Sudan University of science and technology

College of graduate studies

Seroprevalence of African horse sickness in Khartoum state , Sudan

المسح المصلي لمرض طاعون الخيل الافريقي في ولاية الخرطوم - السودان

By

Hind Merghani Hassan Ibrahim

B.V.M. (2007), College of Veterinary Medicine, Sudan University of Science and Technology

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Supervisor: Professor. Mohammed Abd Elsalam Abd Allah

Department of veterinary medicine and animal surgery, college of veterinary medicine, Sudan University of science and technology

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DEDICATION

For my small family, my small angels Dyala , Tala , Yara and my Queen Bana.

To Soule of my beautiful mother

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ABSTRACT

A cross sectional study was conducted on equine to determine the seroprevalence of African Horse Sickness Virus (AHSV) antibodies and identification of potential risk factors in equine population in the three Khartoum localities in Khartoum State namely Khartoum, Khartoum north and Omdurman. A total of 187 serum samples were collected for competitive ELISA test to determine the presence of African Horse Sickness (AHS) antibodies. The seroprevalence of 87, 84 and 100% were found in the Khartoum, Khartoum north and Omdurman localities, respectively. The apparent seroprevalence was found to be 94% in donkey and 84% in horses. The overall seroprevalence of AHS virus was found to be 84%. There was no significant variation between the horse and donkey in the seropositivity ($p>0.05$). Significant variation was not observed in seroprevalence among age groups and sex of equine. All age groups as well as male and female of equine population were equally affected. Knowledge base of equine owner about AHS, *Clucoides* vector and mode of transmission of the disease in the study areas were assessed through structured questionnaire. The survey indicated that almost all equine owners did not know about *Clucoides* vector and mode of transmission. Therefore, there should be awareness reaction about AHS and Clucoides vector among the people through an organized extension package to the present study areas.

الخلاصه

مرض الخيل الافريقي (النجمه) مرض مستوطن فى الدول الواقعه جنوب الصحراء الافريقية ’ و يعتبر واحد من لهم الامراض المهدده للحياة بالنسبه للخيل في بعض من اجزاء العالم .

هذا البحث صمم لمعرفه وضع المرض في ولاية الخرطوم ’ في جمهورية السودان وشمل البحث المحليات الثلاثة داخل الولاية .مائة وسبعه و ثمانون عينة سيرم جمعت من الخيل واستخدمت الاليزا لفحص الاجسام المضادة في دم الخيل وكانت نسبه الوبائية للمرض 84%

وأيضا تم تصميم استبيان لسبعه وستون من مالكي الخيل والسياس لنري مدي معرفتهم بالمرض ’ كيفية التعامل مع الاصابه و مدي السيطرة على الناقل . كل العوامل فى الاستبيان (العمر’ مصدر الحيوان ’ مكان الاسطبل والجنس) لا تعطي قراءة للارتباط بالمرض أو تؤثر علي مناعته . المعدل العالى للوبائيه يتطلب وضع برنامج تحصين متكامل و وضع استراتيجية للتحكم في المرض والقضاء علي الناقل .

Introduction

African horse sickness (AHS) is an economically highly important non contagious, infectious, insect-borne disease of equids caused by a virus of the genus *Orbivirus* of the family Reoviridae. The disease transmitted by a biting midge belonging to the *Culicoides* genus. Although zebra and donkeys rarely exhibit clinical signs, the effects of the disease, particularly in susceptible populations of horses can be devastating and mortality rates for this species may exceed 90%. As a consequence of its severity and because it is able to expand rapidly and without apparent warning out of its endemic areas (Philip and Christopher , 2004) .

There are nine distinct serotypes of AHSV (AHSV-1 to AHSV-9) that can be distinguished in serum neutralisation tests by the specificity of their reactions with neutralizing antibodies. These different serotypes are all classified within the species of African horse sickness virus, genus Orbivirus, family Reoviridae (Katarzyna *etal,* 2014). 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 (Philip and Christopher, 2004).

In horses, AHS is characterized by clinical signs, which develop as a result of damage to the circulatory and respiratory systems giving rise to serous effusion and hemorrhage 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 (which usually affects only mules, donkeys and partially immune horses), the sub acute or cardiac form, the cardio-pulmonary or mixed form and the per acute or pulmonary form. All forms of disease can occur in any one outbreak but in susceptible populations of horses the mixed and pulmonary forms tend to predominate, so mortality rates in these animals will be very high (Philip and Christopher , 2004).

The diagnosis of AHS is based on either the isolation of the virus in mice and/or cell cultures or the demonstration of precipitating viral antigens in infected cell culture, or infected mouse brains, or retrospectively by demonstration of antibodies against the virus in sera of previously infected animals by serum neutralization test (SNT), or complement fixation test (CFT) (Ihsan , 2004).

Since there is no successful treatment for AHS, vaccination is the most important approach to protect horses against AHS. Several AHSV vaccines have been previously developed and used including: a polyvalent live-attenuated (modified-live virus (MLV)) vaccine of adult mouse brain origin, a polyvalent cell culture-adapted MLV vaccine, and inactivated AHSV vaccines. Although new generation vaccines have been described recently, only MLV AHSV vaccines are currently commercially available in southern Africa, and other African countries (Oura , 2012, Crafforda *et al*, 2014).

Clinical AHS is known to have existed in the Sudan since 1903, the first virological confirmation was made in 1957, when samples from infected horses were tested by the Onderstepoort Laboratory in South Africa, and found to contain AHS virus type 3. Horses are the only species vaccinated against the disease in the Sudan (Abu Elzein *et al*, 1989).

Objectives:

1. To determine the prevalence of the disease in unvaccinated horses.
2. To comprise if the management and present of intermediate host can affect on the prevalence of disease.

Chapter One

Literature Review

1.1 Epidemiology:

At present, AHSV is considered to be endemic only in sub- Saharan Africa, with outbreaks involving all nine serotypes of AHSV occurring in South Africa. Until 2007, AHSV-9 was the main serotype circulating in the equine populations of Central Africa. However, other AHSV serotypes have subsequently also been reported in the region, including AHSV-2 in Senegal and Nigeria in 2007, in Ethiopia in 2008 and Ghana in 2010; AHSV-4 in Kenya in 2007, and AHSV-7 in Senegal in 2007. In 2010 an outbreak involving multiple serotypes of AHSV also took place in Ethiopia (Katarzyna, *et al*, 2014).

However, AHSV has caused devastating outbreaks in indigenous horse populations outside of its current endemic zone, including AHSV-9 in the Middle East, India and Pakistan (1959– 1961) and in North Africa and Europe (1965) (Mellor and Hamblin 2004). Importation of an infected zebra from Namibia to Madrid in 1987, led to an outbreak caused by AHSV-4 in Spain that spread to Portugal and North Africa (1987–1990) (Katarzyna , *et al*, 2014).

Since 1998, multiple different serotypes of bluetongue virus (BTV) which are transmitted by similar Culicoides vectors to AHSV have emerged in Southern Europe (Purse *et al*, 2005) leading up to the very severe outbreak caused by BTV-8 which started in 2006, the first ever recorded in northern Europe (Saegerman *et al*, 2008). The virus overwintered and spread over most of Europe causing massive losses to the livestock industries of several countries over a number of years (Carpenter *et al*, 2009, De Koeijer *et al*, , 2011). Phylogenetic analyses showed that the northern European strain of BTV-8 is most closely related to a strain from Central Africa, although the route of introduction has not yet been identified (Maan , *et al*, ,2008, Saeed , *et al*, 2001, Gibbens , 2012). *et al*,

AHSV is endemic in tropical and sub-Saharan Africa, but sporadic cases and short-term epidemics in North Africa and Middle-East have been reported in the mid-20th century. In 1987, an outbreak of AHSV-4 on the Iberian Peninsula, which was

extended for a few years in Spain and spread to Portugal and Morocco indicating that AHSV had overwintered and spread by European Culicoides midges (Mellor and Hamblin 2004, Capela R. *et al,* 2003).

A study was carried out in a farm located in the district of Windhoek (Namibia), where the disease is endemic. A total of 72 cross-breed horses, out of the 150 housed on the farm, were subdivided in six age groups, from 2 to 7 years-old. Each group consisted of 12 heads which were born during the same breeding season and had undergone from four to nine vaccination courses. AHSV specific immune response was evaluated by serum-virus neutralization test. Data about the clinical occurrence of the AHS from 2006 to 2011 were made available. The immune response, in terms of number of seropositive horses and serum neutralizing titers, was quite variable among horses and against different serotypes. Neutralizing antibodies against all serotypes were recorded in all the horses only after eight vaccination courses at 6 years of age onwards. Immune response to AHSV- 5 and 9, which are not included in the LAV formulation, were also established. A severe AHS epidemic occurred in Namibia in 2011. On the farm under study, a total of 32 animals were clinically affected, 12 died, 11 of them were 2 year-old or younger. Our data confirm that vaccination with LAV is a useful tool to reduce the severity of the disease in endemic areas. However, clinical and sometimes fatal AHS can still affect young vaccinated horses, thus highlighting the necessity to better understand the immune response to AHSV and to dispose of more effective vaccines (Umberto , *et al*, , 2015).

A study was conducted with the aims of determining the seroprevalence and risk factors of African Horse Sickness (AHS) in mules and donkeys in selected sites of West Amhara region. A total of 390 (191 mules and 199 donkeys) serum samples were collected from November 2009 to February, 2010. Blocking ELISA was employed to determine the presence of African Horse Sickness Virus (AHSV) antibodies. The overall seroprevalence of AHS in this study was found to be 57.4% (95% CI = 52.35 to 62.39%). The prevalence of AHS was found to be lower in mules (55.5%) than donkeys (59.3%). Statistical analysis of the data showed that there were no significant variation ($P>0.05$) in the prevalence of AHS between the two species of animals examined in this study. The seroprevalence of the disease was higher in mules and donkeys >5 years old when compared to those <5 years

old. But there was no significant variation (P> 0.05). Males were found to be affected by AHS more than female animals do but there were no significant difference (P>0.05) between the two sexes of mules and donkeys examined. The result showed higher seroprevalence of AHS in lowland than the midland agro ecological zones of the study area. Analysis of the data showed statistically significant (P<0.05) variation of AHS between the two agro ecological zones. Of the 100 equine owners interviewed about AHS 25 (25%) were familiar and 75 individuals (75%) of the respondents know nothing regarding the disease AHS. Hence, the knowledge of equine owners about AHS was assessed to be at infancy. This suggests the need to implement strict awareness creation among equine owners on vaccination of their animals and methods of insect control in the study area in order to decrease the prevalence of AHS (Mulualem *et al*, 2012).

A cross-sectional study was undertaken to determine the seroprevalence of African Horse sickness virus (AHSV) antibodies and identify potential risk factors in equine population at selected areas of central high land of Ethiopia from November 2011 to April 2012. A total of 546 sera (506 horses, 18 mules and 22 donkeys) were collected randomly. Competitive Enzyme Linked Immuno Sorbent Assay (c-ELISA) configuration was employed to determine the presence of AHSV antibodies. The apparent prevalence of AHSV was found to be 46 % in horses, 61.1 % in mules and 36.4 % in donkeys. The overall apparent seroprevalence of AHSV in three species equine was found to be 46.2 %. Statistical significant (p<0.05) difference in seroprevalence was observed at the different study areas confirming the existence of agro-ecology based variation in the occurrence of AHS. The highest seroprevalence of AHSV was documented at the mid highland followed by highland areas. There were no significant variations (P> 0.05) among age groups and sexes for seroprevalence of AHSV. In this study, all age groups as well as male and female populations were equally affected by African horse sickness disease. Questionnaire survey also indicated the presence of African horse sickness disease in the study areas. Therefore, control strategies as annual vaccination and appropriate housing system should be targeted at all ages and in both sex (Haji *et al*, 2015).

A cross-sectional serological survey was undertaken in selected districts of different agro-ecology of Jimma zone (Dedo, Yebu, Seka, Serbo, and Jimma town) Ethiopia from November 2009 to February 2010 to determine the seroprevalence of African horse sickness virus and associated risk factors of the disease. Two

hundred seventy-four equids (189 horses, 43 mules, and 47 donkeys) with a history of non-vaccination for at least 2 years were selected randomly from the above areas. Sera samples were collected and assayed for the presence of specific antibody against African horse sickness virus using blocking ELISA. An overall seroprevalence of 89 (32.5%) was found and it was (24 /51.1%) for donkeys, (13 /30.2%) for mules, and 52 (28.3%) for horses. Seroprevalence was significantly ($X^2 = 11.05$, $P < 0.05$) different among the different species of equids. Seroprevalence was also significantly ($X^2 = 11.43$, $P < 0.05$) different among the different agro-ecological areas being higher in highlands 47 (40.5%) followed by midland 30 (34.5%) and lowland 12 (16.9%). Age and sex were not significantly ($X^2 = 3.15$, $P > 0.05$ and $X^2 = 3.38$, $P > 0.05$, respectively) associated with seroprevalence of AHSV. The present study showed that African horse sickness (AHS) is highly prevalent disease for the horses followed by mules and then donkeys in Jimma zone explained by lower seroconversion rate. Therefore, control strategy against AHS should target at high risk species of all age and sex in their locality in the initial stage for better containment of the disease (Molalegne , *et al*, 2011).

A study of the prevalence of African horse sickness in horses was conducted, using records from two private equine practices in Harare, capital of Zimbabwe for the period 1998-2004. Results indicated a higher prevalence of the disease in horses in Zimbabwe in the late rainy season (March - May). Age of the horse was found to be a significant risk factor, with foals or yearlings appearing to be 1.80 times more likely to contract the disease compared with horses older than two years. The case fatality rate in foals or yearlings was also higher than in older age groups, but this difference was not significant. The vaccination status was an important risk factor, with vaccinated horses 0.12 times less likely to die from the disease compared with unvaccinated horses. Young, unvaccinated horses therefore seem to be the most susceptible to the disease and have greater chances of fatality. This study highlights the importance of adequately protecting horses against African horse sickness by providing immunization through vaccination and discusses the need to review current vaccination strategies being practiced in Zimbabwe (Stuart , *et al*, 2013).

A cross sectional study was conducted on equine from November 2010 to February 2011 to determine the seroprevalence of African Horse Sickness Virus (AHSV) antibodies and identification of potential risk factors in equine population in selected areas of Arsi and Bale zones, Ethiopia. A total of 480 serum samples were

collected. Competitive ELISA test was employed to determine the presence of African Horse Sickness (AHS) antibodies. The seroprevalence of 28.63 and 14.23% were found in the Arsi and Bale zones, respectively. The apparent seroprevalence was found to be 24.60% in donkey, 20.34% in horses and 20% in mules. The overall seroprevalence of AHS virus was found to be 21.45%. There was no significant variation between the horse, donkey and mules in the seropositivity ($p>0.05$). Statistically significant difference ($p<0.05$) in the seroprevalence was observed in the different study area, confirming the existence of agro-ecology variation in the occurrence of AHS, thus higher seroprevalence of AHS was documented in midland (31.38%) followed by highland (15.06%). Significant variation was not observed in seroprevalence among age groups and sex of equine. All age groups as well as male and female of equine population were equally affected. Knowledge base of equine owner about AHS, Clucoides vector and mode of transmission of the disease in the study areas were assessed through structured questionnaire. The survey result indicated that almost all equine owners did not know about Clucoides vector and mode of transmission. Therefore, there should be awareness reaction about AHS and Clucoides vector among the people through an organized extension package to the present study areas (Tesfaye , *et al*, 2012).

1.2 Etiology:

African horse sickness virus (AHSV) is a member of the genus *Orbivirus* in the family Reoviridae and as such is morphologically similar to other orbiviruses such as bluetongue virus (BTV) of ruminants and equine encephalosis virus. The virion is an unenveloped particle about 70 nm in diameter and is made up of a two layered icosahedral capsid, which is composed of 32 capsomeres. The genome comprises 10 double stranded RNA segments, each of which encodes at least one polypeptide. The genome is enclosed within the core particle, which comprises two major proteins, VP 3 and 7 which are highly conserved among the nine AHSV serotypes, and three minor proteins, VP 1, 4 and 6, together they make up the group specific epitopes. The core particle is surrounded by the outer capsid composed of two proteins VP 2 and VP 5. VP 2 is the protein that has most responsibility for antigenic variation. At least three non-structural proteins have been identified in infected cells (NS 1, 2 and 3/3a)

To date, nine antigenically distinct serotypes have been identified the last being in 1960. There is considered to be some cross relatedness between 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. 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 (Philip. and Christopher , 2004).

1.3 Host range:

AHSV affects all equine species including horses, donkeys, mules, hinnies and zebras. Morbidity and mortality rates vary between species, horses being most susceptible to the virus. Mortality in horses can reach 95%, while infections in zebras are mostly subclinical (Coetzer and Guthrie, 2004).

In addition to equids, camels, goats, and buffalo can become infected. Additionally, some carnivores such as dogs can become infected via ingestion of contaminated meat. However, there have been no documented cases of transmission of AHSV in carnivores in the wild, and it is considered that they area 'dead-end' host, rather than a reservoir of infection (Geoffrey , 2012).

1.4 Transmission:

Orbiviruses are transmitted to animals mainly by arthropod vectors. The distribution of these viruses is very similar to the distribution of their specific vector and it is therefore influenced by climatic conditions. AHSV is transmitted by midges of the genus *Culicoides* which are also the vectors involved in Bluetongue (BT) transmission (MacLachlan and Guthrie, 2010).

The only proven field vector of AHSV is the biting midge Culicoides imicola Kieffer (= Cpallidipennis) (Diptera: Ceratopogonidae). This species is widely distributed in sub- Saharan Africa, and has been found in Asia as far east as Laos and Vietnam, in North Africa and in Europe, where it has been reported from Spain, Portugal, Cyprus and several Greek islands. However, other species of Culicoides have been shown, under laboratory conditions, to be capable of supporting AHSV replication and AHSV serotype 4 was successfully isolated from mixed pools of parous, non-engorged C pulicaris Linnd and Italic Meigen collected from Spain in 1988. These species must therefore also be considered to be potential vectors of the virus (Baylis *et al*,, 1997). The AHSV was also reported to be transmitted mechanically by biting flies such as stomoxys and tabanus (Ihsan ,

2004). Laboratory experiments have shown that some species of mosquito, most notably Aedes aegypti, *etal*, are capable of AHSV infection and transmission. Although, the virus only underwent multiplication in a limited number of mosquitoes and the maximum virus titre was not appreciably higher than the amounts ingested, an eclipse phase occurred subsequent to infection and virus was recovered for at least five weeks. Additionally, AHSV has also been isolated in the field from samples of camel ticks, Italic in Egypt. However, the prevailing scientific opinion suggests that the role of these species in the transmission of AHSV is likely to be insignificant (Geoffrey ,2012).

The geographic distribution and seasonal incidence of AHSV depend not only on the presence of the virus and susceptible equines but also on the presence and abundance of competent vectors (Mellor ,2000).

1.5 Clinical sings:

AHSV displays itself in the form of four diseases: cardiac form, pulmonary form, mixed form, and horse sickness fever. The cardiac form of the disease is usually characterized by the development of a fever, oedema of the head, neck, chest, supraorbital fossae, petechial haemorrhages in the eyes, ecchymotic haemorrhages on the tongue, and colic. In these cases, mortality of infected animals may exceed 50%. The pulmonary form of the disease is the most serious. It is typically associated with a rapid onset of symptoms which include the development of a fever, depression, severe respiratory distress, severe dyspnoea, coughing, and sweating. Typically, the mortality rates of this form of the disease exceed 95% (Erasmus ,1994).

The mixed form is often the most common form of the disease and as the name suggests, it is a combination of the cardiac and the pulmonary form. Typically, the mortality rate may exceed 70%, and death can often occur within three to six days. Horse sickness fever is the mildest form of the disease, during which, the animal will develop a moderate fever and some oedema of the supraorbital fossae. There is no mortality associated with this form of the disease (Geoffrey, *Italic*, 2012).

1.6 Diagnosis:

Historically, members of the AHSV serogroup/virus-species have been identified by serological methods, including complement fixation (CF) and ELISA tests (Katarzyna , *etal,* 2014). AHSV virus-species specific antibodies can also be detected by ELISA (Kweon *et al*, 2003, Hamblin *et al*, , 1990).

However, these methods are labour intensive and require virus isolation and/or access to standard reagents (antibodies and antigens) that may themselves represent a potential biosecurity risk. AHSV RNA can be detected by amplification in conventional or real-time RT-PCR assays. Different 'conserved' AHSV genome segments have previously been targeted by conventional RT-PCR assays, including the genes encoding VP 3, VP 7, NS 1 and NS 2 (Aradaib , 2009, Stone-Marschat , 1994). Post amplification analyses of conventional PCR assays involve agarose gels electrophoresis (AGE). This could lead to contamination and potentially false positive reactions in subsequent assays using the same laboratory space. In contrast, the design of real-time RT-PCR assays allows detection of the amplified target in a closed tube format, which significantly improves sample throughput and reduces contamination risk (false positives). These assays also offer enhanced sensitivity over the conventional PCR methods. Several real-time RT-PCR assays for detection of different AHSV genome segments have already been described, including those encoding VP 7, VP 7 & NS 2, NS 1 and NS2 genes (Quan , , *et al,* 2010, Monaco , *et al,* 2011).

The ''gold standard'' method for the determination of AHSV serotype is the virus neutralisation test (VNT) where the specificity of reactions between the virus and a panel of reference antisera, representing each of the known serotypes, is tested in tissue cultures (Katarzyna , *et al,* , 2014). However, these serotyping assays are labour intensive, time consuming, require prior virus isolation and can sometimes give inconclusive results. They are also dependent on availability of reference virus strains (as controls) and reference antisera which are highly characterised and may therefore be difficult to obtain. These assays may also require disease-secure laboratory facilities for safe handling the live virus (Sailleau , *et al,* , 2000, Maan , *et al,* 2011).

More than one test should be performed to diagnose an outbreak of AHS, especially the index case. The initial test can be a quick test such as ELlSA or PCR, followed by virus isolation in tissue culture. Virus neutralisation (VN) for serotype identification or RT-PCR with sequencing should be performed as early in the outbreak as possible so that the serotype can be identified and the correct vaccine selected.

At present, there are no international standards for viruses or diagnostic reagents, and there is no standard methodology for the identification of AHSV. However, a viral and antibody panel has been evaluated and comparative studies between

different ELISAs for AHSV antigen and antibody determination have been carried out in different laboratories, including in the EU Reference Laboratory for AHS. The results have demonstrated a high level of correlation for both antigen and antibody determination with an in-house test and commercial kits. Also similar studies have been conducted with several RT-PCR assays also providing a high level of correlation (OIE , 2012).

A neutralising antibody response of foals immunized with this polyvalent MLV AHSV vaccine in South Africa area was evaluated and compared to the response elicited to monovalent MLV AHSV serotypes. Naïve foals were immunized with either the polyvalent MLV AHSV vaccine, or a combination of monovalent MLV vaccines containing individual AHSV serotypes 1, 4, 7 or 8. There was a marked and consistent difference in the immunogenicity of individual virus serotypes contained in the MLV vaccines. Specifically, foals most consistently seroconverted to AHSV-1 and responses to other serotypeswere highly variable, and often weak or not detected. The serotype-specific responses of foals given the monovalent MLV vaccines were similar to those of foals given the polyvalent MLV preparation suggesting that there is no obvious enhanced immune response through the administration of a monovalent vaccineas opposed to the polyvalent vaccine (Crafforda , *et al*, , 2014).

A study was conducted to determine whether subclinical cases, together with clinical cases, of African horse sickness (AHS) occur in immunised horses in field conditions. A whole blood samples were collected and rectal temperatures recorded weekly from 50 Nooitgedacht ponies resident in open camps at the Faculty of Veterinary Science, University of Pretoria, Onderstepoort, during 2008–2010. The samples were tested for the presence of African horse sickness virus (AHSV) RNA by a recently developed real-time RT-PCR. It was shown that 16% of immunized horses in an AHS endemic area were infected with AHSV over a 2 year period, with half of these (8%) being subclinically infected. (Weyer , *et al*, 2013).

A retrospective analysis was carried out concerning 737 AHS outbreaks that occurred during 2007–2010 in Ethiopia. A total of ten outbreaks were investigated in the study period. All four forms of the disease (pulmonary, cardiac, horse sickness fever and the combined form) were observed, with the cardiac form being the most prevalent. Multiple African horse sickness virus serotypes (AHSV- 2, AHSV- 4, AHSV- 6, AHSV- 8 and AHSV- 9) were detected by molecular methods

(type-specific real-time RT-PCR assays), and fourteen isolates were derived from blood and tissue samples collected during 2009–2010. This is the first report of AHSV- 4, AHSV- 6 or AHSV- 8 in Ethiopia (Aklilu N , *et al*, 2014).

Previously, a recombinant modified vaccinia Ankara (MVA) virus expressing the protein VP 2 of AHSV serotype 4 was shown to induce virus neutralising antibodies in horses and protected interferon alpha receptor gene knock-out mice (IFNAR −/−) against virulent AHSV challenge. This study builds on the previous work, examining the protective efficacy of MVA-VP 2 vaccination in the natural host of AHSV infection. A study group of 4 horses was vaccinated twice with a recombinant MVA virus expressing the major capsid protein (VP2) of AHSV serotype 9. Vaccinated animals and a control group of unvaccinated horses were then challenged with a virulent strain of AHSV- 9. The vaccinated animals were completely protected against clinical disease and also against viraemia as measured by standard end-point dilution assays. In contrast, all control horses presented viraemia after challenge and succumbed to the infection. These results demonstrate the potential of recombinant MVA viruses expressing the outer capsid VP 2 of AHSV as a protective vaccine against AHSV infection in the field (Berta , *et al*, 2014).

In Zimbabwe, tests for neutralising (NT) antibodies to the nine serotypes of African horse sickness (AHS) virus on the sera of three groups of horses confirmed that an increasing number of immunizations with vaccine containing attenuated strains of serotypes 1 to 6 of the virus, leads to broader response to the various serotypes and to higher individual titers. Nevertheless some horses failed to respond to one or more serotypes despite receiving numerous immunizations and it was clear that vaccine containing only serotypes 1 to 6 could not be relied upon to induce adequate cross-immunity to serotypes 7 to 9 of the virus. Highest antibody titers and broadest cross-reactivity were recorded in a fourth group of horses which had apparently suffered natural infection recently. The levels of antibody acquired from colostrum by seven foals generally correlated well with the levels of antibody in the sera of their dams and the rate of decline of passively acquired antibody was proportional to initial titre. Antibodies to individual serotypes of virus declined to undetectable levels in two to four months from birth in some instances implying that susceptibility to infection could occur well before the age of six to nine

months which is commonly recommended for initial immunization. Vaccination of eight foals at three to four months of age resulted in weak antibody response but did not adversely affect pre-existing low levels of maternal antibody so that early immunization could be recommended as a means for attempting to control the losses of foals experienced in Zimbabwe (Blackburn and Swanepoel , 1988).

1.7 Treatment and control:

There is no specific treatment for animals suffering from AHS, apart from rest and good husbandry is required. Complicating and secondary infections should be treated appropriately during the recovery period. AHSV is non-contagious and can only be spread via the bites of infected vector species of *Culicoides*. Control may therefore be effected by: (1) Introducing animal movement restrictions to prevent infected animals initiating new foci of infection, (2) Vector control, (3) and Vaccination (Philip. and Christopher , 2004).

Appropriate control measures to prevent movement of animals at risk of being infected should be instituted and this including: (1) completion of effective vaccination protocol against all important serotypes at least 42-60 days before introduction of the horse, (2) positive identification of all horses by microchipping and passport documenting vaccination status, and a veterinary certificate confirming health and issued no more than 48 hours before introduction, and (3) Equids imported from areas in which the disease is enzootic, or from neighboring regions, should be housed in isolation in insect proof enclosures for 60 days (Radostits , *et al*, 2006).

Because of the widespread prevalence and chronic nature of sweet itch, a wide variety of techniques have been employed to reduce the severity of Culicoides biting attacks. These include: application of insecticides and pathogens to habitats where larvae develop; environmental interventions to remove larval breeding sites; controlling adult midges by treating either resting sites, such as animal housing, or host animals with insecticides; housing livestock in screened buildings, and using repellents or host kairomones to lure and kill adult midges (Carpenter , *etal* , 2008).

AHS is typically a highly fatal disease in susceptible horses so vaccination is currently used to prevent the occurrence of disease in endemic areas. Similarly, vaccination will be central to the control of any future incursions of AHSV into previously unaffected areas. Several AHSV vaccines have been previously

developed and used including: a polyvalent live-attenuated (modified-live virus (MLV)) vaccine of adult mouse brain origin, a polyvalent cell culture-adapted MLV vaccine, and inactivated AHSV vaccines

As all AHSV serotypes are present in South Africa and in most parts of sub Saharan Africa, the use of a polyvalent vaccine is necessary to protect horses in these areas. In endemic areas and in regions where AHS occurs almost every year, viz. most parts of Africa south of the Sahara; annual vaccination of horses is a very practical means of control. Although prophylactic immunization against AHS is an efficient method of preventing serious losses, it cannot be relied upon fully to protect horses against infection or disease. However, the majority of horses that have received three or more courses of immunization are usually well protected against the disease. Annual immunization with a live polyvalent attenuated vaccine in late winter or early summer, which is some time before the peak AHS season, is advocated and allows immunized animals to respond adequately to the vaccine before possibly being challenged by natural exposure (Crafforda , *et al* , 2014).

Immunity to either live viscerotropic or attenuated AHSV strains is solid against the homologous virus and probably lasts indefinitely. Cross immunity between serotypes may be enhanced by repeated inoculation of the same virus. In most instances the levels of antibodies acquired by foals from colostrum correlate well with the levels of antibodies in the sera of their dams and determine the duration of their passive immunity. Because of the passive immunity acquired by foals born to immune mares, it is generally recommended that foals should not be immunized before they are six months of age (Coetzer and Guthrie , 2004).

1.8 African horse sickness vaccines:

Two types of vaccines have been described for AHS virus. Attenuated live vaccines (monovalent and polyvalent) and inactivated vaccine. New vaccines, including a subunit vaccine, have been evaluated experimentally (Sánchez-Vizcaíno , 2004).

Polyvalent or monovalent live attenuated AHS vaccines, based on the selection in Vero cell culture of genetically stable macroplaques, have been used for the control of AHSV in and out of Africa (OIE , 2012).

Polyvalent, attenuated vaccines are commercially available from Onderstepoort Biological Products (OPB), Onderstepoort, South Africa. The early vaccines were based on virus strains, attenuated by multiple suckling mouse brain passage. They gave solid immunity but occasionally resulted in serious side effects, including fatal cases of encephalitis in horses and donkeys, particularly after primary vaccination. These problems were minimized by further attenuation of the vaccine virus strains through passage in cell culture. These cell culture adapted viruses still form the basis of the currently available OPB vaccines.

The OPB, AHS vaccines currently used in southern Africa are supplied in two polyvalent vials containing AHSV types 1, 3 and 4, and 2, 6, 7 and 8, respectively. AHSV-5 is currently not included having been withdrawn in 1993 because of reports of severe reactions and deaths in some vaccinated animals. AHSV-9 is also not included because type 6 is strongly cross protective and because type 9 is rarely present in southern Africa and is considered to be of low virulence (Philip and Christopher , 2004).

Immunization with polyvalent attenuated vaccines of AHS virus, leads to broader response to various serotypes and to higher titre but some horses failed to respond to one a more serotypes.

Inactivated vaccine based on the inactivated of the virus with either formaldehyde in concentration of 1:8000, or with B-propilactone at 0.2 percent and in order to enhance the immunogenicity aluminum hydroxide was added to the vaccine as an adjuvant (Ihsan , 2004). Inactivated vaccines have the advantage that they do not contain a live and potentially dangerous agent. However, 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 (Philip and Christopher , 2004).

An inactivated monovalent (serotype 4) AHSV vaccine based on virus purification and inactivation with formalin was produced commercially in the early 1990s. However, these vaccines are not commercially available (OIE , 2012).

Subunit AHSV vaccines based on serotype 4 outer capsid protein VP 2 and VP 5 plus inner capsid protein VP 7, derived from single and dual recombinant baculovirus expression vectors have been used experimentally in different combinations to immunise horses. The protective efficacy of VP 2 in a subunit

vaccine was also evaluated. However, these vaccines are not commercially available (OIE , 2012).

Currently in Sudan, AHS vaccine was produced by veterinary research institutes, Soba, Khartoum which is one of the Animal Resources Research Corporation constituents in Sudan, and it is empower to conduct proper research, quick and reliable diagnosis and vaccines production in Sudan. In spite of that some horse's owners especially those used for race are imparted the AHS vaccine from South Africa.

1.9 African horse sickness in Sudan:

African Horse Sickness disease was reported for the first time in Sudan in (1903) , from Kassala Province when samples from infected horses were tested by the Onderstepoort Laboratory in South Africa, and found to contain AHS virus type 3, the disease was also reported in Sennar and Singa at the same year. Later, an AHS virus isolate was identified as serotype 9 by the Razi Institute, Iran. In later years, only serotype 9 has been isolated repeatedly and serotyped locally from several disease outbreaks in the country. (Eisa , 1974) isolated virus from whole blood and spleen of a mare which died of a disease suspected to be African horse sickness in the Sudan. The virus isolate recognized as (KNT). Later the strain has been identified as type nine of AHSV. Also in (1980) isolated an AHSV in suckling mice-and identified it using immunoelectron microscopy. This isolate was designated as Khartoum 2 (AHSV-K2) and typed as type nine of AHSV. A polyvalent vaccine which was once imported is now produced locally. However, when local production is insufficient, a polyvalent vaccine is sometimes imported. Horses are the only species vaccinated against the disease in the Sudan (Ihsan , 2004; Abu Elzein , , *et al*, , 1989).

A study was performed to determine the prevalence of type specific neutralizing antibodies to African horse sickness virus (AHSV) in equidae and some other animal species in Khartoum State, Sudan. Antibodies (Abs) to AHSV were detected in horses, donkeys, goats, cattle and Dorcas gazelle (Order: *Artiodactyla*). Antibodies to all AHSV serotypes were detected except AHSV-8 because its antigen was not available during this study. The detection of antibodies to AHSV in goats, cattle and Dorcas gazelle points out to the possibility that, these animals might have been exposed to a subclinical infection. The detection of Abs to AHSV

in Dorcas gazelle was the first to be reported in the Sudan; consequently, further studies are required to clarify this finding (Elghazali, and Ali, 2013).

A work was carried out to determine the prevalent serotypes of African horse sickness virus (AHSV) in southern Darfur and Khartoum States. A number of 455 sera were collected from horses, donkeys and camels from Southern Darfur and from horses from Khartoum State. Agar gel precipitin test (AGPT), Counter immunoelectrophorsis (CIEP), passive haemaglutination test (PHA) and serum neutralization test (SNT) were carried out to detect the antibodies against AHSV. AGPT and CIEP showed negative results in all serum sample, while PHA and SNT gave positive results of (42.64%) and (27.75%) respectively. In the present study, three serotypes of AHSV (namely 3, 6 and 9) were found to infect horses on basis of SNT. The prevalence of the three serotypes was found as follows: Types 3 was (6.5%) in Southern Darfur and (0.00) in Khartoum. Type 6 was (48.3%) in Southern Darfur (39.5%) in Khartoum. Type 9 was (45.2%) in Southern Darfur (60.5%) in Khartoum. AHSV was also isolated in this study from spleen and liver of an infected horse from Elgazira area and identified as type 9 of AHSV using SNT (Ihsan ,2004).

1.10 Economic impact of African horses sickness :

Horses are present throughout the world. Horses have been with humans throughout history and have served a variety of practical purposes. These include serving as a means of transport, a work animal in agriculture and in war. Horses were domesticated and utilized by humans since ancient times. Nowadays because of their power, agility, gracefulness and speed, horses are mostly used in sport competitions. However, they are still being used in subsistence agricultural regions particularly in Eastern Europe, Asia, Africa, Central and South America. Draft horses still play an important role in rural life, despite the increased mechanization of agriculture. Pack horses and ponies are still the backbone for the means of transport in some developing countries. Horses have been also used by military forces for expeditions, riding, and transportation (Rupak , 2010).

Recent statistics has estimated the Sudanese equines' population at 788509 horses and 7567928 donkeys (Ministry of Livestock, Fisheries and pastures, 2014). In 2009 the average sale price of donkeys and horses in Sudan was 405 SDG per head

and 1059 SDG per head, respectively. The donkey was on average worth 38% and a horse was worth 98% of the mean sale price for cattle. The capital value of the 784,578 horses were 623 billion and donkeys were worth 2.283 billion, or a combined capital value for equines of 2.906 billion SDG. Assuming that equine owners in Sudan demand a 70% return on their livestock investment comparable to that which they get from ruminants, then the direct use value of the transport services provided by equines amounted to about 2.034 billion SDG in 2009 (Behnke and Osman ,2009).

In Khartoum state the majority of horse owners indicated livestock breeding to be their main activity. Horses have multi-functional roles in different production systems. Sales of animals are important for obtaining regular cash income, in addition to sales of agricultural crops. The serious production constraints which were defined by horse owners include lack of feeds, water shortage, and disease prevalence (Hanan , 2015).

However, many factors contribute to the poor performance of equines, among which the viral disease, African Horse Sickness (AHS) is incriminated as the leading cause of morbidity and mortality in these animals (Mulualem , *et al*, , *et al*, , 2012).

In the Sudan, AHS is frequently reported in imported race and breeding horses, while the native Sudanese horses are much less susceptible. This is quite logical as AHS is known in the country since 1903 (Anon, 1903). It could be pointed out that infected native horses might act as carriers of the AHSV though they are clinically normal and consequently they could affect susceptible horses (Elghazali and Ali, 2013).

A total of 215 serum samples were collected from apparently healthy adult working donkeys, from different localities in Khartoum Province. Samples were inactivated at 56°C for 30 min and stored at - 2 0 ° C until used. The micro AGID test, using standard microscope slides, was carried out as described by Abu Elzein , *et al,,(1989).* One percent Noble agar (Difco Labs, USA) was used in phosphate buffered saline (PBS) at pH 7.4. Antibody to African horse sickness virus was present in 211 (98%) of 215 apparently healthy Sudanese donkeys. No case of clinical disease had been seen in donkeys. Little was known about the role of donkeys in the epidemiology of the disease.

Chapter two

Materials and methods

2.1 Study area:

The study was conducted in Khartoum state, capital of Sudan. It lies between latitude 15-16 N and longitude 21-24 East with a total area of 20,736 km². Most of Khartoum State falls within the semi-desert climatic zone while the northern part of it falls within the desert climatic zone. The state is prevailed with a hot to very hot climate with rainy season during the summer and warm to cold dry in winter. Rain fall ranges between 100-200 mm at the North Eastern parts to 200-300 mm at the Southern parts with 10-100 mm at the North Western parts. The temperature in summer ranges between 25-40° C during the months of April to June and between 20-35° C during July-October period. Temperature degrees continue to fall during the winter period between November-March to the level of 15-25° C. Khartoum State is divided to seven localities (Karari, Umbadda, Omdurman, Bahari, Sharg El-Nil, Khartoum, and Jebal Awlia), with each locality there are numbers of administrative units. Also each Administrative Unit consists of numbers of Popular Committee. The last statistics of horse's population conducted by Ministry of Livestock, Fisheries and pastures for 2014 estimated 1577 horse In Khartoum State which represent about 0.20% of total number of horses in Sudan (Ministry of Livestock, Fisheries and pastures 2014).

2.2 Study population:

The target population for the serological survey was all the horses'(187) complexes in Khartoum State. The selected horses' complexes were: (1) Equestrian Club and donkeys market in Khartoum locality ((2) horses' stables in Omdurman locality (3) and Police horses' stables (called Sawari) of Alhaji Yusuf and Almazad city in Bahari locality. The study included unvaccinated horses either used for work or

race. The study also included both sexes of horses above six months of age. The horse died during the study was excluded from the study. The horses in the Khartoum state are kept for working, Police working, individual riding or racing. The horse breeds in this State are crosses of Arab horses and the English thoroughbred and other local breeds.

2.3 Study design and sample size:

A cross–sectional study was carried out to determine the prevalence of African Horse sickness during the period from August 2016 to may 2017, on three localities of Khartoum State out of seven localities selected randomly. Specific study sites included: Khartoum locality(123 samples), Bahari locality (47samples), and Omdurman locality (17 samples). The sample size was determined by calculating 12% of total number of horse founded in Khartoum State which was 1577 horses according to Ministry of Livestock, Fisheries and pastures in 2014.

2.4 Samples collection:

A total of 187 serum samples of horses and 50 of donkeys were collected. From each selected animal whole blood of 7 to 10 ml was collected by vein puncture using sterile venoject needles and plain vacutainer tubes including needle holder under aseptic conditions. Each sample was labeled with identification number, and then it was transported to the Veterinary Researches Institute (VRI), Soba, and Khartoum. The blood was allowed to clot over night at room temperature. The recovered serum was decanted in to another tube and labeled. If the serum is not separated, then the samples were centrifuged at 1000 rpm for 5 min to allow for proper separation of serum from the clotted red blood cells. The samples were kept at -20°C until tested.

A total of 67 horses was be in the questionnaire

2.5 Samples analysis:

The collected samples from the field for the diagnosis of African horse sickness were shipped to the Veterinary Researches Institute (VRI), Soba, Khartoum. The serological test was performed using Indirect – ELISA to identify AHSV antibodies.

2.5.1 Indirect – ELISA (I-ELISA):

The indirect ELISA was performed according to protocol provided by the c-ELISA kits manufacturing company,(INGENASA) .

The antigen was fixed in a solid support (polystyrene plate). After incubation with sera samples, an AHSV specific monoclonal antibody (Mab peroxidase conjugated) was added. If the sample contains antibodies specific of the virus, they was not allow the binding of the labeled Mab to the antigen whereas if it did not contain specific antibodies, the Mab was bind to the antigen coating the plate. After washing the plate to eliminate all non-fixed material, presence or absence of labeled Mab can be detected by adding the substrate which, in presence of the peroxidase, will develop a colorimetric reaction. If there had been color development, the conjugate had bound to the antigen, being the sample negative. On the other hand, if there were antibodies specific (Abs) of AHSV in the sample, they was block the binding of conjugate and there was not be color development. The antigen used in this kit was VP 7 recombinant protein from the AHSV (serotype 4) obtained using the baculovirus expression system. The use of serotype 4 confers many advantages to the assay. From the safety point of view, it was due to the total absence of infectivity and from the accuracy point of view, VP 7 is one of the major proteins from AHSV, as well as the most antigenic one and the most conserved with in the 9 different serotypes. Besides to this it had not been found any infected or vaccinated animal without antibodies to this protein.

Test procedure used to identify AHSV antibodies was: first, the reagents were brought to room temperature before use. Second, 100 µl of diluted sample was dispensed in to appropriate wells (dilution1/5), 100 ml of positive control was dispensed into two wells, and 100 ml of negative control was dispensed into two wells. Third, the plates were covered and incubated for 1 h at 37 °C, and then washed 5 times. Fourth, 100 µl of conjugate per well was added and incubated for 30 min at 37° C, and then washed 5 times. Fifth, 100 µl of substrate solution in each well was dispensed using a multichannel pipette and incubated for 10 min at room temperature. Sixth, 100 µl of stop solution was dispensed to each well. Finally, the optical density (OD) was read at 405 nm within 5 minutes after addition of stop solution.

The optical density (OD) of the positive control must be lower than 0.2 and the OD of the Negative control must be higher than 1.0. Samples showing Blocking

percentage (BP) value lower than 45% were considered negative for antibodies to AHSV where as samples showing BP value higher than 50% were considered as positive to antibodies to AHSV. Samples with BP value between 45% and 50% were considered doubtful and were retested. Interpretation: Blocking percentage of each sample was determined using the following formula:

$$BP = \frac{\text{Abs (control}-\text{)}-\text{Abs (sample)}}{\text{Abs (control}-\text{)}-\text{Abs (control}+\text{)}} \times 100$$

2.6. Questionnaire:

Was used to collect information about the knowledge of disease and the control of the vectors , nutrition other animal in the stable and the resource of animals

2.7 Data analysis:

The prevalence proportion was determined by considering the total number of animals tested and positive reactors by AHS, using the formula given by Thrusfield (2007).

$$P = \frac{\text{number of individuals having a disease at a particular point in time}}{\text{number of individuals in the population at risk at that point in time}} \times \mathbf{100}$$

Chapter three

RESULTE

The results showed that 84%(157/187) of horses was positive to African horse sickness using ELAISA test . while 94% (47/50) of donkeys were positive the test .

The overall prevalence of the infected horses was 12% .

Table (1) – Sero prevalence of African horse sickness virus in different localities in Khartoum state .

locality	Number of tested	Number of positive	Number of negative	Percentage of prevalence
-Khartoum locality	123	99	24	80%
Omdurman	17	17	0	100%
Bahri	47	41	6	87%
Total	187	157	30	84%

As shown in table (1) the high percentage in Omdurman locality (100%) and low percentage was found in Khartoum locality (80%) .

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Table (2) Descriptive statistics of risk factors associated with AHS in Khartoum state:

The Factor		% Within ELISA		
		Positive	**Negative**	**Total**
Gender	Male	39 (67.2)	5 (55.6)	44 (65.7)
	Female	19 (32.8)	4 (44.4)	23 (34.3)
Stable	Khartoum	31 (53.4)	5 (55.6)	36 (53.7)
	East Nile	27 (46.6)	4 (44.4)	31 (46.3)
Mixing with others animals	Yes	15 (25.9)	2 (22.2)	17 (25.4)
	No	43 (74.1)	7 (77.8)	50 (74.6)
Age (Years)	< 5	8 (13.8)	2 (22.2)	10 (14.9)
	5-10	27 (46.6)	4 (44.4)	31 (46.3)
	> 10	23 (39.7)	3 (33.3)	26 (38.8)
Animal Origin	Khartoum	32 (55.2)	5 (55.6)	37 (55.2)
	West Sudan	25 (43.1)	3 (33.3)	28 (41.8)
	Foreign	1 (1.7)	1 (11.1)	2 (3)
Total		**58 (100)**	**9 (100)**	**67 (100)**

All the factors (gender, origin, mixing with other animals, age and the stable) were no significant .

Chapter four

DISCUSSION

African horse sickness represents a major health concern and negatively impacts the equine industry, mainly in those countries, such as Sudan, where high quality horses are bred and vaccination is not compulsory. A freeze-dried, polyvalent, live attenuated vaccine against AHS (Onderstepoort Biological Products, OBP) is currently used in Africa. Horses should be inoculated 3 times – at 6, 9, and 12 months of age – and then annually re-vaccinated, before the rainy season, to become immune to all the serotypes in the vaccine. As a result, the immune status of Sudanese horses is likely to be variable, such speculation reasonably explaining the occurrence of severe, sometimes fatal AHS, in a number of immunized horses. Furthermore, a growing body of evidence indicates that prophylactic immunization against AHS is useful to prevent serious losses, but cannot fully protect horses from infection and disease under natural condition (Coetzer *et al.* 2004, Crafford *et al.* 2013, Weyer *et al.* 2013, Molini *et al.*2015, forthcoming).The prevalence of AHSV antibodies detected by ELISA in the horse stables in Khartoum state, Sudan, was estimated at 84%. This high prevalence is in harmony with previous serological studies in Sudan Ihsan . (2012) showed that the percentage of the antibodies were 74% and 79.4 % by using SNT and ELISA techniques respectively , and that may be an indication of continual exposure of the horses regardless of age, sex and breed. Our study area Khartoum state extends in three localities, with thick vegetation and hot and dry climatic conditions which favor vector propagation and virus transmission. In Nigeria, no preventive vaccination is routinely applied against AHSV, particularly in indigenous and local crossbred horses. There appears to be a form of innate resistance to infection by AHSV as corroborated . It could explain the probable absence of reported outbreak of the disease in the region, despite the high prevalence of antibodies. In contrast, newly imported horses, particularly from AHS-free areas, are susceptible to infection and are therefore usually vaccinated before importation. But once established in the country (the animals of exotic breeds examined during the study had been introduced more than ten years ago or were born in the country), they are seldom revaccinated (Adeyefa and Hamblin, 1995) and therefore vulnerable to AHSV infection.(Ehizibolo *etal* ., 2014) . From the study it can be conclude that, the

seroprevalence of AHSV was 87%, 84 and 100% in Khartoum, Khartoum north and Omdurman localities, respectively. From the total sample tested the seroprevalence of 84% in donkeys and 94% horses was obtained, the overall prevalence with 84%. These findings showed that the African horse sickness affects equally almost all age groups and sexes, hence there is no significant variation in the seropositivity among the equine. The questionnaire survey result indicates that the knowledge base of equine owners about AHS, mode of transmission and clucoides vector was not satisfying. They are unaware of culicoides vectors and mode of transmission of AHS. Generally AHSV exists in all localities and affect the two types of equidae in the study areas. Based on the above conclusion following recommendation are forwarded: There should be awareness reaction about AHS and Clucoides vector among the people through an organized extension package to the present study areas. Appropriate equine enclosure system to avoid insect bite by stabling them some hours before sunset and letting them out a few hours after sun rise as Clucoides are nocturnal in nature and are not inclined to enter equine stables and also control of insect using chemical or biological method. All equine greater than 6 month in the study area should be vaccinated with polyvalent vaccine in the study areas. There is a need of deeper understanding of the epidemiology of African horse sickness in the study areas by an integrated approach of serotyping and identification of the clucoides vectors as well as other potential vectors.

CONCLUSION

This study corroborates previous studies and could suggest a potential threat of AHS to the equine industry in Sudan , and a continual prevalence of the disease. Annual vaccination of horses is advocated. Vector control and good stable management practices may assist in minimizing incidence. Suspected outbreaks should be investigated to ascertain the circulating serotypes in the region.

RECOMMENDATION

- CONTROL MUST BE INCLUDE MOVEMENT CONTROL AWARENESS CRESTION AND VACCINATION .
- UPDATE OF HUSBANDRY SYSTEM .
- UPDATE INFORMATION KNOWLEDGE ON TRANSMISSION AND CONTROL POINTS .
- SURVELANCE OF DISEASE EVERY YEAR IN ALL SUDAN .
- VECTOR SURVEILLANCE .
- VECTOR CONTROL (INSECTICIDES) .
- MOVEMENT OF ANIMALS BETWEEN STSTS MUST BE QUARANTINE .

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APPENDEX

Table (1)

		Age Groups			
		Less than 5 Years	**5-10 Years**	**More than 10 Years**	**Total**
Negative	Count	2	4	3	**9**
	% within ELISA	22.2%	44.4%	33.3%	**100.0%**
	% within Age Group	20.0%	12.9%	11.5%	**13.4%**
positive	Count	8	27	23	**58**
	% within ELISA	13.8%	46.6%	39.7%	**100.0%**
	% within Age Group	80.0%	87.1%	88.5%	**86.6%**
Total	Count	10	31	26	**67**
	% within ELISA	14.9%	46.3%	38.8%	**100.0%**
	% within Age	**100.0%**	**100.0%**	**100.0%**	**100.0%**

Taple(2)

		Origin			
		Khartoum	**West Sudan**	**Foreign Country**	**Total**
Negative	Count	5	3	1	**9**
	% within ELISA	55.6%	33.3%	11.1%	**100.0%**
	% within Origin	13.5%	10.7%	50.0%	**13.4%**
positive	Count	32	25	1	**58**
	% within ELISA	55.2%	43.1%	1.7%	**100.0%**
	% within Origin	86.5%	89.3%	50.0%	**86.6%**
Total	Count	37	28	2	**67**
	% within ELISA	55.2%	41.8%	3.0%	**100.0%**
	% within Origin	**100.0%**	**100.0%**	**100.0%**	**100.0%**

Table (3)

		Stable		
		Khartoum	**East Nile**	**Total**
Negative	Count	5	4	**9**
	% within ELISA	55.6%	44.4%	**100.0%**
	% within Stable	13.9%	12.9%	**13.4%**
positive	Count	31	27	**58**
	% within ELISA	53.4%	46.6%	**100.0%**
	% within Stable	86.1%	87.1%	**86.6%**
Total	Count	36	31	**67**
	% within ELISA	53.7%	46.3%	**100.0%**
	% within Stable	**100.0%**	**100.0%**	**100.0%**

Table (4)

		Gender		
		Female	**Male**	**Total**
Negative	Count	4	5	**9**
	% within ELISA	44.4%	55.6%	**100.0%**
	% within Gender	17.4%	11.4%	**13.4%**
positive	Count	19	39	**58**
	% within ELISA	32.8%	67.2%	**100.0%**
	% within Gender	82.6%	88.6%	**86.6%**
Total	Count	23	44	**67**
	% within ELISA	34.3%	65.7%	**100.0%**
	% within Gender	**100.0%**	**100.0%**	**100.0%**

Table (5)

		Mixing		
		No	**Yes**	**Total**
Negative	Count	7	2	**9**
	% within ELISA	77.8%	22.2%	**100.0%**
	% within Mixing	14.0%	11.8%	**13.4%**
positive	Count	43	15	**58**
	% within ELISA	74.1%	25.9%	**100.0%**
	% within Mixing	86.0%	88.2%	**86.6%**
Total	Count	50	17	**67**
	% within ELISA	74.6%	25.4%	**100.0%**
	% within Mixing	**100.0%**	**100.0%**	**100.0%**

Figures of risks Factors associated with African hoers sickness in Khartoum state:

Figure (1)

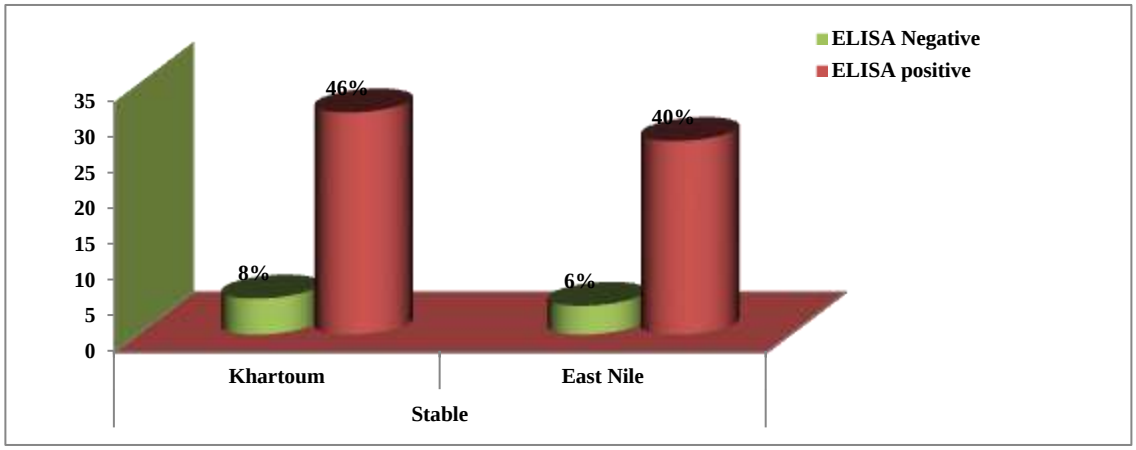

Figure (2)

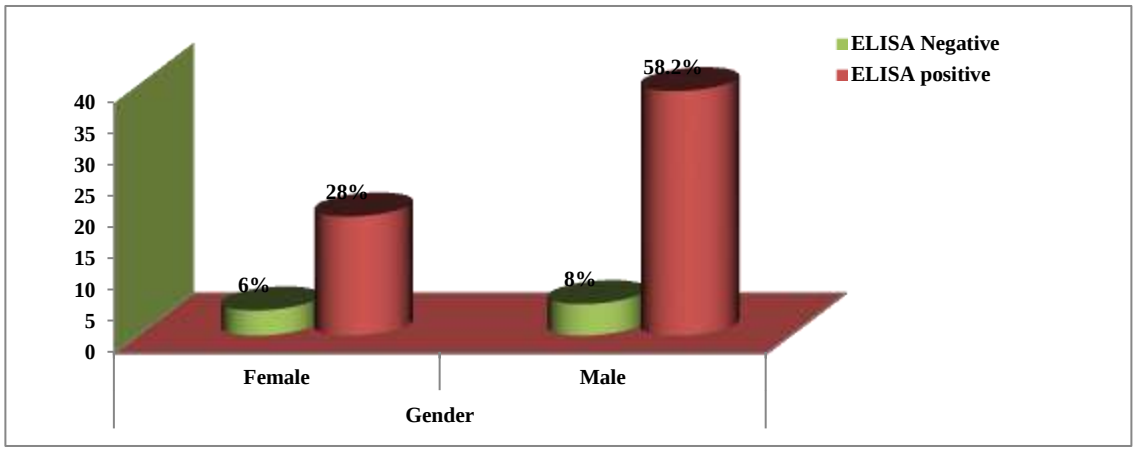

Figure (3)

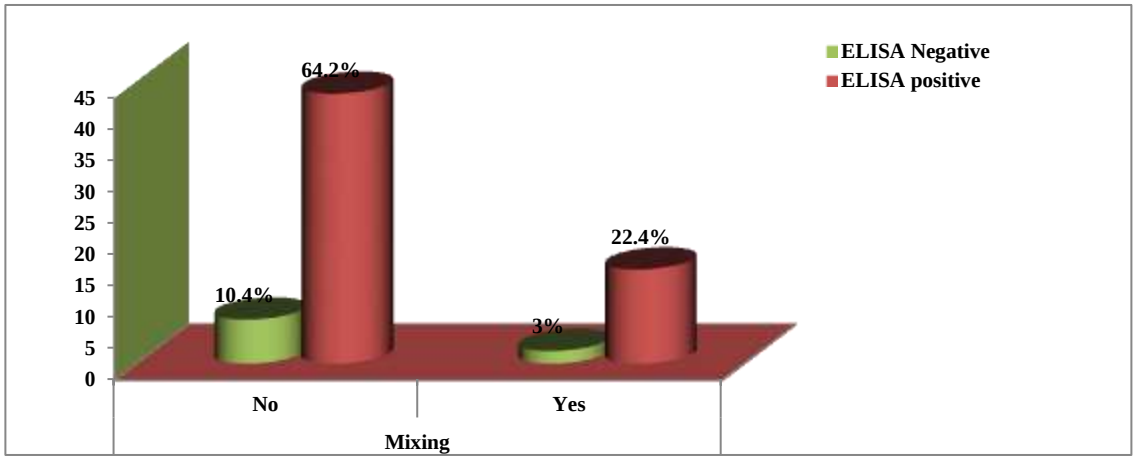

Figure (4)

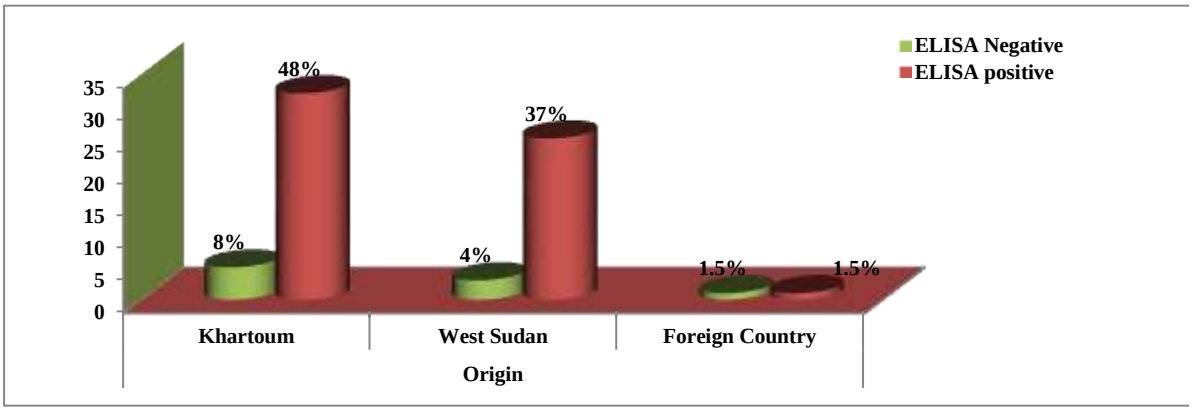

Figure (5)

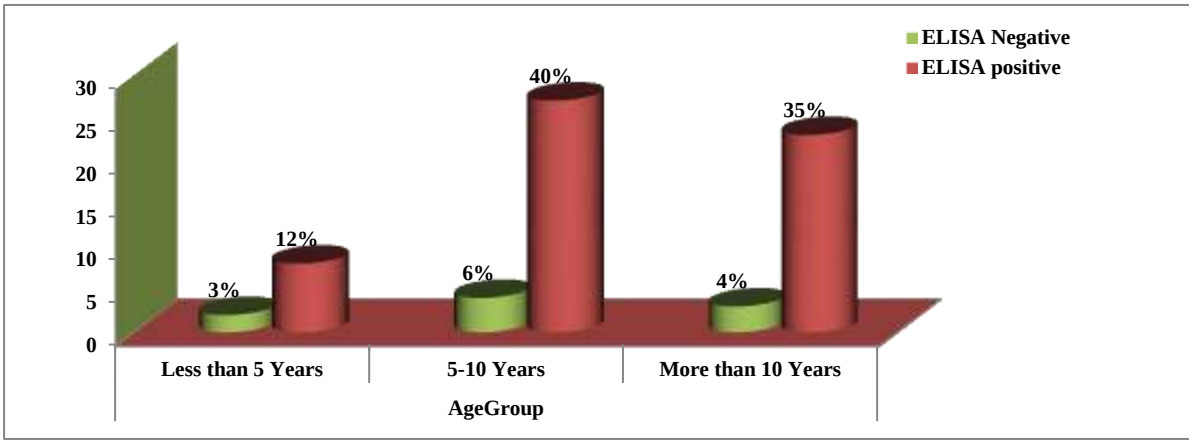
ELISA Negative
ELISA positive
30
25
20
15
10
5
0
3%
12%
6%
40%
4%
35%
Less than 5 Years
5-10 Years
More than 10 Years
AgeGroup

Table (6) Univariate analysis of risk factors associated with AHS in Khartoum state using Chi – square (χ^2) test:

	The Factor	Positive (%)	χ^2	P-Value
Gender			0.472	0.492
	Male	39 (67.2)		
	Female	19 (32.8)		
Stable			0.014	0.906
	Khartoum	31 (53.4)		
	East Nile	27 (46.6)		
Mixing with			0.055	0.815
others animals	Yes	15 (25.9)		
	No	43 (74.1)		
Age (Years)			0.459	0.795
	< 5	8 (13.8)		
	5-10	27 (46.6)		
	> 10	23 (39.7)		
Animal Origin			2.478	0.290
	Khartoum	32 (55.2)		
	West Sudan	25 (43.1)		
	Foreign	1 (1.7)		
Total		**58 (100)**		

الاستبيان

التاريخ

المحليه

الاسطبل : **رقم الحصان :**

العمر : **الجنس :**

مصدر الحيوان : -

ماهي الامراض الموجوده في الاسطبل ؟ طفيليات اصابات اخري -

هل تعرف مرض النجمه ؟ -

اذا كانت الاجابه نعم : **هل اصيب أحد خيلك بالمرض ؟** -

كم عدد الخيل المصابه ؟ -

ماهي الاعراض ؟ -

متي ظهر المرض ؟ وفي اي فصل ؟ -

صيف شتاء خريف -

هل تم تشيخيص المرض ؟ -

ماهي الحيوانات التي تم الاختلاط بها ؟ حمير كلاب -

هل يوجد باعوض ؟ -

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تشخيص عادي تشخيص معمل -

هل تم تطعيم الحصان ؟ -