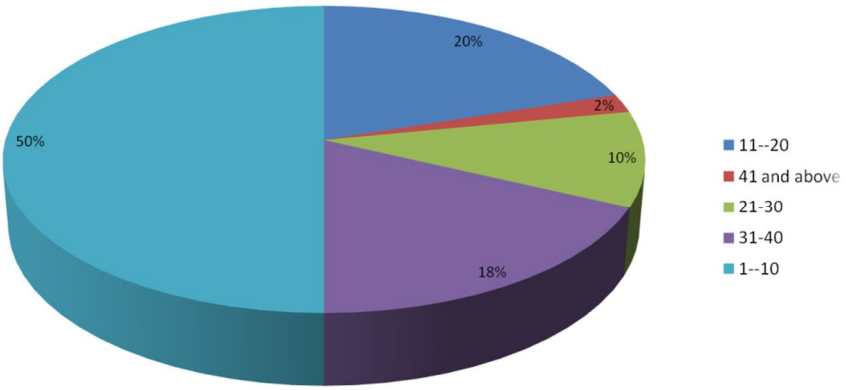


Figure 4: Number of Horses in the Facilities

Table 4.10: Farm Attributes based on purpose of keeping animals

S/No	Purpose of keeping Horses	No	Frequency (%)
1.	Polo	25	50
2	Racing	2	4
4	Pleasure	22	44
	Total	**50**	

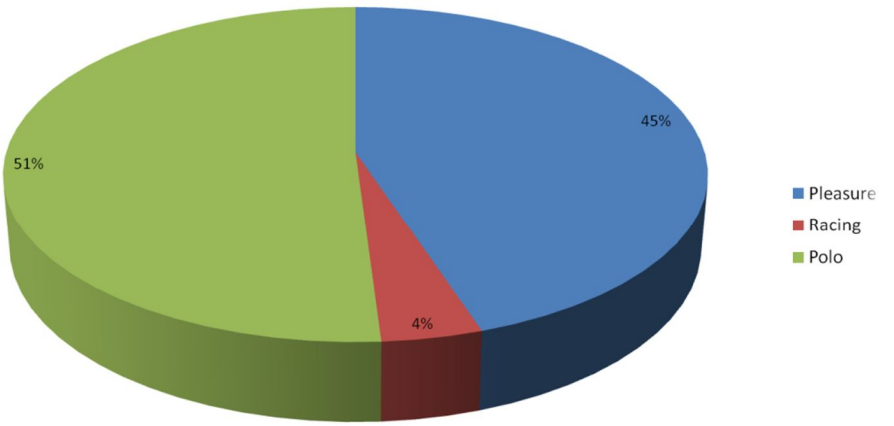


Figure 5: Use of horses

Among the respondents in the facilities interviewed 20 reported having exotic horses while 30 had indigenous horses (Table 4.11).

Table 4.11: Distribution of Horses Based on Type and Transportation of Horses within the past 2-years.

S/No	Horse Breeds	No	Frequency (%)
1.	Exotic horses	20	40
2.	Local horses	30	60
3.	Horses transported over a distance	25	50
4.	Sedentary horses	25	50

4.3.2 Husbandry practices

Stables were found to have facilities such as paddocks, stables, and water provision facilities for managing horses. (Table 4.12). These watering facilities include drilled wells, traditional wells and bore holes. One respondent reported practicing boarding of horses for owners to pay monthly rates. Some horses were found to be kept outside the stables while others were within darkened stables and it was common to find cracks and crevices inside the stables (Plates II, III, IV and VI).

Table 4.12: Frequency based on facilities

Variables	Local Government Area							Total
	Zaria	S/Gari	Igabi	K/North	Lere	Ikara	Makarfi	
Paddock	-	-	2	-	-	-	-	2
Stables	4	4	5	5	2	2	3	25
Hay Production			1					
Hay Management	2	-	-	-	3	2	2	9
Boarding	-	-	1	-	-	-	-	1
No Visible facility	2	-	-	-	3	2	2	9
Drilled well	2	2	4	2	2	2	1	15
Traditional well	5	4	2	4	5	5	5	30
Bore hole	-	1	2	2	-	-	-	5

Key: S /Gari = Sabon Gari; K/North = Kaduna North



Plate II: A typical polo horse shelter without window in Igabi Local Government Area



Plate III: A typical polo horse shelter with window. It also has horse manure and a damp horse bedding which could be a breeding sight for biting flies



Plate IV: A well lighted stable with holes in the wall which could serve as a hiding place for insect.



Plate V: A modern stable with open ends and fans to improve ventilation

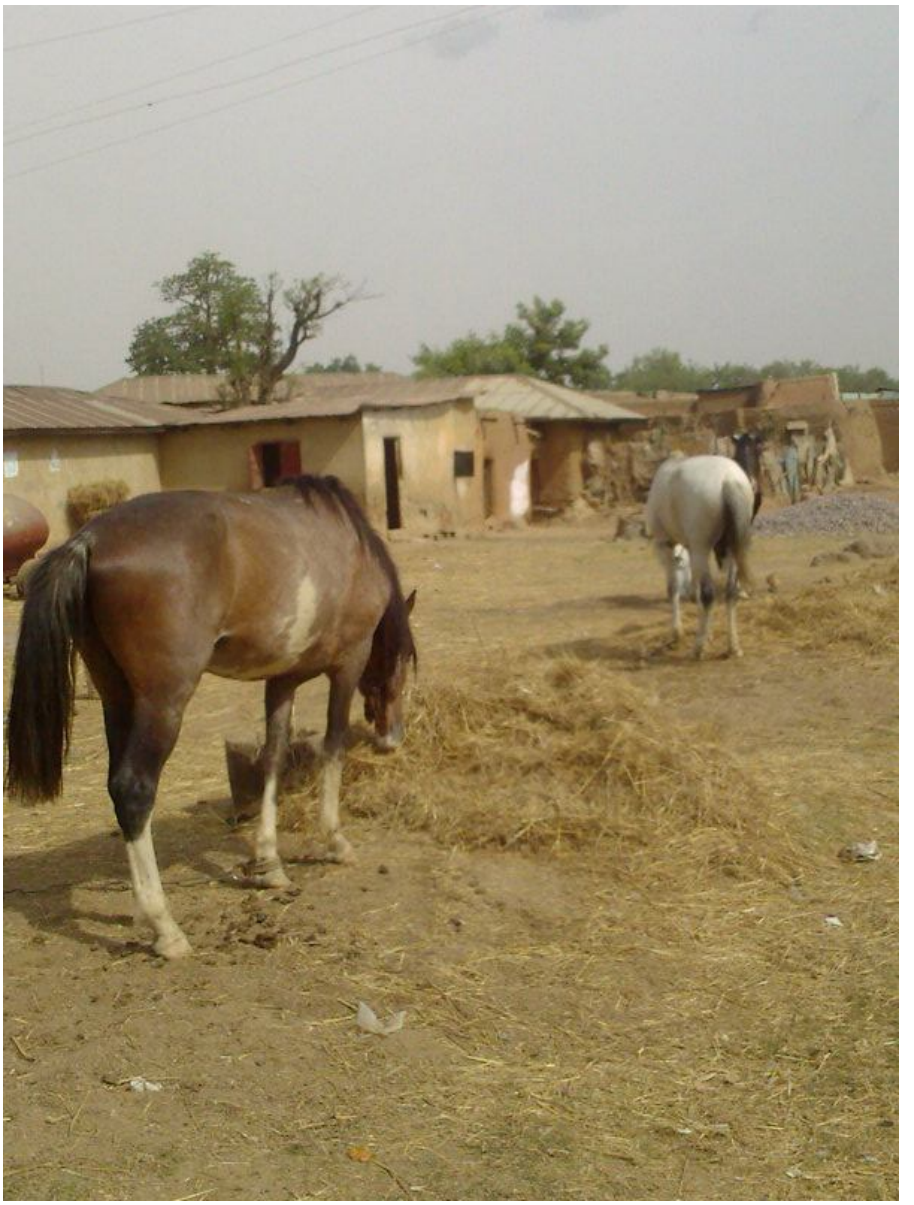


Plate VI: Horses tethered in an open field (No-House), typical of a traditional setting in Zaria City, Zaria L.G.A

4.3.3 Disease Prevention and Control Practices

Of the 50 Stable managers interviewed 10 of them reported being aware of AHS and even applied insecticides at their premises to drive insects away (Table 4.13). Five of the respondents reported restricting horse movement in their facilities while 20 others practiced routine stable disinfection. Further 35 of them reported introduction of new horses into their stables since the last 2 years. Also a number of respondents reported having water logged areas around their stables, wooded areas, and open pastures near their stables and water logs. (Table 4.13). On the use of prophylactic measures in the stables four of the respondents reported using insecticides to drive away insects in stables while and two each of the respondents reported using repellants, vaccination or humanely euthanizing horses respectively (Table 4.14). Horses were kept near water pools and where they were in stable it was possible to find insects attacking them in those stables, especially those that did not apply prophylactic measures (Plates VII and VIII).

Ten veterinarians were found providing veterinary service to the stables (Table 4. 15; Fig VI). Among them only one was found to have practiced in this regard for over 10 years. Furthermore six (60%) out of the ten veterinarians reported encountering AHS during their practice. The clinical forms of the disease they reported encountering includes pulmonary, cardiac, mixed and horse sickness fever forms.

From the study various types of AHS vaccines were in use in the areas studies. Two (20%) veterinarians each reported using polyvalent and monovalent vaccines respectively (Table 4.16; Fig. VI). Two of other veterinarians reported experiencing

vaccine failure (a situation whereby a horse was vaccinated with AHS vaccine a suddenly comes down with the disease), and none of the veterinarians interviewed reported screening all horses following importation.

Table 4.13: Farm Attributes based on Disease Prevention/Control Measures

	No.	Frequency (%)
Disease Prevention and Control Measures		
Horse Movement restriction in and out of facility	5	10
Routine stable disinfection	20	40
Introduction of new horses in past 2 years	35	70
Stabling Horses late noon to mid morning	0	0
Stabling horses throughout the day	0	0
Covering of all access points to stables	5	10
Awareness about AHS	10	20
Premises Insecticide application	10	20
Exclusion devices:		
• Ear Nets	0	0
• Fly blankets	0	0
• Face masks	2	40
• Others	30	60
Hay and Manure Management:		
• Hay in pasture	5	10
• Routine sanitation	35	70
• Presence of other animals/Manure on farm	35	70
Farm/Stable Terrain:		
• Presence of body water in the area	35	70
• Wooded areas	20	40
• Open pastures	5	10
• Water logged	20	40

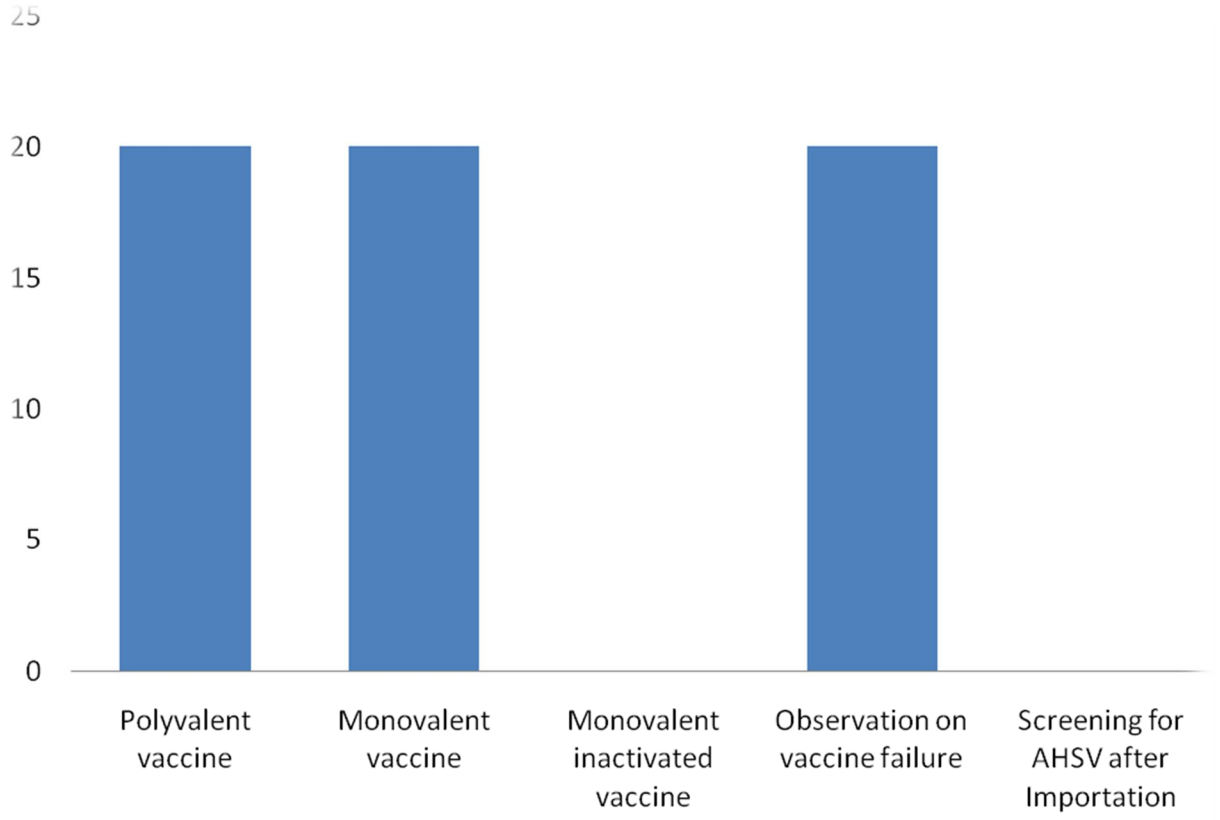


Figure VI: Type of AHS Vaccines used in the Study area

Table 4.14: Prophylaxis/Vector control Practices by Stables under the Study

Sanitary Prophylaxis/Vector Control	No	Frequency (%)
Slaughtering of affected horses	2	20
Insecticides	4	40
Repellants	2	20
Screen	0	0
Medical prophylaxis		
Identification of Vaccinated horses	0	0
Vaccination of non-infected horses	2	20

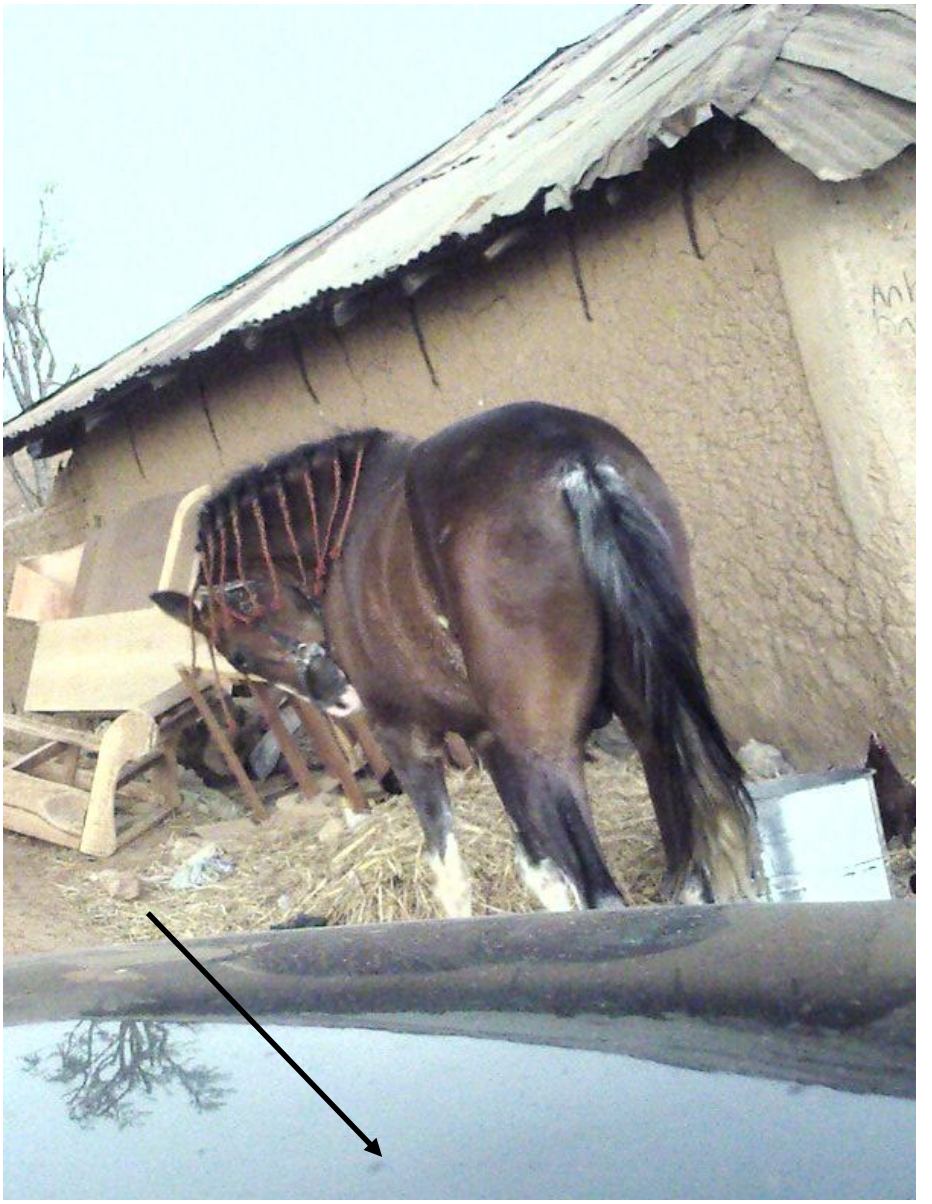


Plate VII: A horse stabled outside with a water cistern (arrow) which could encourage breeding of flies around the horse's environment.



Plate VIII: The forelimb of West African Barb horse infested with Biting flies (arrow). This could serve as a cofactor in the infection of AHS.

Table 4.15: Length of Service in Veterinary Practice

Variable		No	Frequency (%)
1	Period (years)		
	0 – 5	5	50
	6 – 10	4	40
	11 and above	1	10
2	Encountered AHS in practice		
	Yes	6	60
	No	4	40
3	Forms of AHS Encountered		
	Pulmonary Form	3	30
	Cardiac Form	1	10
	Mixed Form	3	30
	Horse Sickness Fever	3	30

Table 4.16: Types of AHS Vaccines used in the Study area

Type of AHS vaccine used/Observation on vaccination failure	No. of Vets Interviewed	Frequency (%)
Polyvalent (Live attenuated)	2	20
Monovalent (Live attenuated)	2	20
Monovalent inactivated	0	0
Observation on vaccine failure	2	20
Screening for AHSV after importation	0	0

CHAPTER FIVE

DISCUSSION

From the study more horses were found in Zaria, Sabon gari, Igabi and Kaduna North LGAs than in the other LGAs studied. This could be understandable as traditionally Zaria has been known as a centre for polo, racing and ceremonial activities. This could also explain the low number of horses in Lere, Ikara and Makarfi LGAs as there were no polo and racing horses found in these areas.

Though no reason was given by the respondents as to why there was few (2) racing horses, it could be due to the increasing cost of horse maintenance and the fact that polo rather than racing is increasingly being more popular. Further, the high number of stables holding either polo or pleasure horses could be accounted for as elites and particularly the traditional rulership are increasingly being more interested in keeping these horses. The increasing awareness of youths to take up horse keeping for pleasure and hire during religious festivals and marriage durbars that take place weekly could also account for the high population of these horses in some of the study area.

The age range for horses under the stables in the study revealed that more horses between the ages of 6-10-years were kept. This could possibly be due to economic reasons, since horses of this age range would be more useful for polo and pleasure as discussed above.

The number of horses per stable was found to be low in most of the stables. This could be due to the high cost of maintenance, especially for the horses kept under traditional setting. The horses kept for polo and pleasure could be said to partially pay for their

maintenance as some revenue could be seen to return from their keeping. This is visible for those kept for pleasure as there could be weekly hire fees. Furthermore, youths routinely give out their horses on hire during religious festivals and occasionally during durbar.

Many of the stables were found to have good husbandry practices to the level of establishing paddocks, feeding their horses with hay along with provision of efficient watering facilities. This can be accounted for due to the fact that polo is being continuously regarded as a business rather than leisure while those who keep horses for pleasure were operating at near commercial level in contrast to those kept under traditional rulership. This could also be explained by the fact that some of the stables operated boarding with a view to raising revenue in the process. However, it was evident that good husbandry practices were seen mainly in polo horse stables.

The practice of some stables to keep horses outside stables could be due to ignorance of the disease (AHS) as keeping horses outside could increase their chances of coming in contact with insects, especially *Culicoides spp* that transmit AHSV, should these insects be harbouring the virus. AHS Trust (2012) reported that stabling of horses from late afternoon to mid morning has proven to reduce the midges activity inside the stable up to14 times. Further the cracks and crevices seen in some of the stables could serve as a hide out for insects. Similarly, the none prompt removal of manure from stables as seen in some of the stables could also encourage the breeding of these insects, with consequent increase in chances of horses coming down with the disease should these insects be infected/carrying the virus.

The fact that there are water pools and such other areas favourable for the breeding of these insects could mean that more infections could occur in these horses and this agrees with the observations of Henning, (1956); Rafyi,(1961); Reid,(1961); Laaberki,(1969) and Sers,(1965). Further, Adeyefa and Dipeolu (1993) reported the presence of Culicoides breeding sites around horse stables in Nigeria. They also reported the presence of *Culicoides. quinquelineatus, C. imicola and C. schulzei* as possible vectors of AHS in Nigeria. Similarly, Fasina *et al* (2008) also reported the presence of biting midges in stables during a field investigation in various stables across Nigeria.

From the study, 20% of the stable managers/grooms were aware of AHS. This could be through their interaction with veterinarians who provided clinical services to them or through previous exposure of their horses to the disease. Further allowing horses to mix with imported ones could expose the imported ones to infection, should they not have been vaccinated in the exporting countries, especially as they did not indicate screening imported horses. The following mentioned can also explain the high seroprevalence of 92.7% as observed in this study. Further allowing their horses near wooded trees and water pools could similarly expose their horses to infection with AHSV, should the organism be harboured by insects in the area since such facilities could encourage the breeding of these insects.

The use of insecticides and repellants by some of the respondents could be said to decrease the chances of spread of infection only in such stables. However, in view of the general high seroprevalence, the use of these insecticides and repellants could be said to be in-effective particularly as some of the horses were kept outside the stables.

Regardless of the cause, it is worthy of note that in this survey, 3 (4.11%) of vaccinated horses which have received both primary and secondary vaccination were seronegative. Though, the apparent absence of AHSV antibodies in such animals may not necessarily indicate a total lack of immunity within the stable studied (Hamblin *et al.,* 1991).

The fact that some of the practicing veterinarians reported encountering AHS means that the disease is very much around in Kaduna state and that all the four forms of the disease could be seen. It is possible that the disease is endemic in the State.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The presence of antibodies in the vaccinated horses could be an indication that the vaccine coverage within the population of vaccinated horses in the few stables that were studied was adequate to put AHS under control. The apparent absence of AHSV antibodies in such animals may not necessarily indicate a total lack of immunity within the stable studied. The detection of seroconversion with production of antibodies to AHSV with prevalence of 92.7% in non-vaccinated horses in the study area is an indication of infection since none of the horses sampled showed clinical signs consistent with AHS. Also the presence of the various horse management and environmental factors associated with AHS viral infection were seen in all the facilities studied.

6.2 Conclusion

From this work it was concluded that:

1) AHSV infection is prevalent in the study area.

2) The horse management and environmental factors that favours AHSV infection are available in the various areas studied.

6.3 Recommendations

- Further work should be carried out to determine the role played by predisposing factors to the disease in the study area
- Studies should also be carried out to determine the role(s) of African donkeys in the epidemiology of AHS in the study area.
- Further work should also be carried out to determine the specific serotypes circulating in the study area.
- The insect vectors should similarly be closely studies with a view to determine their infection rate with the AHSV.

REFERENCES

Abdulkadir, A.L. (1989). Infectious Diseases of Livestock in Nigeria; An Outline. First Edition. Ahmadu Bello University Press, Zaria, Nigeria. pp. 43.

Abegunde, A. (1975). Epizootiology of African horse sickness: the situation in Nigeria. *Bulletin Office International des Épizooties,* 83: 1091-1095.

Adeyefa, C.A and C. Hamblin (1995). Continuing prevalence of African horse sickness in Nigeria *Revue d'élevage et de médecine vétérinaire des pays tropicaux*, 48(1):31-3.

Adeyefa, C.A.O. and Dipeolu, O.O. (1993). Studies on *Culicoides* species (latreille, Diptera: Ceratopagonidae) of Nigeria. XI species caught around horse stables. *Insect science application,* 14 (2): 211 – 214.

AHS Trust(2013). African horse sickness information booklet. African horse sickness trust secretariat, Durban. Pp5-7.

Alhaji, I., Hamburg, J.M. and Kemp, G.E. (1974). African horse sickness in Nigeria- A Case report. *Tropical Bulletin Epizootic Diseases in Africa*, 22: 311-314.

Anderson R.M., and May R.M. (1991). Infectious diseases of humans: dynamics and control, Oxford University Press, Oxford.

Anon. (1987). Status of African horsesickness (AHS), *Bulletin Office International des Épizooties*, 99: 46-48.

Anon. (2006). African horse sickness trust (CSFPH) Available at http : //www.africanhorse sickness.co.za [Accessed 17 July, 2012].

Anwar, M. and Qureshi, A.A.A. (1972). Control and eradication of African horse sickness in Pakistan, in: Lawrence M.M. (Ed.), Control and Eradication Viral Diseases in the CENTO region, Central Treaty Organisation, Ankara, pp. 110—112.

Awad, F.I., Amin, M.M., Salama, S.A. and Khide, S. (1981). The role played by *Hyalomma dromedarii* in the transmission of African horse sickness in Egypt, *Bulletin Animal Health and Production in Africa.* 29: 337—340.

Baba, S.S., Olayele, O.D. and Ayanbadejo, O.A. (1993). Haemagglutimation-inhibiting antibodies against African horse sickness virus in domestic animals in Nigeria. *Veterinary Research*. 24: 483-487.

Barnard, J.H. (1998). Epidemiology of African horse sickness and the role of the zebra in South Africa. *Archive of Virology*, 14: 13-19.

Baylis, M., Mellor, P.S. and Meiswinkel, R. (1999). Horse sickness and ENSO in South Africa. *Nature,* 397-574.

Best, J.R., Abegunde, A. and Taylor, W. P. (1975). An outbreak of African horse sickness in Nigeria. *Veterinary Records*, 97:394.

Botha, W.J.S., Jaeger, H.G., Aitchison, H., Stoltsz and W.H. (1996). Vaccines against viral diseases. Onderstepoort Biological Products. National Department of Agriculture, pp. 28–42.

Bouayoune, H., Touti, J., El Hasnaoui, H., Baylis, M. and Mellor, P.S. (1998). The *Culicoides* vectors of African horse sickness virus in Morocco: distribution and epidemiological implications, *Archives of Virology Supplement,* 14: 113-125.

Bourdin, P. (1973). Ecology of African Horse Sickness. Proceedings of the 3rd international conference on equine infections diseases, Paris. Pp. 12 – 30.

Braverman Y., (1989). Control of biting midges *Cullicoides* (Diptera: Ceratopogonidae), vectors of bluetongue and inducers of sweet itch: a review, *Israeli Journal of Veterinary Medicine,* 45: 124-129.

Bremer, C.W., Huismans, H. and Van Dijk, A.A. (1990). Characterisation and cloning of the African horse sickness genome, *Journal of General Virology,* 72: 793-799.

Burrage, T.G., Trevejo, R., Stone-Marschat, M. and Laegreid, W.W. (1993). Neutralising epitopes of African horse sickness virus serotype 4 are located on VP2. *Virology,* 196, 799-803.

Capela, R., Purse, B.V., Pena, I., Wittmann, E.J., Margarita, Y., Capela M, Mellor, P.S. and Baylis, M. (2003). Spatial distribution of *Culicoides* species in Portugal in relation to the transmission of African horse sickness and bluetongue viruses, *Medical and Veteterinary Entomology*, 17: 165-177.

Coetzer, J.A.W. and Erasmus B.J. (1994). African horse sickness, in: Coetzer, J.A.W., Thomson, G.R., Tustin, R.C. (Eds.), Infectious diseases of livestock with special reference to southern Africa, Vol. 1, Oxford University Press, Cape Town, pp. 460-475.

Coetzer, J.A.W. and Guthrie, A.J. (2004). African horse sickness, in: Coetzer, J.A.W., Tustin, R.C. (Eds.), Infectious diseases of livestock, 2nd ed., Oxford University Press Southern Africa, Cape Town,pp. 1231–1246.

Crafford J.E., Guthrie A.J., van Vuuren M., Mertens P.P.C., Burroughs J.N., Howell P.G., Hamblin C. (2003). A group-specific, indirect sandwich ELISA for the detection of equine encephalosis virus antigen. *Journal Virological Methods,* 112: 129-135.

Davies F.G. and Lund L.J. (1974). The application of fluorescent antibody techniques to the virus of African horse sickness. *Research in Veterinary Science,* 17: 128-130.

Dent, A.A. and Hendricks, Bonnic. (2001). International encyclopedia of horse breeds, Norman: university of Oklahoma Press, ISBN 0-8061-388 4 –x.

Devaney, M.A., Kendall, J. and Grubman, M.J. (1988). Chatacterisation of a phosphoprotein of two orbiviruses. *Virus Research,* 11: 1G51 – 164.

Diaz Montilla, R. and Panos Marti P. (1967). Epizootologia de Ia peste equina en Espana, *Bulletin Office International des Épizooties*. 86: 705-714.

Diaz Montilla, R. and Panos Marti, P. (1968). La Peste Eqwn. *Bulletin Office International des Épizooties*. 70: 647 – 662.

Diaz Yubero, M.A. (1987). Situacion de Ia Peste Equina, Epizootiological Infections. No. ESP. 87/6/ 145.

Du Plessis, D.H., van Wyngaardt, W. and Bremer, C.W. (1990). An indirect sandwich ELISA utilising F(ab')2 fragments for the detection of African horse sickness virus, *Journal of Virological Methods*, 29: 279-289.

Du Toit, P.J. (1924). Horse-sickness in 1923, *Journal of Department of Agriculture*, April.

Du Toit, P.J. (1924). Horse-sickness in 1923. *Journal of Department of Agriculture*, April.

Dudourget P., Preaud J.M., DetrazF., Lacoste A.C., Erasmus B.J.. Lombard M. (1992). Development, production and quality control of an industrial inactivated vaccine against African horse sickness virus serotype 4, in: Walton T.W., Osbum B.I. (Eds.), Bluetongue, African horse sickness and related orbiviruses, CRC Press, Boca Raton, pp. 874-886.

Edwards, E.H. (1993). Horses. Dorling Kindersley Handbooks, London, pp.11-17.

Erasmus, B.J. (1972). The pathogenesis of African Horse Sickness, in: Bryans J.T. Gerber, H. (Eds.), Equine Infectious Diseases III, *Proceedings of the 3rd International Conference on Equine Infectious Diseases.* Pans, France. Karger, Based, 1973, pp. 1 – 11.

Erasmus, B.J. (1976). A new approach to polyvalent immunisation against African horse sickness, in: Bryans, J.T., Gerber, H. (Eds.), Equine infectious diseases IV. *Proceedings of 4th Conference on Equine Infectious Diseases*. Princetown, New Jersey, 1978, pp. 401-403.

FAO (2003). Economic and Social Department, The statistical division, on line data base FAOSTAT 2003. http://www/Fao.org

Fasina, F., Potgieter, A.C., Bako, B., Bwala, D., Kumbish, P. (2008). First report of an outbreak of African horse sickness virus serotype-2 in the northern hemisphere. *Journal of equine science.* Vol. 28. No. 2.

Garba, U .M. (2006). Babesia in royal horses in Niger state. MSc thesis presented to Faculty of Veterinary Medicine A.B.U., Zaria.

Gilfoyle D (2006).Veterinary immunology as colonial science: Method and quantification in the investigation of horse sickness in South Africa,C.1905-1945.J *History of Medicine Allied Science*, 61:26-65.

Gohre D.S., Khot J.B., Paranjpe V.L., Manjrekar S.L. (1965). Observations on the outbreak of South African horse sickness in India during 1960-1961. *Bombay Veterinary College Magazine*, 5-15.

Greiner, M. and Gardne, L.A. (2000). Application of Diagnostic tests in Veterinary epidemiological studies. *Preventive Veterinary Medicine*. 45, 43 – 59.

Grimes, J., Basak, A.K., Roy, P. and Stuart, D.J. (1995). The crystal structure of bluetongue virus VP7. *Natur*e, 373: 167 – 170.

Grubman, M.J. and Lewis, S.A. (1992). Identification and characterisation of the stuctural and non- structural proteins of African horse sickness virus and determination of the genome coding assignments, *Virology* 186, 444-451.

Guthrie, A.J. (2007). African horse sickness, in: Sellon D.C., Long M.T. (Eds.), Equine infectious diseases, Saunders Elsevier, St. Louis. pp. 164–171.

Guthrie, A.J. and Quan, M. (2009). African horse sickness, *Equine Veterinary Education Manual* 8:120–128.

Hamblin, C., Mellor, P.S., Graham, S.D., Hooghuis, H., Montejano, R.C., Cubillo, M.A. and Boned, J. (1991a). Antibodies in horses, mules and donkeys following monovalent vaccination against African horse sickness. *Epidemiology of Infections*. 104: 365-371.

Hamblin, C., Mertens, P.P.C., Mellor, P.S., Burroughs, J.N. and Crowther, J. R. (1991b). A serogroup specific enzyme-linked immunosorbent assay for the detection and identification of African horse sickness viruses, *Journal of Virological Methods* 31, 285-292.

Hamblin, C., Salt, J.S. Mellor, P.S., Graham, S.D., Smith, P.R. and Wohisein P. (1998). Donkeys as reservoirs of African horse sickness, *Archive of Virology*, 14: 37 – 47.

Hazrati, A. and Ozawa, Y. (1965). Serologic studies of African horse sickness virus with emphasis on neutralisation test in tissue culture, *Canadian Journal of Comparative Medicine*, 29: 173-178.

Hazrati, A., Mirchamsy, H. and Bahrami S. (1972). Comparative studies on the serological responses of horses to African horse sickness virus, in: Bryans, J.T., Gerber, H. (Eds.), Equine infectious Diseases III, Proceedings of the third International. *Conference on Equine Infectious Diseases.* Paris, France, Karger, Basel, 1973, pp. 69-80.

Henning, M.M. (1956). African horse sickness, perdesiekte, Pestis equorum, in: Animal diseases in South Africa, Central News Agency, South Africa 3rd ed., pp. 785-808.

Henning, M.W. (1956). Animal diseases in South Africa, 3rd ed., pp. 785-808.

Hess, W.R. (1988). African horse sickness, in: Monath T.P. (Ed.), The Arboviruses: epidemiology and ecology, Vol. II, CRC Press, Boca Raton, pp. 1-18.

Hewat, E.A., Booth, T.F., Loudon, P.T. and Roy, P. (1992). Three dimensional reconstruction of baculovirus expressed bluetongue virus core-like particles by cryo-electron microscopy. *Virology,* 189: 10-20.

House C., Mikiciuk P.E., Berringer M.L. (1990). Laboratory diagnosis of African horse sickness. Comparison of serological techniques and evaluation of storage methods of samples for virus isolation, *Journal Veterinary Diagnosis and Investigation*. 2, 44-50.

Howell, P.G. (1960). The 1960 epizootic in the Middle East and South West Asia. *Journal of South African Veterinary Medical Association*. 31, 329-334.

Howell, P.G. (1962). The isolation and identification of further antigenic types of African horse sickness virus, *Onderstepoort Journal of Veterinary Research*, 29: 139-149.

Howell P.G. (1963). African horse sickness, In: Emerging diseases of animals, FAO, Rome FAO Agricultural Studies, No.61, pp.7 1-108.

Howell, P.G., Guthrie, A.J. and Cuetzer, J.A.W. (2004). Equine Encephalosis, in: Coetzer, J.A.W., Justin R.C. (Eds.), Infectious diseases of livestock, 2nd ed., Oxford University Press Southern African, Cape town, 2004, pp. 1247-1251. http:/www.bluetonguesymposium.it/index.htm.

Huismans, H. and Els, H.J. (1979). Characterization of tubules associated with the replication of the different orbiviruses. *Virology,* 92: 397 – 406.

Kassa, D. (2006). African Horse Sickness: seroprevalence and identification of risk factors in equidae at selected sites in Ethiopia. Unpublished MSc Thesis, Addis Ababa University.

Keith, R.P. (2005). Survey in to 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 Sanctury, sidmouth, Devon, England, UK, pp. 68–81.

Kemp, G.E., Humburg, J.M., Alhaji, I. (1971). Isolation and identification of African horse sickness virus in Nigeria. *Veterinary Records*, 89:127-128.

Kwanashie, G and Abdullahi, S.U. (1991). African horse sickness in imported horses in Kaduna and Kano States of Nigeria,2nd International Symposium on Bluetongue, African horse sickness and Related Orbiviruses 17-21 June 1991.Paris, France.

Laaberki, A (1969). Evolution d'une épizootie de P.E.A. au Maroc. *Bulletin Office International des Épizooties*,***71:*** 921-936.

Laegiad, W.W., Skowronek, A., Stone – Marschat, M and Burage, T. (1993). Characterization of virulence Variants of African Horse Sickness Virus, *Virology* 195, 836 – 839.

Laegreid W.W., (1996). African horse sickness, in: Studdert M.J. (Ed.), Virus infections of vertebrates, Vol. 6, Virus infections of equines, Elsevier Press, Amsterdam, pp. 101-123.

Laviada, M., Roy, P. and Sanchez-Vizcaino, J.M. (1992). Adaptation and evaluation of an indirect ELISA and immunoblotting test for African horse sickness antibody detection, in: Walton T.W., Osburn B.I. (Eds.), Bluetongue, African horse sickness and related orbiviruses, CRC Press, Boca Raton, pp. 646-650.

Laviada, M.D., Roy, P., Sanchez-Vizcaino, J.M. and Casal J.1. (1995). The use of African horse sickness NS3 protein expressed in bacteria, as a marker to differentiate infected from vaccinated horses, *Virus Research*. 38, 205-2 18.

Lubroth J. (1988). African horsesickness and the epizootic in Spain 1987, Equine Practice 10:26–33.

MacLachlan, N.J. and Osburn, B.I. (2004). Epizootic hemorrhagic disease of deer, in: Coetzer, J.A.W., Tustin, R.C. (Eds.), Infectious diseases of livestock, 2nd ed., Oxford University Press Southern Africa, Cape Town, pp. 1227–1230.

Maclachlan, N.J. and Guthrie, A.C. (2010). Re-emergence of bluetongue, African horse sickness, and other orbivirus disease. *Veterinary Research*, 41:35.

Mare, S. and Paweska J.T. (2005). Preparation of recombinant African horse sickness virus VP7 antigen via a simple method and validation of a VP7-based indirect ELISA for the detection of group-specific IgG antibodies in horse sera. *Journal of Virological Method*, 125:55-65.

Martinez-Torrecuadrada, J.L. and Casal, J.1. (1995). Identification of a linear neutralisation domain in the protein VP2 of African horse sickness virus. *Virology,* 210: 391-399.

Maurer, R.D. and McCully, R.M. (1963). African horse sickness with emphasis on pathology, *American Journal of Veterinary Research*, 26: 235-266.

Maurice, Y. and Provost, A. (1976). Fapeste equine a type of en Afrique Centrale enquete serologique, *Rev. Elev. Med. Vet Pay Trop*. 20, 21 – 25.

McIntosh B.M. (1956). Complement fixation with African horse sickness, Onderstepoort *Journal of Veterinary Research*, 27: 165-169.

Mclntosh, B.M. (1958). Immunological types of horse sickness virus and their significance in immunization, *Onderstepoort Journal of Veterinary Research*, 27: 465 – 538.

Meiswinkel R., Baylis M., Labuschagne K. (2000). Stabling and the protection of horses from Culicoides bolitinos (Diptera: Ceratopogonidae), a recently identified vector of African horse sickness, *Bullettin of Entomology Research*, 90: 509-515.

Mellor, P.S. (1993). African horse Sickness; transmission and epidemiology, *Veterinary Research,* 24: 199 – 212.

Mellor, P.S. (1994). Epizootiology and Vectors of African horse sickness virus. Comparative Immunology and Microbiology of Infecious Diseases, 17: 287 – 296.

MeIlor, P.S., Capela, R., Hamblin, C., Hooghuis, H., Mertens, P.P.C., Plateau, E. and Vizcaino, J.M. (1994). African horse sickness in Europe, epidemiology, in: Nakajima N.,

Plowright W. (Ed.),7th *Conference on equine Infectious Disease,* Tokyo, Japan, 8-11 June, R & W Publications, Newmarket, pp. 61-64.

Mellor, P.S. and Boorman J. (1995).The transmission and geographical spread of African horse sickness and bluetongue viruses, Annal Tropical Medicine Parasitology, 89: 1-15.

Mellor, P.S. and Hamblin, C. (2004). African Horse Sickness. Veterinary Research 35; 445 - 446.

Meseret, B. (2007). Cross sectional seroepidemiological study of African Horse Sickness in selected site of two regional state of two regional states of Ethiopia. (Unpublished DVM Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre zeit).

Mirchamsy H. and Hazrati A. (1973). A review of the aetiology and pathology of African horse sickness, *Archive of the Institute Razi*, 25: 23-46.

Mornet, P., Toma, B. and Sers, J.L. (1967). Une nouvelle maladie réputée légalement contagieuse: Ia pesteéquine, Rec. Med. Vet.143, 119-139.

Moulay S., Zientara S., Sailleau C., Cruciere C. (1995). Detection of African horse sickness viruses by dot blot hybridisation using a digoxigenin labelled probe, Mol. Cell Probes 9 233-237.

Moule, L. (1896). Histoire de Ia Médecine Vétérinaire, Maulde, Paris, pp. 38.

Mshelia,W.P., Abdussamad, A.M., Lazarus. D.D and Sambo, K. (2008). African horse sickness vaccine failure in a five year old mare. *Veterinary clinical practice bulletin* 1: 44-50.

Mshelia,W.P., A.M. Abdussamad, Y.J. Atuman, S.M. Samdi and G. Kwanashie (2010). A pictorial review of injuries and disease conditions in foreign and part-barb horses in northern Nigeria: Part I. *Research Journal of Veterinary Science*, 3: 1-12.

Mshelia,W.P., Isreal Ikani Onoja., Saidu Queen Victoria, Edeh Emmanuel Richard. (2012). Husbandry and Management Practices in Domestic Horses (*Equus caballus*) in Northern Nigeria and their clinical Implications. Proceedings of the 2nd International Equine science Meeting (IESM) Regensburg, Germany. ISBN 978-3-9808134-3-3.

Newsholme, O., Bedford, G.A.H. and Du Toit, R.M. (1983). A morphological study of the lesions of African horse sickness, *Onderstepoort Veterinary Research*, 50:7-24.

Nwathe, D.R., Synage E., Okoh, A.E. and Abegunde A. (1981). Persistence of African Horse Sickness in Nigeria. *Tropical Animal Health and Production*, 13: 167 – 168.

Oellerman, R.A., Els, H.J., Erasmus, B.J. (1970). Characterisation of African horse sickness virus, *Archives Gesamte Virusforsch*. 29(163-174.

Office International des Epizzoties, (2000). African horse sikness. In, OIE Manual of Standards for Diagnostic Tests and Vaccines. OIE, Paris, France, pp. 178–188.

OIE (Office International des Epizooties / World Organization for Animal Health), (2008). African Horse Sickness. Technical Card Database. Available at: http://www.oie.org [Accessed 10 April, 2009].

OIE (Office International des Epizooties / World Organization for Animal Health), (2012). African Horse Sickness. Technical Card Database. Available at: http://www.oie.org [Accessed 10 Jan, 2013].

Oladosu, L.A., Olaleye, O.D., Baba, S.S., Omilabu, S.A. (1993). Isolation and identification of African horse sickness virus during an outbreak in Lagos, Nigeria. Revue d'élevage et de medicine, 12: 873-877.

Parker, J., Armstrong, R.M., Abegunde, A and Taylor.W.P. (1977). African horse sickness virus in northern Nigeria, 1974 – 1975 Research Journal of veterinary science, 22: 274 – 280.

Pilo – Moron, E. Vincent, J. A.T. – Mesbah, O. and Forthomme, G. (1969). Origine de la peste equine en Afrique du Nord; resultants d' une enquete sur les leites du sahara Algerien, Archive Institute of Pasteur Alger. 47, 105 – 118.

Prasad, B.V.V., Yamaguchi, S. and Roy, P. (1992). Three- dimensional structure of single shelled blue- tongue virus, Journal of Virology, 66: 2135-2142.

Quan, M., Van Vuuren, M., Howell, P.G., Groenewald, Dand Guthrie, A.J. (2008). Molecular epidemiology of the African horse sickness virus 510 gene, Journal of General Virology, 89:1159 – 1168.

Radostits, O.M., Gray, C.C., Blood, D.C. and Hinchdiff, K.W. (2003). African horse sickness, Veterinary Medicine – Textbook of the diseases of Cattle, sheep, pigs, goats and horses. pp 1038 – 1040.

Rafyi, A. (1961). Horse sickness, Revue d'élevage et de medicine, 56:216-250.

Reid, N. R. (1961). African horsesickness. The 1959-1969 epizootic of African horsesickness in the Near East region and India and a note on technical assistance provided by the F.A.O. of the United Nations. British Veterinary Journals, 117: 137-142.

RIM. (1992). Nigerian National Livestock Resource Survey. Report by Resource Inventory and management Limited (RIM) to FDL and PCS, Abuja, Nigeria.Vol.6.

Rodriguez, M. Hooghuis, H and Castano, M. (1992). African horse sickness in Spain. *Veterinary Microbiology*, 33:129.

Roy, P., Hirasawa, T.,Fernandez, M., Blionov, V.M., Sanchez-Viscaino J.M. (1991). The complete sequence of group-specific antigen, VP7, of African horse sickness virus serotype 4 reveals a close relationship to bluetongue virus, Journal of General Virology (72)1237-1241.

Roy P., French T., and Erasmus B.J. (1992). Protective efficacy of virus-like particles for bluetongue disease, Vaccine 10, 28-32.

Roy P., CaIlis J., Erasmus B.J. (1993).Protection of sheep against bluetongue disease after vaccination with core-like and virus-like particles: evidence of homologous and partial heterologous protection, Proceeding of the Annual Meeting of US Animal Health Association, 97: 88-93.

Roy P.. Bishop B.H.L., LeBlois H., Erasmus B.J. (1994). Long lasting protection of sheep against bluetongue challenge after vaccination with virus-like particles: evidence for homologous and partial heterologous protection, *Vaccine* 12805-811.

Roy, P and Sutton G. (1998). New generation of African Horse sickness virus vaccines based on structural and molecular studies of virus particles,Arch. Virol. Suppl. 14 (1998) 177-202.

Sailleau C.,Hamblin C.,Paweskai.Y., Zientara, S. (2000) Identification and differentiation of the nine African horse sickness virus serotypes by RT-PCR amplification of the serotypespecific genome segment 2, Journal of General Virology, 81: 831-837.

Sakamoto, K. Punyahotra, R., Mizukoshi, N; Ueda, S., Imagawa, H., Sugiura, T., Kamada, M. and Fukusho, A. (1994). Rapid detection of Africa horse sickness by the reverse transciptase polymerase chain reaction (RT-PCR) using the amplimer for segment 3 (VP3 gene), Arch, Virol. 136, 87 – 97.

Selvin, S. (1991) Statistical Analysis of epidemiological Data, 2nd Edition. Oxford University Press Inc. New York pp 83.

Sers, J. L. (1965). La peste équine en Algérie; Etude sur l'épizootie d'automne 1965; these doct. vét., Alfort.

Spence, R.P., Moore, N.F. and Nuttal, P.A. (1984). The biochemistry of orbiviruses, Archive o Virology, 82: 1-18.

Stanley, N.F. (1981). Reoviridae: Orbivirus and reovirus infections of mammals and birds, in: Kurstak E., Kurstak C. (Eds.), Comparative Diagnosis of Viral Diseases, Vol. 4b, New York, Academic Press, pp. 67-104.

Theal,G.M. (1899). Records of South-Eastern Africa collected in various libraries and archieve departments in Europe,Gov. Cape Colony, pp. 224.

Theiler A. (1921). African horse sickness (Pestis equorum), South Afr. Dept. Agricultural Science Bulletin No. 19, pp. 30.

Trochim, W. M (2004). The Research Methods Knowledge Base, 2nd Edition. Internet WWW page, at URL: <http://trochim.human.cornell.edu/kb/index.htm> [Accessed on 2 February, 2013].

Van Dijk, A.A (1998). African horse sickness vaccine development. In: Wade, J.F. (Ed.), Proceedings of the Eighth International Conference of Equine Infectious Diseases. R & W Publications, New Market, UK, pp. 261–265.

Van Rensburg, L.BJ., De Clerk, J., Groenewald, H.B. and Botha, W.S. (1981). An outbreak of African horse sickness in dogs, *Journal of South African Veterinary Association*, 52: 323-325.

Venter, E.H., Van Dijk, A.A., Huismans, it and Van der Lugt, J.J. (1992). The detection of bluetongue and African horse sickness wing dotspot and conventional insitu hybridization, in: Walton T.E., Osburn B.I. (eds). Bluetongue, African horse sickness and related orbiviruses, CRC Press, Boca Raton, Pp. 671 – 678.

Venter, G.J., Groenewald, D., Venter, E., Hermanides, K.G. and Howell, P.G. (2002). A Comparison of the Vector Competence of the biting midges, Culicoides (Avaritia) bolitinos and C. (A.) imicola, for the Bryan Ston serotype of equine encephalosis virus. *Medical and Veterinary Entomology*, 16: 372 – 377.

Verwoerd, D.W., Huismans, H. and Erasmus B.J. (1979). Orbiviruses, in: Fraenkel-Conrat, H., Wagner, R.R. (Eds.), Comprehensive Virology, Vol. 14, Plenum Press, London, pp. 285-345.

Vreede, F.T. and Huismans, H. (1994). Cloning, characterisation and expression of the gene that encodes the major neutralisation-specific antigen of African horse sickness virus serotype 3, J. Gen. Virol. 75 3629-3633.

Wohlsein, P., Pohlenz, J.F., Salt J.S. and Hamblin, C. (1998). Immunohistochemical demonstration of African horse sickness antigen in tissues of experimentally infected equines. Archive of Virology, 14: 57-65.

Zientara, S., Sailleau, C., Moulay, S. and Cruciere, C. (1993). Diagnosis and molecular epidemiology of the African horse sickness virus by the polymerase chain reaction and restriction patterns, Veterinary Research, 24: 385-395.

APPENDIX I

RESEARCH QUESTIONAIRE

TO DETERMINE VETERINARY STAFF KNOWLEDGE AND EXPERIENCE ON AFRICAN HORSE SICKNESS

As one of the vital aspects of an on-going MSc. Project work, findings from this questionnaire are intended for assessing the prevalence and co-factors of infection in African Horse Sickness.

A. Demographic Variable

- Date _____
- Location area _____
- Respondent _____ (optional)
- Stable name _____
- Horse breed(s) on farm _____

B. Farm Attributes

- Number of horses in stable/farm: _____
- Source of Horses: Local/imported/both local and imported
- Purpose of horses: Polo/Racing/Draught/Pleasure
- When was the stable established? (Tick appropriately)

  1 – 3 months ago [ ]

  4 – 6 months ago [ ]

  7 months ago and above [ ]

- How often are horses imported into the stable?_____
- Migratory activity of horses: Places Visited for tournament/other equestrian event for the past two (2) years. (Tick appropriately)

  Ibadan [ ]

  Yola [ ]

  Maiduguri [ ]

Minna [ ]

Bauchi [ ]

Katsina [ ]

Lagos [ ]

Port – Harcourt [ ]

Kano [ ]

Kaduna [ ]

Neighboring countries specify for others _____

- Facilities on the farm (Tick appropriately)

  Paddock [ ]

  Stables [ ]

  Track room [ ]

  Fans [ ]

  Nets [ ]

  Quarantine/Isolation stable [ ]

  Boarding facility [ ]

  Polo field [ ]

  Training field [ ]

- Do you carryout employee education (tick appropriately)

  [ ] yes No [ ]

C. Veterinary Staff Experience

- For how long have you been serving in this stable? _____
- How often do you come across horse diseases in the stable (specify) _____
- Among these diseases which is the most prevalent? _____
- Have you encountered African Horse Sickness in your practice especially in the stable? (Tick appropriately) [ ] Yes [ ] No
- If yes above, when last did you encounter it? _____

D. Health – Management Interaction

VACCINATION

- Where the Horses Vaccinated against African Horse Sickness in home country before importation to Nigeria? (Tick appropriately) Yes [ ] No [ ]
- If yes above, what type of vaccine was used? (specify) _____
- Are the vaccines imported or locally produced? (specify) _____
- Do you employ strategic vaccination program Yes [ ] No [ ]
- Do you vaccinate them after arrival? (Tick Appropriately) [ ] Yes No [ ]
- If yes, What type of vaccine was used? (Specify) _____
- Do you often notice diseases following importation? Yes [ ] No [ ]
- If Yes above, which disease(s) are often noticed? Specify _____

DISEASE MANAGEMENT

- How many suspected cases of African Horse Sickness have you had in the past five (5) years? Specify _____
- Which form do they often show? (Tick appropriately)

  Pulmonary form [ ]

  Cardiac form [ ]

  Mixed form [ ]

  Horse sickness form [ ]
- How many horses were infected out of the total horse population on the farm/stable? _____
- How many horses died of African Horse Sickness _____
- How do you handle horse infected with African Horse Sickness? _____
- Which horse breeds are mostly affected? (Tick appropriately)

  Local [ ]

  Exotic [ ]
- What sample(s) were taken from such cases? Specify _____

- Were the samples analysed? _____
- Were the samples positive for African Horse Sickness? Yes [ ] No [ ]

PREVENTION/CONTROL

- Which insect(s) are commonly found around horse stables? (Tick)

  House flies [ ]

  Sand flies [ ]

  Mosquitoes [ ]

  Others (specify)_____

- Do you use quarantine facilities for imported horses? (Tick) Yes [ ] No [ ]
- Do you usually get import permit for horses? (Tick) Yes [ ] No [ ]
- How often do you supervise cleaning of the stable? (Tick)

  Daily [ ]

  Weekly [ ]

  Forthnightly [ ]

- How is African horse Sickness generally prevented/controlled in your Station? State: _____ _____ _____ _____

APPENDIX II

RESEARCH QUESTIONAIRE

TO DETERMINE STABLE MANAGERS KNOWLEDGE AND EXPERIENCES ON AFRICAN HORSE SICKNESS

As one of the vital aspects of an on-going MSc project work, findings from this questionnaire are intended to be used in assessing the prevalence, control measures and possible co-factors of infections in AHS.

The information you provide will be treated as confidential.

A. DEMOGRAPHIC VARIABLE

i. Date _____

ii. Stable _____

iii. No of Males _____ No of Female _____

iv. Use – Polo [ ] Racing [ ] Draught [ ] Pleasure [ ]

v. Breeds of horses on facility _____

_____

_____

B. FARM/STABLE ATTRIBUTES

i. Location of facility – L.G.A _____ District _____

ii. Source of animals _____

iii. When was the stable established (Tick as appropriate)

1 – 3 months ago [ ]

4 – 6 months ago [ ]

7 months ago and above [ ]

iv. Do you have a resident veterinarian in the facility? Yes [ ] No [ ]

v. How long have you been serving as the head groom/Manager? (Tick as appropriate)

1 – 5 years [ ]

6 – 10 years [ ]

11 – 15 years [ ]

16years and above [ ]

vi. How many horses are in the facility:

1 – 10 [ ]

11 – 20 [ ]

21 – 30 [ ]

31 – 40 [ ]

41 – 50 [ ]

51 and above [ ]

Migratory activity of horses

vii. Place visited for tournament/other equestrian event within the year (2011).

Ibadan [ ]

Yola [ ]

Maiduguri [ ]

Minna [ ]

Bauchi [ ]

Katsina [ ]

Lagos [ ]

Port Harcourt [ ]

Kano [ ]

Kaduna [ ]

Housing

viii. Facilities on the farm: (Tick as appropriate)

Paddock [ ]

Stables [ ]

Tack room [ ]

Fans [ ]

Nets [ ]

Quarantine/isolation Stable [ ]

Boarding facility [ ]

Polo field [ ]

Training field [ ]

ix. Stable /stall size: (tick as appropriate)

Less than 10 by 10 [ ]

10 by 10 [ ]

12 by 10 [ ]

12 by 12 [ ]

Others (specify)_____

x. List the three (3) most common diseases you encounter:

a) _____

b) _____

c) _____

xi. Among this diseases which is/are the most common _____

xii. Are you aware of African horse sickness? (Tick as appropriate) Yes [ ] No [ ]

xiii. If yes what is it called locally _____

xiv. Have you ever seen it on your facility? (Tick) Yes [ ] No [ ]

If yes when was the last encounter? _____

C. DISEASE PREVENTION AND CONTROL MEASURES

i. Do you restrict Horse movement in and out of your facility? Yes [ ] No [ ]

ii. Do you perform routine disinfection of the stables? Yes [ ] No [ ]

iii. How often do you change the beddings? 3months [ ] 6months [ ]

others (specify) ……..

iv. Have you introduced new horses in the past 1year? Yes [ ] No. [ ]

Fly control

A. Stabling

[ ] Morning

[ ] Afternoon

[ ] Evening

B. Exclusion Device

[ ] Ear nets

[ ] Fly blankets

[ ] Face masks

[ ] others (specify) _____

C. Hay and manure Management

[ ] Hay in pastures?

[ ] General sanitation?

D. Management of other species on farm

Presence of cattle and manure on farm? Yes [ ] No [ ]

Presence of animal on farm? (specify) _____

E. Farm Terrain

Presence of body of water in the area: Yes [ ] No [ ]

Wooded areas: Yes [ ] No [ ]

Open breezy pastures: Yes [ ] No [ ]

F. Premises Insecticide Application

Do you apply insecticide on your premises?

Yes [ ] No [ ]

If yes, tick type and forms

Types of insecticide_____

Forms of Repellents and Insecticide_____

- Sprays [ ]
- Stabilized sprays [ ]
- Face lotion [ ]
- Roll-ons for face and head [ ]
- Wound treatment gels or ointments [ ]
- Fly collars, leg bands [ ]
- Wipe –on and toweletes [ ]

Appendix III: Serum Titre Value of AHSV Based on Breed of horses from seven LGAs

LGA	Indigenous	Argentine	South African
Zaria	62.67	103.61	122.00
Igabi	87.76	103.94	194.80
Sabon Gari	81.50	86.33	93.00
Kaduna North	96.06	98.89	119.50
Lere	75.60	-	-
Ikara	64.83	-	-
Markarfi	71.75	-	-
Overall mean	80.35	97.51	117.60
Overall SEM *	5.28	5.73	21.58

Significant $P < 0.05$ (0.0003) * Standard error of the mean

Appendix IV: Serum Titre Value of AHSV Based on purpose of Horses in seven LGAs

LGA	Pleasure	Racing	Polo
Zaria	41.50	75.62	113.68
Igabi	67.40		93.00
Sabon Gari	57.17	-	-96.76
Kaduna North	82.67	-	105.56
Lere	75.60	-	-
Ikara	71.75	-	-
Markarfi	64.83	-	-
Overall mean	70.74	41.50	102.95
Overall SEM*	4.97	25.50	5.45

Significant $P < 0.05$ (0.018)

Appendix V: Mean serum titre value of AHSV based on Sex of horses from seven LGAs

LGA	Male	Female
Zaria	86.71	86.83
Igabi	79.11	118.46
Sabon Gari	73.72	101.06
Kaduna North	95.13	103.00
Lere	75.40	123.00
Ikara	58.00	70.90
Markarfi	79.62	83.00
Overall mean	80.82	100.09
Overall SEM	49.04	53.60

(Highly Significant at 1%) P value 0.008

Appendix VI: Mean serum titer value of AHSV based on Age of horses from seven LGAs

LGA	1 month – 5years	6 – 10 years	11 – 15 years	16 – 20 years
Zaria	86.00	98.65	102.75	175.00
Igabi	86.63	111.62	71.75	56.00
Sabon Gari	79.71	95.38	66.33	104.33
Kaduna North	97.20	90.00	111.11	11.00
Lere	67.50	95.00	52.50	-
Ikara	15.50	101.60	51.33	-
Markarfi	46.50	62.00	87.00	-
Overall mean	77.72	95.07	88.51	92.50
Overall DEM	9.83	5.03	6.57	23.42

(Not significant) P value 0.460

Appendix VII: Mean serum titer value of AHSV based on Vaccination Status of animals

LGA	NHV	Vaccination
Zaria	95.04	108.68
Igabi	85.00	91.21
Sabon Gari	71.52	107.41
Kaduna North	98.43	100.06
Lere		
Ikara		
Makarfi		
Overall mean	84.70	100.10
Overall DEM	53.38	48.57

(Significant at 5%) P value 0.043

Appendix VIII: Seroprevalence of AHSV based on purpose in the Zaria LGA

Variance	Racing	Polo	Pleasure
Total no tested	2	25	13
No positive	2	23	12
Prevalence (%)	100.0	92.0	92.30

Appendix IX: Seroprevalence of AHSV based on purpose in Sabon Gari Local Government Area

Variance	Racing	Polo	Pleasure
Total no tested	0	25	12
No positive	0	24	11
Prevalence (%)	0	96.0	91.67

Appendix XI: Seroprevalence of AHSV based on purpose in Igabi LGA

Variance	Racing	Polo	Pleasure
Total no tested	0	25	10
No positive	0	23	9
Prevalence (%)	0	92.0	90

Appendix XII: Seroprevalence of AHSV based on purpose in Kaduna North LGA

Variance	Racing	Polo	Pleasure
Total no tested	0	25	12
No positive	0	25	12
Prevalence (%)	0	100	100

Appendix XIII: Seroprevalence of AHSV base on purpose in Lere LGA

Variance	Racing	Polo	Pleasure
Total no tested	0	0	10
No positive	0	0	10
Prevalence (%)	0	0	10

Appendix XIV: Seroprevalence of AHSV based on purpose in Ikara LGA

Variance	Racing	Polo	Pleasure
Total no tested	0	0	12
No positive	0	0	11
Prevalence (%)	0	0	91.6

Appendix XV: Seroprevalence of AHSV based on purpose in Makarfi LGA

Variance	Racing	Polo	Pleasure
Total no tested	0	0	12
No positive	0	0	10
Prevalence (%)	0	0	83.3

APPENDIX XVI- I-ELISA Results

S/No	Sample Reference	I. Elisa	Vaccistatus	sex	Breed	Use	Age (year)	LG
1	1	40	NVH	M	Idg	Pleasure	7	SG
2	2	16	NVH	F	Idg	Racing	5	ZA
3	3	101	NVH	M	Idg	Pleasure	7	IG
4	4	105	NVH	M	Idg	Pleasure	5	KN
5	5	97	NVH	M	Idg	Pleasure	7	ZA
6	6	67	NVH	M	Idg	Racing	8	ZA
7	7	24	NVH	M	Idg	Pleasure	6	SG
8	8	155	NVH	M	Idg	Pleasure	6	IG
9	9	68	NVH	M	Idg	Pleasure	7	KN
10	10	41	NVH	M	Idg	Pleasure	7	ZA
11	11	32	NVH	M	Idg	Pleasure	6	SG
12	12	89	VACC	F	Arg	Polo	10	ZA
13	13	Neg	VACC	M	Arg	Polo	2	IG
14	14	194	VACC	M	Arg	Polo	3 ½	SG
15	15	109	VACC	F	Arg	Polo	15	KN
16	16	Neg	VACC	M	Arg	Polo	7	ZA
17	17	83	VACC	F	Arg	Polo	20	SG
18	18	78	VACC	F	Arg	Polo	12	IG
19	19	175	VACC	F	Arg	Polo	20	ZA
20	20	119	VACC	F	Arg	Polo	9	SG
21	21	124	VACC	F	Arg	Polo	15	IG
22	22	98	VACC	F	Arg	Polo	8	KN
23	23	123	VACC	F	Arg	Polo	15	ZA
24	24	83	VACC	M	Arg	Polo	5	SG
25	25	111	VACC	F	Arg	Polo	7	KN

26	26	88	VACC	F	Arg	Polo	5	IG
27	27	131	VACC	F	Arg	Polo	10	SG
28	28	60	VACC	M	Arg	Polo	10	ZA
29	29	82	VACC	F	Arg	Polo	6	KN
30	30	121	VACC	F	Arg	Polo	8	SG
31	31	117	VACC	F	Arg	Polo	11	ZA
32	32	88	VACC	F	Arg	Polo	7	KN
33	33	130	VACC	F	Arg	Polo	5	IG
34	34	80	VACC	F	Arg	Polo	7	SG
35	35	22	VACC	F	Arg	Polo	11	ZA
36	36	52	VACC	F	Arg	Polo	11	KN
37	37	91	VACC	F	Arg	Polo	7	IG
38	38	42	VACC	M	Arg	Polo	8	KN
39	39	63	VACC	F	Arg	Polo	8	ZA
40	40	72	VACC	F	Arg	Polo	5	IG
41	41	66	VACC	F	Arg	Polo	7	SG
42	42	131	VACC	F	Arg	Polo	11	ZA
43	43	137	NVH	M	Idg	Pleasure	9	SG
44	44	82	VACC	F	Arg	Polo	11	IG
45	45	104	NVH	M	Idg	Pleasure	12	ZA
46	46	132	NVH	F	Arg	Polo	15	SG
47	47	83	VACC	F	Arg	Polo	12	IG
48	48	125	VACC	F	Arg	Polo	11	ZA
49	49	123	NVH	M	Idg	Pleasure	9	ZA
50	50	107	VACC	F	Arg	Polo	5	SG
51	51	82	VACC	F	Arg	Polo	14	KN
52	52	58	VACC	F	Arg	Polo	11	IG
53	53	102	VACC	F	Arg	Polo	12	ZA

54	54	83	VACC	F	Arg	Polo	6	KN
55	55	84	VACC	F	Arg	Polo	11	SG
56	56	85	VACC	F	Arg	Polo	11	IG
57	57	59	VACC	F	Arg	Polo	11	ZA
58	58	106	VACC	M	Arg	Polo	14	SG
59	59	118	VACC	M	Arg	Polo	11	KN
60	60	190	VACC	F	Arg	Polo	15	ZA
61	61	36	NVH	M	Idg	Pleasure	10	ZA
62	62	66	NVH	M	Idg	Pleasure	13	SG
63	63	97	VACC	F	Arg	Polo	6	IG
64	64	176	VACC	F	Arg	Polo	9	SG
65	65	93	VACC	F	Arg	Polo	11	KN
66	66	Neg	VACC	F	Arg	Polo	11	ZA
67	67	143	VACC	F	Arg	Polo	6 1/2	KN
68	68	42	VACC	M	Arg	Polo	11	IG
69	69	66	VACC	F	Arg	Polo	15	SG
70	70	112	VACC	F	Arg	Polo	11	ZA
71	71	54	VACC	F	Arg	Polo	5	KN
72	72	63	VACC	M	Arg	Polo	5	IG
73	73	89	VACC	F	Arg	Polo	7	SG
74	74	102	VACC	F	Arg	Polo	8	ZA
75	75	97	VACC	F	Arg	Polo	10	IG
76	76	77	VACC	F	Arg	Polo	6	SG
77	77	205	VACC	F	Arg	Polo	7	ZA
78	78	89	VACC	F	Arg	Polo	13	KN
79	79	38	VACC	F	Arg	Polo	11	IG
80	80	132	VACC	F	Arg	Polo	20	SG
81	81	187	VACC	F	Arg	Polo	11	ZA

82	82	42	VACC	F	Arg	Polo	14	KN
83	83	122	VACC	F	Arg	Polo	15	IG
84	84	Neg	NVH	F	Idg	Polo	11	SG
85	85	242	VACC	F	Arg	Polo	12	KN
86	86	243	NVH	F	Idg	Polo	15	ZA
87	87	204	VACC	F	Arg	Polo	11	IG
88	88	84	NVH	M	Idg	Pleasure	9month	ZA
89	89	90	NVH	F	Idg	Pleasure	12months	KN
90	90	98	NVH	M	Idg	Pleasure	17	SG
91	91	83	NVH	M	Idg	Pleasure	15	MK
92	92	118	NVH	M	Idg	Pleasure	6	ZA
93	93	86	NVH	F	Idg	Pleasure	11	LR
94	94	52	NVH	F	Idg	Pleasure	8	MK
95	95	75	NVH	M	Idg	Pleasure	5	IG
96	96	Neg	NVH	M	Idg	Pleasure	8	ZA
97	97	Neg	NVH	F	Idg	Pleasure	3 1/2	SG
98	98	11	NVH	M	Idg	Pleasure	7 1/2	KN
99	99	113	NVH	M	Idg	Pleasure	7	SG
100	100	114	NVH	M	Idg	Pleasure	7	ZA
101	101	56	NVH	M	Idg	Pleasure	18	IG
102	102	39	NVH	M	Idg	Pleasure	13	ZA
103	103	102	NVH	M	S/Africa	Polo	11	IG
104	104	19	NVH	M	Arg	Polo	11	SG
105	105	105	NVH	F	Arg	Polo	12	KN
106	106	194	VACC	F	S/Africa	Polo	7	ZA
107	107	204	NVH	F	Idg	Polo	9	IG
108	108	119	VACC	F	S/Africa	Polo	15	KN
109	109	113	NVH	F	Idg	Polo	15	SG

110	110	39	NVH	M	Idg	Pleasure	14	IG
111	111	13	NVH	M	Idg	Pleasure	13	ZA
112	112	43	NVH	M	Idg	Pleasure	12	IG
113	113	112	VACC	M	S/Africa	Polo	9	SG
114	114	61	VACC	M	S/Africa	Polo	6	IG
115	115	154	VACC	F	Idg	Polo	15	KN
116	116	89	NVH	M	Idg	Polo	11	SG
117	117	75	NVH	F	Idg	Polo	10	IG
118	118	56	NVH	F	Idg	Pleasure	10	ZA
119	119	11	NVH	F	Idg	Pleasure	12	SG
120	120	41	NVH	F	Idg	Polo	5	SG
121	121	151	NVH	M	Idg	Polo	7	KN
122	122	96	NVH	M	Idg	Polo	7	ZA
123	123	113	NVH	F	Idg	Polo	6	SG
124	124	86	NVH	M	Idg	Polo	7	SG
125	125	52	NVH	F	Idg	Polo	10	ZA
126	126	Neg	NVH	F	Idg	Polo	15	IG
127	127	92	NVH	F	Idg	Polo	8	ZA
128	128	211	NVH	M	Idg	Polo	5months	IG
129	129	75	NVH	F	Idg	Polo	12	KN
130	130	137	NVH	F	Idg	Polo	11	KN
131	131	226	NVH	F	Idg	Polo	6	ZA
132	132	30	NVH	M	Idg	Polo	8	KN
133	133	136	NVH	F	Idg	Polo	11	KN
134	134	188	NVH	F	Idg	Polo	10	IG
135	135	158	NVH	M	Idg	Pleasure	5	ZA
136	136	204	NVH	M	Idg	Polo	7	KN
137	137	68	NVH	M	Idg	Polo	11	ZA

138	138	Neg	NVH	M	Idg	Pleasure	11	IG
139	139	166	NVH	M	Idg	Pleasure	11	KN
140	140	70	NVH	M	Idg	Pleasure	5	SG
141	141	80	NVH	M	Idg	Pleasure	5 1/2	MK
142	142	32	NVH	F	Idg	Pleasure	9	SG
143	143	48	NVH	M	Idg	Pleasure	11	IG
144	144	43	NVH	M	Idg	Pleasure	10	KN
145	145	106	NVH	M	Idg	Pleasure	9	KN
146	146	63	NVH	M	Idg	Pleasure	4	SG
147	147	54	NVH	M	Idg	Pleasure	4	IG
148	148	11	NVH	F	Idg	Pleasure	16	KN
149	149	83	NVH	F	Idg	Pleasure	10	MK
150	150	103	NVH	M	Idg	Pleasure	9 1/2	IG
151	151	151	NVH	F	Idg	Pleasure	11 1/2	KN
152	152	Neg	NVH	F	Idg	Pleasure	7 1/2	MK
153	153	173	NVH	M	Idg	Pleasure	9 1/2	MK
154	154	129	NVH	M	Idg	Pleasure	10 1/2	KN
155	155	30	NVH	M	Idg	Pleasure	7 1/2	MK
156	156	106	NVH	M	Idg	Pleasure	10 1/2	MK
157	157	94	NVH	M	Idg	Pleasure	11 1/2	KN
158	158	82	NVH	M	Idg	Pleasure	10	LR
159	159	24	NVH	M	Idg	Pleasure	12	IK
160	160	18	NVH	M	Idg	Pleasure	11	KN
161	161	88	NVH	M	Idg	Pleasure	9 1/2	LR
162	162	93	NVH	M	Idg	Pleasure	7 1/2	IK
163	163	121	NVH	M	Idg	Pleasure	5 1/2	LR
164	164	59	NVH	M	Idg	Pleasure	6 1/2	IK
165	165	35	NVH	M	Idg	Pleasure	5	LR

166	166	38	NVH	M	Idg	Pleasure	7 1/2	MK
167	167	19	NVH	M	Idg	Pleasure	10 1/2	LR
168	168	77	NVH	M	Idg	Pleasure	12	IK
169	169	40	NVH	M	Idg	Pleasure	4	MK
170	170	25	NVH	M	Idg	Pleasure	9	LR
171	171	75	NVH	M	Idg	Pleasure	9	IK
172	172	100	NVH	M	Idg	Pleasure	5	LR
173	173	109	NVH	M	Idg	Pleasure	10	IK
174	174	129	NVH	M	Idg	Pleasure	9	LR
175	175	71	NVH	M	Idg	Pleasure	8	LR
176	176	143	NVH	M	Idg	Pleasure	7	IK
177	177	93	NVH	M	Idg	Pleasure	11 1/2	MK
178	178	53	NVH	M	Idg	Pleasure	12	IK
179	179	Neg	NVH	M	Idg	Pleasure	5 1/2	MK
180	180	65	NVH	M	Idg	Pleasure	6 1/2	IK
181	181	Neg	NVH	M	Idg	Pleasure	5	IK
182	182	132	NVH	M	Idg	Pleasure	10	IK
183	183	31	NVH	M	Idg	Pleasure	3 1/2	IK

KEY

ZA	Zaria
SG	Sabon gari
LR	Lere
IK	Ikara
KN	Kaduna North
IG	Igabi
MK	Makarfi
Idg	Indigenous
Arg	Argentine polo pony
NVH	No Vaccination history
Vacc	Vaccinated