Introduction

The equid population is important in Khartoum state, being estimated at 1579 horses and 7541 donkeys, (Ministry of Livestock, Fisheries and Rangelands, Information Center, 2009) , and it used for working, racing, and breeding. Exportation and importation movements at the border create a high risk for the equid population, particularly if the status of infections is not known.

The aim of this study was first to determine whether or not Equine Infectious Anaemia (EIA) is present in the Khartoum state, hence there are no records or previous published study showing the status, prevalence and potential risk factors for the disease, and to help the

government to establish a compulsory monitoring scheme for all horses including horses held on location, being moved, imported or exported, in order to control the disease. It is suggested that the national EIA program could be improved by standardization and wider application of uniform active surveillance measures, and improved documentation of EIA status of horses on acquisition and transfer records.

Definition:

Equine infectious anemia (EIA),also known as Swamp Fever, Mountain Fever, Slow Fever, Equine Malarial Fever, Coggins Disease is caused by the equine infectious anemia virus (EIAV), classified in the sub family Lentivirinae of the family Retroviridae (APHIS, 2006).

Equine infectious anemia (EIA) is a reportable, eradicable epizootic chronic disease, (Kaiser, et al,.1984) that affects equidae (such as horses, mules, donkeys ,ponies and zebra), (Yapkic, et al., 2007).The disease is characterized by recurrent febrile episodes, thrombocytopenia, anaemia, rapid weight loss, and oedema of the lower parts of the body. If the acute clinical attack does not provoke

death, the infected horse can remain a viraemic carrier and potentially transmit the infection to susceptible horses by means of bloodsucking horseflies or iatrogenically. In uteri infection of the fetus may also occur, (Marenzoni, et al., 2013).

The virus can be transmitted through secretions and excretions of acutely or chronically infected animals, blood transfusions, blood–contaminated materials and blood sucking insects such as horseflies, deer flies and Stomoxys calcitrans, (Kirmizigul, et al., 2009).

The highest number of cases occurs in warm climates because of the prevalence of blood sucking insects that are the primary transmitters of this disease, (Camargo, 2011) and in non-flooded and flooded farm areas, (Vilamiu, et al., 2012).

Acute: Seen within one to two weeks after the horse's first exposure to the virus, this phase is the most detrimental. It may be difficult to accurately diagnose acutely infected horses, as antibodies are not immediately produced and anemia is not present at this stage. However, the virus is active, replicating and damaging the immune system and other organ systems.

Chronic: If the horse survives the acute phase, a sub acute or chronic phase may occur. The classic signs of EIA, such as fever, depression, weight loss, anemia and petechial (pinpoint sized) hemorrhages on the mucous membranes, are most likely seen in this phase. Repeated flare-ups of clinical signs often occur. Such episodes are seen with recrudescence of the virus and viremia during periods of stress or the administration of corticosteriods.

In-apparent: Over time, the periodic episodes decrease in severity and frequency. Within a one-year period many horses begin to control the infection and show no clinical signs. These inapparent carriers are infected for life and may be a source of infection for other horses. (AAEP, 2006).

The persistent carriers have played the major epidemiological role in spreading of EIA infection.

The agar-gel immunodiffusion (AGID) test, formerly named the Coggins test is commonly used for the diagnosis of EIA , (Turan, et al., 2001). The test is a reference test and is used in many countries**,** during importation and exportation since EIA is on List-B as indicated by the Office International des Epizooties (OIE). Other tests such as enzyme linked immunosorbent assay (ELISA) and competitive ELISA (C-ELISA) have also been introduced for the diagnosis of EIA, (OIE, 2013).

Recently, molecular biological techniques have been applied to detect EIAV in tissues and blood (OIE, 2008).

No specific treatment is available. Supportive treatment, including blood transfusions can be considered. However, because recovered horses can become carriers, if the disease occurs in a previously free area it is generally better to destroy affected horses.

In endemic areas, risk of infection can be reduced by protecting horses from insect vectors where practicable. For example

- Keeping horses away from low lying areas
- Draining swamps
- Use of insecticides
- Insect-proof stabling (*Merck Veterinary Manual*, Eight ed, 1998., *Veterinary Medicine*, Saunders, Eight ed, 1997.)

Rationale

a. Equines are considered source of transportation, prestige, or racing.

b. Scarce information about the disease prevalence in Khartoum State.

a. Ignorance about the disease prevalence leads to ignorance about its control and prevention, consequently spreading of the disease, leading to reduce equines population and production.

b. No previous studies for prevalence of the disease in equine, in Khartoum State, although the vector is present.

Research significance

a. Knowing the disease prevalence will help in implementing the proper strategic plans for control and prevention.

b. Control and prevention of disease reduce losses, increase equine population and production, and encourage equine industry.

c. Local, regional and global cooperation for control and prevention.

Research objectives

The objectives of this study were :

1. To estimate the prevalence of Equine Infectious Anaemia in Khartoum state.
2. To estimate potential risk factors associated with the disease.

Chapter one

Literature Review

1.1. Definition

Equine infectious anemia (EIA), also known as swamp fever, is a persistent infection caused by equine infectious anemia virus, a member of the lentivirus subfamily of retrovirus, affecting all members of the Equidae, including horses, mules, and donkeys and may develop fatal viremia, but most survive and remain viremic for life, (Bicout, et al., 2006).

The disease is characterized by recurrent febrile episodes, thrombocytopenia, anaemia, rapid loss of weight and oedema of the lower parts of the body. If death does not result from one of the acute clinical attacks, a chronic stage develops and the infection tends to become in-apparent, (Marenzoni,et al., 2013). Another characteristic of the EIA virus is that it is a retrovirus. Because retroviruses lack proofreading ability, a number of mutations occur with each replication.

Therefore, during the course of infection, a number of viral variants will develop, and each horse will be infected with one of these mutant variants. One horse can be infected with more than one variant of the virus as well. HIV also replicates in this manner, which is one of the reasons why researchers have not been able to develop a vaccine against either HIV or EIA, (Camargo, 2011).

The incubation period is normally 1–3 weeks, but may be as long as 3 months.

Antibodies usually develop in infected horse blood 7-14 days after infection and last for life, (Keenan, et al.,)

1.2. Causative agent

EIA virus (EIAV): a lentivirus in the family Retroviridae, subfamily Orthoretrovirinae. Other members ofthe genus Lentivirus include: bovine immunodeficiency virus; caprine arthritis encephalitis virus;

feline immunodeficiency virus; human immunodeficiency virus 1; human immunodeficiency virus 2; and maedi/visna virus, (Turan, et al., 2001.

1.3. Introduction to retroviruses

Retroviruses (family Retroviridae) are enveloped, single stranded RNA viruses that replicate through a DNA intermediate using an RNA –dependent DNA polymerese (reverse transcriptase). This large and diverse family include members that are oncogenic, are associated with a variety of immune system disorder, and cause degegenerative and neurologic syndromes.

Until the discoveryc of these viruses it had been dogma that the transfer of genatic information always occur in the direction of DNA to RNA, so finding that some viruses carry out transcripion backwards (reverse transciption) caused something of arevolution, (Donovan 1999).

1.3.1 General Features of Retroviruses

Many of the features of retroviruses are known in great details because of the extensive work done on the oncoviruses in cancer research. The members of the family Retroviridae share many common features in their composition, organization, and life cycle, although the details of individual retroviruses vary (Donovan,1999)

1.3.2. Components of Retroviruses

A typical retovirus virion is composed of 2% nucleic acid (RNA), 60% protein, 35% lipid , and 3%(or more) carbohydrate , its buoyant density is 1.16 to 1.18 g/ml, (Donovan,1999).

1.3.3. Retrovirus virion

The virion contain tow copies **of** the RNA genome, hence the virion can be described as diploid. The tow molecules are present as a dimer, formed by base pairing between complementary sequences.

The region of interaction between the two RNA molecules have been described as kissing loop complex.

As well as the virus RNA, the virion also containsmolecules of host cell RNA that were packaged during assembly. This host RNA includes a molecule of transfer RNA (tRNA) bound to each copy of the virusRNA through base pairing. The sequence in the virus RNA that binds a tRNA is known as the primer bindingsite (PBS).

Each retrovirus binds a specific tRNA .A number of protein species are associated with the RNA. The most abundant protein is the nucleocapsid(NC) protein, which coats the RNA, while other proteins, present in much smaller amounts, haveenzyme activities , (Carter,et al., 2007).

1.3.4. Proviral DNA

Within a cell, the retroviral RNA genome is reverse transcribed into a DNA copy, and it is the proviral DNA from which serves as the intracellular retroviral genome. The retroviral DNA is several hundred bases longer than the retroviral RNA genome due to duplication of repeated and unique terminal sequences present in the RNA genome during the reverse transscripion process.These sequences form the long terminal repeats (LTR) that flank the genes in the retroviral DNA (Fig 74.2B). The proviral DNA is covalently integrated in the DNA of the infected host cell,(Donovan, 1999).

1.3.5.Retroviral Nucleic Acid Structure and Sequence.

The sequence of structural genes of retroviruses, from the 5 end to the 3 end of genomic RNA, is Gag-pol-Env. Some retroviruses, such as the Ientiviruses and spuma viruses, have additional genes that regulate expression of the retroviral genome and other accessory functions. Highly oncogenic retroviruses often have an oncogene in place of a portion of the pol and /or Env gene (Donovan,1999) .

1.3.6. Retroviral proteins

Retroviral structural proteins are coded for by the Gag gene and the Env. Gag (group specific antigen) proteins from the core of the virus and consist of three major proteins. The nucleo-capsid (NC) is a small protein (about 5 kd to 10kd) that interacts with retroviral RNA. The capsid (CA) protein (about25kd) forms the major structural element of the retroviral core. The matrix (MA) protein (about15kd) serves to join the retroviral core with the retroviral envelope. In some retroviruses, there are additional small core proteins, (Donovan 1999).

The Env (envelope) gene is responsible for the synthesis of two glycol-protiens that are linked to form adimer. The glycol-protiens outside the retrovirus (US, surface) is knob-like glycol-protiens (about 100 kd) that is responsible for binding the retrovirus to its cellular receptor during infection. The other glycoprotein (TM, trans membrane) is a spike-like structure (about 50 kd) that attach the SU protein to the retroviral envelope, (Donovan,1999).

1.3.7. Retroviral lipids

Retroviral lipids are mainly phospholipid and ocuur in the virion envelope. They form a bilayered structure similar to the outer cell membrane from which the retrovirus envelope is derived, (Donovan 1999).

1.3.8. Retroviral Enzymes:-

The Pol gene codes for several proteins with enzymatic activities that are important for replication of retroviruses . These enzymatic proteins are found within the retroviral particle , but in a much lower molar concentration than the retroviral structural proteins .

The reverse transcriptase (RT) enzyme is responsible for production of retroviral DNA genome from the retroviral RNA genome. To accomplish this , reverse transcriptase possesses several catalytic functions, including an RNA – requires DNA polymerase and an

RNase H activity. RT requires the presence of a divalent caution to function, and the type of divalent caution (magnesium or manganese) that particular retrovirus requires is useful in retroviral classification. The measurement of reverse transcriptase activity is one of the principal laboratory methods for the detection and assay of retroviruses.

The Pol gene also codes for other enzymes. The retroviral protease (PR) mediates cleavages of Gag and Pol polyproteins during retroviral assembly and maturation. The retroviral integrase (IN) functions to covalently link the retroviral DNA into the host cells , DNA as an integrated provirus (Donovan ,1999).

1.3.9. Retroviral Replication:-

A general scheme of retroviral replication is shown in figure 74.4. A retroviral particle binds to a specific receptor on the surface of a target cell via the SU protein. The retrovirus penetrates the cell and the retroviral core undergoes specific structural changes. The retroviral RNA within the modified core is reverse transcribed by RT using the associated tRNA primer, first to an RNA/ DNA hybrid form, then to a linear double- stranded DNA form with long terminal repeats.

replicate when the cellular DNA is replicated by the cell.

New retroviral particles are produced by budding from cellular membranes. Immature retroviral Gag polyprotein and genomic RNA assemble and acquire envelopes as they exit infected cells by budding through the plasma membranes into which retroviral SU and TM envelope proteins have been inserted.

In the final step, the retroviral protease (PR) cleaves the Gag polyprotein into the mature structural proteins of matrix, capsid , and nucleocapsid.

1.3.10.Immunologic Characteristics of Retroviruses:-

Retroviral proteins possess various types of antigens that define the envelope glycoproteins. Groupspecific antigens are shared by related

viruses and, in general, are associated with the virion core proteins. There are also interspecies antigens that are shared by other wise unrelated viruses derived from different host species. Reverse transcriptase (RT) is also antigenic and contains type-, group-, and interspecies-specific determinations,*(* Donovan,1999).

1.4. EIAV properties

1.4.1. Physical , chemical and Antigenic properties

EIAV is composed of tow envelope-encoded glycoprotiens (gp90=SU and gp45=TM) and four major non glyco-sylated proteins (p26=ca,p15=ma,p11=nc.and p9).the p26is the major core protein and demonstrates group specificity, while the envelope –associated glycol-proteins demonstrate hemagglutination activity and are type specific (Donovan, 1999). the EIAV genome is highly mutable. When the virus is placed under selective pressure by the host immune system, genomic point mutations produce novel new antigenic variants of the gp 45 and 90 envelope proteins. These antigenic variants cause EIA s characteristic episodic recurrence. In cell culture (where there is no immune selection).antigenic types remain stable and by serum anti bodies from the horse from which the virus was isolated. When introduced in to new horse. These same strains produce new antigenic viral variants that can no longer be neutralized by the original antibodies (Donovan,1999).

1.4.2. Resistance for other species and culture system

Horses ,ponies ,donkeys ,and mules are susceptible to infection by EIAV-there is only one report of human infection ,and no cases of EIA-like disease have been identified .attempts to propagate the virus in lambs, mice ,hamsters ,guinea ,pigs, and rabbits have failed .

Primary isolates of EIA can be propagated only in equine leukocyte cultures, where it grows in cells of the monocyte /macrophage lineage laboratory strains of EIA can be propagated in a variety of cell lines from several species, including human fetal lung fibroblasts, (Donovan 999).

1.5. Identification of the agent:

Virus from a horse can be isolated by inoculating suspect blood into a susceptible horse or on to leukocyte cultures prepared from susceptible horses. Recognition of infection in horses that have been inoculated experimentally may be made on the basis of clinical signs, haematological changes and a positive antibody response determined by an immunodiffusion test or enzyme-linked immunosorbent assay (ELISA) or by molecular techniques.

Successful virus isolation in horse leukocyte cultures is confirmed by the detection of specific EIA antigen, by immunofluorescence assay, polymerase chain reaction, reverse-transcriptase assay, or by the inoculation of culture fluids into susceptible horse.

Virus isolation is rarely attempted because of the time, difficulty and expense involved. (OIE, 2013).

Virus isolation is usually not necessary to make a diagnosis.

Isolation of the virus from suspect horses may be made by inoculating their blood on to leukocyte cultures prepared from horses free of infection.

Virus production in cultures can be confirmed by detection of specific EIA antigen by ELISA (Shane, et al., 1984), by immunofluorescence assay (Weiland et al., 1982), by molecular tests or by sub inoculation into susceptible horses.

Virus isolation is rarely attempted because of the difficulty of growing horse leukocyte cultures.

When the exact status of infection of a horse cannot be ascertained, the inoculation of a susceptible horse with suspect blood may be employed. In this case a horse that has previously been tested for antibody and shown to be negative is given an immediate blood transfusion from the suspect horse, and its antibody status and clinical condition are monitored for at least 45 days. Usually, 1–25 ml of whole blood given intravenously is sufficient to demonstrate infection, but in rare cases it may be necessary to

use a larger volume of blood (250 ml) or washed leukocytes from such a volume (OIE, 2013).

1.6. The host:

Equine infectious anemia virus (EIAV) is a non-primate lentivirus that does not cause human disease (Shu Kachi., et al., 2009).

EIA infects equidae only, which then remain infected for life.

There is no evidence that other animals are susceptible to infection with EIA there is no risk to public health, (Kaiser , et al .,1984) .

Horses naturally infected with EIA develop a persistent infection, (Hammond, et al., 2000).

It is estimated that more than 30% (Issel, et al., 1985) to more than 90% of these horses remain clinically healthy (i.e. latent carriers) and show no signs of the disease ((Issel, et al ., 1982),(Issel, et al., 1988)). Persistent infection results from the ability of the virus to undergo antigenic variation and avoid the host immune responses, (Cullinane , et al., 2006).

Persistently infected horses are considered to have a lower level of the virus in their blood than during viraemia (Oaks et al, 1998). Some persistently infected horses may develop the acute form of the disease (Issel, et al, 1982) and, in this event, most are likely to die. In the acute form of the disease, horses frequently have very high levels of the virus during viraemia (Issel, et al., 1988).

1.7. Distribution:

Equine infectious anemia virus is distributed worldwide and exists in an enzootic form in about 23% of countries and had been diagnosed in all continents except Antarctica. The virus is endemic to the Americas, parts of Europe and Far East, Russia and South Africa, (Yapkic, et al., 2007).

1.8. History of EIA:

The equine infectious anaemia was observed for the first time in France in1843 (Ligné, 1843). In 1903, Torrance described the disease in the USA and called it “swamp fever”. Its viral etiology was supposed in 1904 (Vallé & Carré,1904) but the pathogenesis of EIA and the features of its agent were a mystery for more than 50 years as the attempts to select a culture, sensitive to it were not successful until 1964, (Shuljak, 2006).

EIA was first described in France in 1843by Ligné and was associated with infection with a “filterable agent” in 1904. This makes EIA the first animal disease to be assigned a viral etiology, preceding by several years the major discovery of the first tumor virus by Rous, (Leroux, et al., 2004).

For years, knowledge of the molecular biology of EIAV has been retarded by the lack of a tissue culture system. Development of in vitro systems and production of viral particles led to the classification of EIAV as a member of the and opened the possibility of biochemical and molecular studies, until the mid-1990s, 92 percent of test positive samples have originated from horses located in what is referred to as the “hot zone.

In Brazil, the first confirmed EIA case was reported in Minas Gerais State (MGS) in 1968 and the AGID has been used as the official diagnostic test for EIA since 1974. In 1998, 131,991 horses out of 8,391,942 were tested in all Brazil for EIA, and 3,689 of them were classified as positives, resulting in a prevalence of 3%. According to the local office of the Brazilian Ministry of Agriculture, the EIA prevalence in MGS was 0.88% from 1973 to 1991, (Bicout. ,et al,2006)

1.9. Prevalence and Previous Studies:-

A serological investigation of equine infectious anaemia (EIA) was conducted on the sera of 346 Turkish horses using a combination of tests in series (ELISA and agar gel immunodiffusion) for EIA. No positive samples were detected, (Marenzoni , et al., 2011).

Another cross sectional study was conducted in Konya city. A total of 406 serum samples were examined by agar gelimmunodiffusion (AGID) and

enzyme-linked immunosorbent assay (ELISA) for antibody to equine infectious anaemia virus (EIAV) and no positive result was detected, (Yapkic, et al., 2007).

Also, a total of 404 horses was examined from the selected cities (Istanbul, Bursa, Bal›kesir) of the Marmara region of Turkey.Blood was collected from all horses and the sera were analyzed for the presence of antibodies to equine infectious anemia virus (EIAV) using an agar gel immunodiffusion (AGID) test. The results revealed that none of the horses were positive for antibodies to EIAV (Turan,et al., 2002).

A retrospective study was conducted to examine the prevalence of equine infectious anemia (EIA) in horse populations in the northern part (comprising 89 cities) of Minas Gerais State, Brazil, from January 2002 to December 2004.Data on 8,981 agar gel immunodiffusion test results from the region were used as input for a statistical and autoregressive analysis model to construct a city-level map of the distribution of EIA prevalence. The following EIA prevalence (P) levels were found: 49 cities with 0,P#0.5%, 26 with 0.5%,P#1.5%,10with 1.5%,P#5%, and 4 with 5%,P#25% ,(Bicout, et al., 2002).

Another seronegative study consisted of 476 equids including 400 horses and 76 donkeys from Kars and Ardahan provinces, North-Eastern of Turkey. Blood was collected from all horses and donkeys and the sera were analyzed for the presence of antibodies to equine infectious anemia virus (EIAV) using an agar gel immunodiffusion (AGID) test, (Kirmizigul, et al., 2009).

As well**,** whole blood and serum samples (n=310) from horses over one year old from different regions of Iran were examined. Samples were initially checked in the agar gel immunodiffusion (AGID) test and 9 cases (2.90%) had antibody against EIAV. Then, positive serum samples and 301 apparently healthy horses (negative in the AGID test) were examined by nested PCR to detect proviral DNA of EIA.

PCR results showed that all positive sera and 2 of AGID-negative sera were positive, (Motaz.et al, 2010).

In Punjab, Pakistan, A cross-sectional epidemiological survey was conducted and 430 blood and serum samples wererandomly collected from 332 donkeys, 65 horses, and 33 mules along with epidemiological information.. Serum samples were analyzed for the presence of antibodies against EIA virus througha commercial ELISA. Although, erythrocyte indices indicated towards presence of anemia in equines, no serum sample was found positive on ELISA. It was a first ever study in Pakistan where presence of EIA was investigated over a wide geographic region and indicated towards the possible disease free status of the selected equine population, (Hussain, et al., 2012).

1.10. Transmission:

Equine infectious anemia virus is transmitted from an infected horse to a susceptible one mechanically on the mouthparts of biting insects. In horses, this virus persists in blood leukocytes for life, and also occurs in plasma during febrile episodes. Symptomatic horses are more likely to transmit the disease than animals with inapparent infections; after visiting an asymptomatic carrier, only one out of every 6 million flies is likely to become a vector. High levels of viremia have also been reported during the early stages of the infection in mules. Significantly lower titers have been reported in donkeys inoculated with certain horse-adapted strains (OIE, 2009).

Although other insects including stable flies (*Stomoxys calcitrans*) can transmit EIAV, the most effective vectors are biting flies in the family *Tabanidae*, especially horse flies *(Tabanus* spp. and *Hybomitra* spp.) and deer flies (*Chrysops* spp.), (oie, 2009). The bites of these flies are painful, and the animal's reaction interrupts feeding. The fly attempts to resume feeding immediately, either on the same animal or on another nearby host, resulting in the transfer of infectious blood. EIAV survives for a limited

time on the mouthparts of insects, and it is less likely to be spread to more distant hosts, (OIE, 2009). Mosquitoes do not transmit EIA (Fernanda, 2011).

1.11. Important factors for EIA transmission by insects

Horses with a high amount of virus in their blood (high viremia) and presenting clinical signs of disease are more likely to transmit EIA than inapparent carriers with low levels of virus in their blood.

Behavior of feeding insects also plays an important role. For example, for transmission to occur, an insect needs to feed from an infected horse, be interrupted in that feeding, and then, within a short period of time, find and feed on another horse. Horseflies are not able to transmit EIA if the subsequent feeding does not occur within four hours. Horseflies and deerflies have a painful bite that contributes to their efficiency as vectors because the horse interrupts their feeding with defensive movements, so the insect often moves on to another horse.

The distance between the infected horse and the other susceptible horses is another important factor in transmission.

When a horsefly or deerfly feeds off a horse and is interrupted, it will return to the same horse 99% of the times if the other horses are over 160 feet away.

A mare can transmit EIA to the foal in uterus ,during parturition(foaling),or th rough the colostrums or milk. These transmissions are more likely to occur if the mare develops acute clinical disease and viremia during gestation.(Fernanda .,2011)

Finally ,this virus can also be transmitted in blood transfusions , teeth floats and by Needles and equipment contaminated with blood from an infected horse can also spread the virus when used on unexposed horse (Issel, et al., 1988) .

Horses demonstrating clinical signs of EIA pose the greatest risk of spreading the virus because they have the greatest concentrations of

circulating virus. However, even in-apparent carriers pose a risk to other horses**, (**APHIS, 2006).

Other, minor routes of transmission might be possible. EIAV does not appear to be shed in saliva or urine. However, it can be found in milk and semen, and horses can be infected by inoculating these secretions subcutaneously, and possible transmission through milk has been reported in some nursing foals. Although venereal transmission does not seem to be a major route of spread, one stallion appears to have transmitted the virus to a mare with a vaginal tear during breeding. The possibility of aerosol transmission by infectious material during close contact was raised during the 2006 outbreak in Ireland, (OIE 20090).

1.12. Transmission cycle:

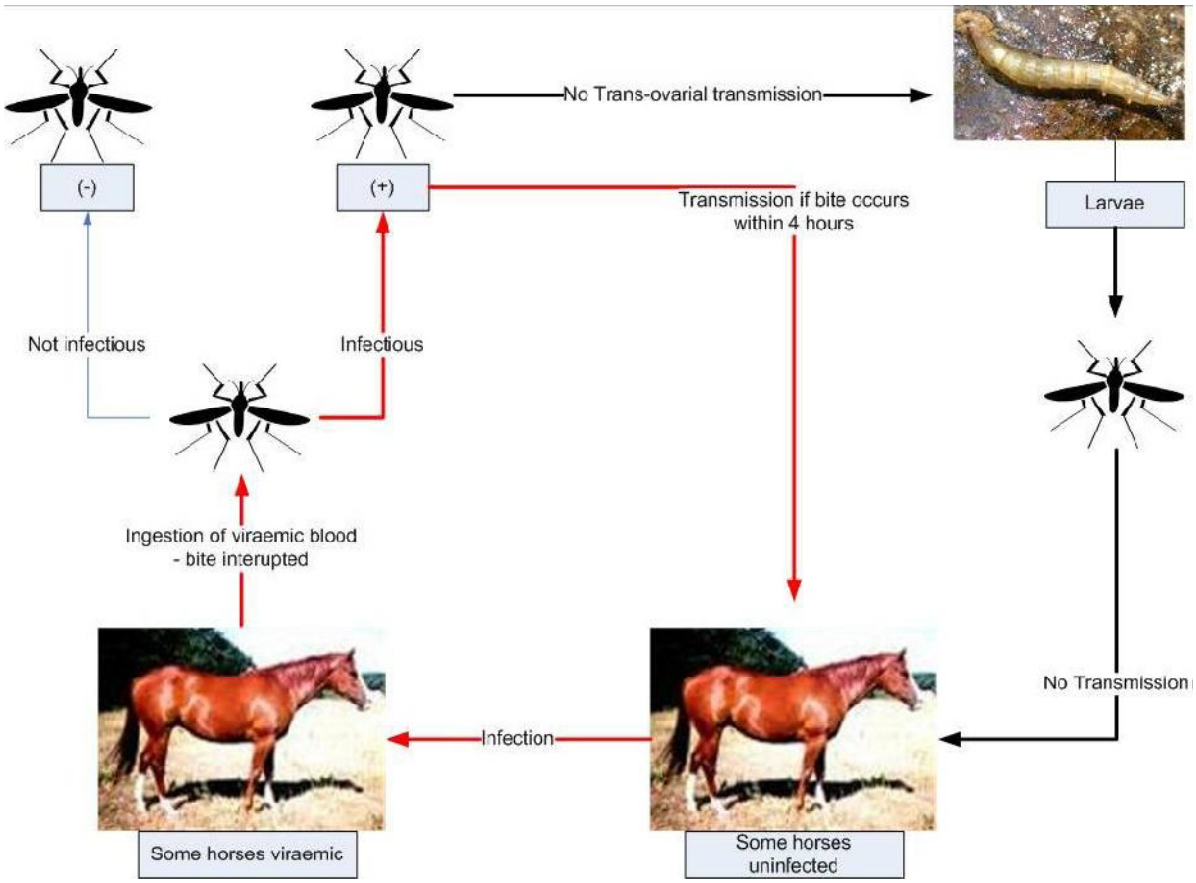

Figure 1:Transmission cycle of Equine Infectious Anaemia (Sabirovic, et al., 2010)

1.13. Pathogenicity*:*

Equine infectious anemia is a potentially fatal viral disease, the EIAV reproduces in white blood cells that circulate throughout the body. The immune system, via antibodies, may attack and destroy red blood cells, leading to anemia. Inflammation associated with the viral infection may damage vital organs, such as bone marrow, liver, heart and kidney. Secondary infections (e.g. pneumonia) may occur due to subsequent immunosupression. EIAV-infected horses may die from the direct effects of the virus or from Moreover, high number of uncontrolled animal movements pose another significant threat factor for the spreading the infection to the free areas.(Kirmizigul, et al., 2008).

1.14. Clinical EIA:

Equine infectious anemia should be among the differentials in individual horses with weight loss, edema and intermittent fever. It should also be considered when several horses experience fever, anemia, edema, progressive weakness or weight loss, particularly when new animals have been introduced into the herd or a member of the herd has died, (OIE, 2009).

1.15. Clinical features of the EIAV-induced disease:

EIA can cause a variety of clinical signs, which include fever, lethargy, inappetence, thrombocytopenia (low platelet count in the blood), anemia, splenomegaly (enlarged spleen), hepatomegaly (enlarged liver), weight loss, edema (swelling), and hemorrhage. The severity of the disease will depend upon the viral load, the virulence of the virus (the strength of the particular virus strain), and the susceptibility of the horse.

Therefore, severity will vary from horse to horse.

The three clinical stages of EIA infection are acute, chronic, and in apparent.

The acute stage occurs after initial infection. Five to 30 days after exposure, viremia can occur, leading to fever, thrombocytopenia, lethargy,

and in appetence. These signs can be mild and may not even be noticed by the owner. The fever will usually subside in a few days, although a small percentage of horses develop high viremia and severe anemia, a combination that can be fatal. After the initial acute stage of the disease, most horses experience recurrent episodes of the acute clinical phase.

These horses become permanently infected, and most of the time they are inapparent carriers of the virus. Another characteristic of the EIA virus is that it is a retrovirus. Because retroviruses lack proofreading ability, a number of mutations occur with each replication. Therefore, during the course of infection, a number of viral variants will develop, and each horse will be infected with one of these mutant variants. One horse can be infected with more than one variant of the virus as well. HIV also replicates in this manner, which is one of the reasons why researchers have not been able to develop a vaccine against either HIV or EIA.

1.16. Clinical Forms:

EIA has three common clinical forms: acute, subacute, and choronic form ,

(Crisman, D).

In the acute or early form, the horse will be depressed, uncoordinated and feverish. Horses are rarely anemic during this stage. This phase may last several days and is the stage during which the horse is most likely to transmit the disease to nearby horses.

The second phase is characterized by weight loss, recurring fevers and general weakness. Anemia is likely to be present, and mares can abort during this stage.

If horses survive the first two stages, they enter the final or chronic stage, where they often appear normal. An owner may report that a horse is a poor keeper, and the animal may be mildly anemic. Infected mares can transmit the disease to their foals.

A horse infected with the EIA virus will be a carrier of the disease for life. Although horses in the chronic stage appear normal, they become ill again if subjected to stress, such as shipping or severe weather. Carriers pose a

health threat to the equine community. Since the early 1960's, several outbreaks of EIA have occurred at either race tracks or large breeding farms, resulting in the deaths of many horses.

1.17. Diagnosis:

1.17.1. Laboratory test:

The monitoring of EIA is currently based on serologic detection of anti EIA virus antibodies, once an animal is infected, it becomes a carrier for life. The two most commonly used serological tests are the agar gel immunodiffusion (AGID or Coggins) test and enzyme-linked immunosorbent assays (ELISAs). Horses are usually seronegative in the AGID test during the first 2-3 weeks after infection; in rare cases, they may not develop antibodies until 60 days. ELISAs can detect antibodies earlier than the AGID test and are more sensitive, recently, PCR had been also used.(OIE, 2009)

1.17.2. Polymerase chain reaction:

A nested polymerase chain reaction (PCR) assay to detect EIA proviral DNA from the peripheral blood of horses has been described. The nested PCR method is based on primer sequences from the *gag* region of the proviral genome. It has proven to be a sensitive technique to detect field strains of EAV in white blood cells of EIA infected horses; the lower limit of detection is typically around 10 genomic copies of the target DNA.

A real-time reverse-transcriptase PCR assay has also been described. To confirm the results of these very sensitive assays, it is recommended that duplicate samples of each diagnostic specimen be processed. Because of the risk of cross contamination, it is also important that proper procedures are followed.

The following are some of the circumstances where the PCR assay maybe used for the detection of EIAV infection in horses:

• Conflicting results on serologic tests;

• Suspected infection but negative or questionable serologic results;

• Complementary test to serology for the confirmation of positive results;

• Confirmation of early infection, before serum antibodies to EIAV develop;

• Ensuring that horses that are to be used for antiserum or vaccine production or as blood donors are free of EIAV;

• Confirmation of the status of a foal from an infected mare (OIE., 2009)

Table 1.17.3:. Test methods available for the diagnosis of equine infectious anaemia and their purpose (Source: OIE 2013)

Method	Purpose			
	Population freedom from infection / efficiency of eradication policies	Individual animal freedom from infection	Confirmation of clinical cases	Prevalence of infection – surveillance
AGID	++	++	++	++
ELISA	++	++	+	+
Immunoblot	–	++	++	–
PCR	–	+/–	+	–
Virus isolation/horse inoculation	–	–	+	–

Key: +++ = recommended method; ++ = suitable method; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose.

Although not all of the tests listed as category +++ or ++ have undergone formal standardisation and validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

1.18. Pathological Findings and Post Mortem Lesions :

The spleen, liver and abdominal lymph nodes may be enlarged; Histologically these organs are infiltrated with nests of immature lymphocytes and plasma cells. Kupffer cells in the liver often contain haemosiderin or erythrocytes. The enlarged spleen may be felt on rectal examination.

The mucous membranes can be pale. In chronic cases, *em*aciation may also be noted. Edema is often found in the limbs and along the ventral abdominal wall. Petechiae may be observed on internal organs, including the spleen and kidney. Mucosal and visceral hemorrhages and blood vessel thrombosis have also been reported. Chronically infected horses that die between clinical episodes usually have no gross lesions, but some animals may have proliferative glomerulo-nephritis or ocular lesions, (OIE, 2009)

1.19. Differential diagnosis

The differential diagnosis includes other febrile illnesses including equine viral arteritis, purpura hemorrhagica, leptospirosis, babesiosis, *Anaplasma phagocytophilum,*othrombocytopaenia/ecchymoses severe strongyliasis or fascioliasis, phenothiazine toxicity, autoimmune hemolytic anemia and other diseases that cause fever, edema and/or anemia. B205 Equine Infectious Anaemia,

Animals generally recover from either the acute or chronic form of the clinical disease, but will remain lifelong carriers of the virus.

1.20. Morbidity and Mortality

The infection rate varies with the geographic region. Virus transmission is influenced by the number and species of flies, their habits, the density of the horse population, the level of viremia in the host and the quantity of blood transferred. Infections are particularly common in humid, swampy regions. Seroprevalence rates as high as 70% have been seen

on farms where the disease has been endemic for many years. The morbidity rate and severity of the clinical signs are influenced by the strain and dose of the virus, and the health of the animal. Horses are more likely to develop clinical signs than donkeys or mules, but many horses are infected subclinically. The presence of EIAV in a herd often goes unnoticed until some horses develop the chronic form of the disease or routine testing is done. Epizootics with high morbidity and mortality rates have been reported, but deaths are otherwise uncommon in naturally infected horses. Experimental inoculation with a high viral dose can result in mortality rates as high as 80%, (OIE 2009).

1.21.Epidemiology of the Disease

EIA has been diagnosed in several different continents. In Europe, it is most prevalent in the northern and central regions. It has appeared in most states in the United States and the provinces of Canada but the principal enzootic areas are the Gulf Coast region and the northern wooded sections of Canada (Altaeb, 2004)

Diagnosis of the disease was made in Australia in 1959, but the incidence appears to be very low. The only area of Australia in which EIA could be regarded as being endemic is along the inland river systems of central and western Queensland. In a serological survey in this area in 1978, 21.7% of horses and 23% of properties were positive for EIAV . The disease was also reported once in Thailand in 1996 and Mongolia.

The morbidity varies considerably and depends on the strain of the virus, and the inoculum delivered by the biting insects (Coggins, 1984). Extensive serological surveys over large areas, using the agar gel immunodiffusion (AGID) Coggins test have shown the prevalence rates, ranging from 1.5 - 2.5 % in the United States, to

19 -50% in Brazil. The prevalence of infection varies depending on the population of horses, the proportion of carrier and the density of

insect vectors. Large-scale movements of horses during wartime

have been responsible for extensive dissemination of the disease. The possible detection of the infection at present is due to obligatory testing carried out. Rapid expansion of 'pleasure horse' activity in affluent countries may lead to more movement of horses and opportunities for spread of the infection from relatively few donors (Altaeb.,2004).

1.22. Risk Factors:-

There are management and geographic factors that put horses at greater risk for contracting EIA. These include:

- Close proximity to regions where EIA outbreaks have been identified.
- Stabling or pasture environments that have a steady influx of new horses, especially if negative Coggins certificates are not required.
- Exposure to horses at shows, sales or events, especially where stringent health care regulations are not enforced and verification of a current negative Coggins test is not required.
- Pasturing horses in swampy areas and in areas where all horses have not been regularly tested for EIA, (AAEP, 2006).

1.23. Treatment and Prevention:

There is no specific therapy for EIA infection. This disease is reportable, and federal law prohibits the interstate transport of infected horses. If an owner chooses to keep the horse alive, treatment will include isolation from other horses, non-steroidal anti-inflammatory drugs for the fever, leg wraps for the swelling, and blood transfusion for severe anemia.

Since there is no vaccine to prevent EIA infection, prevention is achieved through removal of the infected horse from the population. Humane

euthanasia is the most common method of removal. Horses that are not euthanized must be permanently identified with a brand or lip tattoo applied by a USDA representative, after which an infected horse may be quarantined on the premises with a 200-yard separation distance from all other horses. The horse and premises will be monitored periodically to ensure that the provisions are not being violated. The infected horse can also be moved to a federally approved research facility.

Other methods to prevent infection include the annual testing of horses, the testing of horses as part of a pre -purchase evaluation, testing of blood and plasma donors, no sharing of the same syringe and needle between horses, and proper sterilization of dental and surgical equipment, between procedures.

Finally, vector control in your property will minimize the transmission of EIA to susceptible horses.

1.24. Control measures

Minimizing or eliminating contact between non-exposed horses and the secretions, excretions, and blood of EIAV-infected horses can help control the spread of EIAV. This is accomplished in most areas of the world by testing horses for EIAV and either euthanizing or segregating test–positive horses from test-negative horses. Once the reservoirs of EIAV are identified, separated, and maintained at a safe distance from non exposed horses, the transmission of EIAV is broken. The major regulatory actions to control EIAV are carried out by individual States. However, States' rules, while encompassing a broad scope of EIA concerns, are not consistent. In 2002, USDA created a Uniform Methods and Rules to facilitate the development of a uniform control program for EIA (APHIS2006).

EIA has no known cure or vaccine. Infected horse sproduce antibodies, which cannot rid the body of the disease. The viruscan hide and "drift" into variousforms, making complete recovery impossible and hampering efforts tocreate a vaccine (BOAH, 98).

1.25. Requirements for Vaccines

Inactivated and subunit EIAV vaccines were tested in different laboratories and proved to protect infections of homologous prototype strains only. An attenuated live vaccine, developed in the early 1970s, was extensively used in China (People's Rep. of) between 1975 and 1990 and was effective in controlling the prevalence of EIA.

With low prevalence since 1990, the strategy for EIA control has shifted from vaccination to quarantine to avoid the interference of vaccine antibodies with diagnostic tests.

Although no safety concerns arose with the use of attenuated EIAV vaccine in China, it should be noted that, like other lentiviruses, EIAV is highly mutable and can integrate into host genomes. The use of a live EIAV vaccine needs to be very cautious and carefully evaluated. (OIE, 2013)

Chapter Two

Materials and Methods

2.1. Study area:

The study was conducted in Khartoum , political capital of sudan. The State lies between longitude 31.5 – 34 east and latitude 15 – 16 north in an area about 28,165 square kilometres. It is bordered to the north and the east side by River Nile State, to North Western by the North State, and to the east , south-eastern and south by Kassala, Gedaref and Gezira State respectively and to the west by North Kordofan. Most of the Khartoum State lies in the semi-desert climatic region, while northern areas lie in desert zones. The climate of the state is ranging from hot to very hot. The weather is rainy in summer, cold and dry in winter. Average rainfall reaches 100 – 200 mm in the north-eastern areas and 200-300 mm in the North Western areas. Temperature ranges in summer between 25 – 40 degrees in the months of April to June, and 20 – 35 in the months from July to October. In winter, however, temperatures continue to decline between November to March from 25 – 15 degrees. Geographically, Khartoum State is divided into three blocks:-

A. first block: it starts from the Mugran, i.e the confluence of the two rivers (the Blue and White Niles). Being confined between them, this block extends southwards to the boundaries of the Gezira State. Administratively, it is divided into two localities, Khartoum and Gabalawlia localities.

B. second block: is limited between the Blue Nile and the River Nile. It includes the localities of Khartoum North and east of the Nile, North or Khartoum North represents a largest one of the towns of this block.

C. third block: namely, the one located west of the White Nile and the River Nile and includes three Um Badda and Karari localities.

According to 2008 population census, the population of Khartoum state is estimated to be about five million people who are a mixture of tribes of the Sudan .As to the activity of the population of Khartoum state ,it can be said that most of the population are workers and personnel in the state champers ,the private sector ,and banks .In addition ,there is a large segment of capitalists dealing in trading and another segment represented by migrants and displaced people working in marginal activities .As to fellow citizens ,they are engaged in agriculture ,grazing and thus supply the capital ,with vegetables , fruits , and diary.

There are also some residents who live on the banks of the river engaged in the river-related works such as pottery, brick, and fishing (WWW.Krt.gov.sd).

2.2. Test animals:

The target test animals are horses (*Equus ferus caballus*) and donkeys (*Equus asinus).*

2.3. Questionnaire:

A pre-tested structured questionnaire with the primary objective of elucidating the multi factorial background of EIA was conducted in an interactive manner. Animals were visited in each unit and examined and filling out the questionnaire by asking the owner. The animals attribute included species, breed, age, color, sex, pregnancy, body condition, and infection with other disease. The farm attributes included vet service, type of work, presence with other animals, source of water and type of pasture. The general management factors

included housing, and presence of the vector; the Horse-fly (*Tabanus*) and/or the Stable-fly (*Stomoxys calcitrans*).

Clinical examinations were performed by checking the mouth, nacres, eyes, lymphnodes (axillar, mandibular and prescapular) and skin.

2.4. Type of samples collected from test animal:

Whole blood sample (5 – 12 ml) were collected, during the period 31 August 2013 to 23 September 2013, using disposable syringes, from the Jugular vein, of the target animals.

2.5. ELISA for detection of EIA antibodies:

Commercial test kit (The INGEZIM ANEMIA EQUINA kit (INGENASA, Madrid, Spain) is a double recognition enzymatic immunoassay for detection of antibodies in serum specific for Equine Infectious Anemia virus (EIAV)**)** was used following the manufacturer instruction. Optical density values were at 450 nm using ELISA plate reader at 450 nm wave length.

2.6. Dentition:

The age of sampled animals was carried out according to The Merck veterinary manual 8th edition, (Aiello., et al., 2000) and Equine internal medicine, (Reed, et al., 2004) as shown in table (3.3.5.1) (Appendix 1).

2.7. Sample Size:

The actual sample size for determining the prevalence rate of Equine Infectious Anaemia in the Khartoum State was calculated based on the following parameters: 95% level of confidence, ±5% desired level of precision, the expected prevalence rate of EIA was calculated using the formula described by Martin, et al. (1987) as follow:

Prevalence rate = **No. of equines infected with EIA*100**

Total No. of equines at a particular point in time

Sample size determination:

The sample size was calculated according to the following formula (Martin, et al., 1987):-

$$N = \frac{(1.96)^2 * P * Q}{L2}$$

N= sample size

L= desired absolute precision

Q= (I-P).

The records of veterinary clinics in the State show that the prevalence of EIA wasn't calculated before, thus, according to the study on Detection of proviral sequences of equine infectious anemia virus horses in Iran the prevalence was estimated about2.90% (Momtaz, et al., 2010) consequently the sample size was calculate as follows:

$$N = \frac{(1.96)^2 * (0.029) * (1-0.029)}{(0.0025)} = 43.27$$

This number of sample size was inflated 4 – fold to account according to Thrusfield theory (Thrusfield, 2007) for the effect of randomness and representativeness in multistage sampling strategy with more than two levels. Thus, the total sample size was 174 serum samples from Khartoum State, additionally, more ten sera sample were added that the ELISA plate perform 184 samples.

2.8. Processing of whole blood specimens collected:

Serum samples were collected in the period from 31 August 2013 to 23 September 2013. One serum sample (5 – 12 ml) was taken from each animal using disposable syringes from Jugular vein. After that, syringes were kept in a slant position and protected from direct sunlight until the blood was clotted and thereafter the serum was separated. The separated sera were transferred to sterile labled cryovials and were kept at -20^{o}C at Veterinary Research Institute (VRI) for test and analysis. Blood sample were collected from different spiecies (horses, donkeys), and from different ages. From a total of 220 sera collected only 184 samples were tested and analyzed, as shown in table 2.8.1 in appendix (3)

Table 2.8.1: Total number of samples collected from different equines in the selected localities

Locality	**Horses**	**Donkeys**	**Total**
Khartoum	29	36	65
Bahry	17	20	37
Omdurman	8	13	21
Gebel Awlia	18	43	61
Total	72	112	184

2.9. Sampling Strategy and Study Design:

Across-sectional type of epidemiological studies was conducted with a multistage sampling strategy to estimate the prevalence of EIA ,and to investigate the risk factors associated with the disease. A single visit was made to collect samples and filling out the questionnaires. Using the probability sampling methods t o select the equines. Four localities (Khartoum, Bahry. Omdurman and Gebelawlia) were selected randomly from the seven localities of the state, each locality has a many of administrative units .The epidemiological units in this study are equines markets, assembling of equines worked at dairy farms and equines living in camps for rent. All animals found on their expected usual working or living areas were selected expect those whose owners refuse to combine the study. To encourage owners to participate the study, Multivitamin, Ivomec and Antibiotics were offered.

Random serum samples were collected from 184 equines, 72 were horses and 112 were donkeys ,65 from Khartoum: 29 were horses and 36 were donkeys, 39 from Bahry:17 were horses and 22 were donkeys,19 from Omdurman: 8 were horses and 11 were donkeys, 61 from Gebel Awlia: 18 were horses and 43 were donkeys. Serum samples were tested in the period from 22 September to 24 September 2013.

2.10. ELISA KITS:

2.10.1 Description of the kits:

Microwells are coated with NP (Gene GAG) recombinant antigen.

Specimens to be tested and controls are added to the microwells. Anti-EAIV antibodies if present form an anti-body-antibody-antigen complex.

plates were washed and a NP antigen peroxidose (Po)conjugate was added to the microwells .

After washing, in order to eliminate the excess conjugate, the substrate solution (TMB) was added. The resulting coloration depends on the quantity of specific present antibodies.

In the presence of antibodies, a blue solution appears which became yellow after addition of the stop solution.

In the absence of antibodies, no coloration appeared.

The micro-plate was read at 450nm.

Table: 2.10.2. Components

*Reagents
Microplates coated with NP recombinant antigen
GAG Ag –Po Conjugate (tox)
Positive Control
Negative Control
Dilution Buffer 13
Dilution Buffer 19
Wash Concentrate (20X)
Substrate Solution
Stop Solution (H_2SO_2)

2.10.3. Test Procedure:

-50 pt of Dilution Butter 13 were added to all wells of ELISA plate

-50 pt of the Negative Control were added to wells A1 and B1

-50 pt of the Positive Control were added to well c1 and D1

-50 pt of each sample to be tested to the remaining wells and incubated for 45 min at room temperature.

2. The wells were emptied and washed three times with approximately 300 µl of the wash solution avoiding drying of the wells between washings.

Then 100 conjugate were added to all wells and incubated for 30 min

The plates were washed 3 times using approximately 300µ of the

wash solution.

100 of substrate were added to all wells then incubated for 15 min at room temperature in the dark .Then, 100 of stop solution as a last step of the test. The plate was put in ELISA reader, mounted and then read at 450 nm.

10.4 . Interpretation of results :

Table: 2.10.4.1. Plate1 Layout of ELISA for EIA :

Control	<>	1	2	3	4	5	6	7	8	9	10	11	12
ve+	A	17950	690	850	560	570	4520	870	600	580	1080	690	640
ve+	B	26220	750	1030	550	570	600	540	580	510	600	750	630
ve-	C	930	650	740	600	720	670	22030	37250	560	700	580	660
ve-	D	860	650	720	630	560	730	710	560	840	1260	650	670
	E	730	660	620	920	630	760	660	620	780	650	670	840
	F	700	650	720	1080	640	1830	960	640	570	520	810	590
	G	760	630	1480	1350	610	630	31740	530	730	560	540	700
	H	14410	14610	37490	15160	14960	810	1150	780	830	1050	610	810

Table: 2.10.4.2. Plate 2 Layout of ELISA for EIA :

Control	<>	1	2	**3**	4	5	6	7	8	9	10	11	12
ve+	A	16700	660	**650**	680	730	20700	16700	660	650	680	730	20700
ve+	B	15900	770	**730**	830	720	740	15900	770	730	830	720	740
ve-	C	950	1280	**1170**	650	610	590	910	1280	1170	650	610	590
ve-	D	950	3650	**560**	590	600	910	700	3650	560	590	600	910
	E	660	1550	**700**	500	760	850	660	1550	700	500	760	850
	F	850	680	**730**	600	29960	740	850	680	730	600	29960	740
	G	860	3030	**630**	740	640	710	860	3030	630	740	640	710
	H	880	1530	**850**	1450	850	690	880	1530	850	1450	7090	690

2.11. Data Management and Analysis:

All collected data like age, breed of individual animals and locations during sampling and the laboratory results were entered, coded, and stored electronically in a Microsoft® Excel for Windows® 2007 data base. The Statistical Package for Social Sciences (SPSS) for Windows® version 20 was used for all appropriate statistical analysis.

Descriptive statistics of the variables were obtained. For each variable (locality, animal species, color, sex age, animal body condition, presence of other disease, veterinary service, type of work, housing, presence with other animals, source of water, type of grass and presence of vectors), frequencies (number of observations within variable) and prevalence rates by cross-tabulation (number of positive valid samples/number of individuals sampled in the variable) were obtained.

Hypothesis testing for association between disease and potential risk factors were first tested by the univariate analysis by means of the 2-tailed Chi-square test. Risk factors found associated with the disease at significance level of P-value ≤ 0.25 were entered in the multivariate analysis. In a second step, a logistic regression model was used to assess the associations and strengths between the potential risk factors and the disease. Associations in the logistic regression model were deemed significant when $p \leq 0.05$.

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Chapter Three

Results

3.1 ELISA results:

The results showed that the prevalence of EIA was 8.7% in tested sera collected from equines in Khartoum state as shown in table (3.2).

Table 3.1: Results of distribution of EIA in Khartoum state

Disease

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	disease negative	168	91.3	91.3	91.3
	disease positive	16	8.7	8.7	100.0
	Total	184	100.0	100.0	

3.2. Summary of the results:

3.2.1 Locality:

Result of frequency of EIA in locality is shown in table (3.2), 37 of equines from Bahry, 61 from Gabalawiia, 65 from Khartoum and 21 from Omdurman. It was higher in Khartoum (35.3%) compared with other localities .

For distribution and prevalence of EIA in locality, the results were shown in table (3.3). One of equines from Bahry , 7 from Gabalawlia, 2 from Khartoum and 6 from Omdurman. Infection

was higher in Omdurman (28.6%) compared with Gabalawlia (11.5%), Khartoum (3.1%) and Bahry (2.7%) .

The results of association between locality and ELISA for EIA seropositivity is depicted in table (3.4). A significant association was observed between Locality and EIA (P-value =0.002).

3.2.2 Animal species:

Results of frequency of EIA in animal species is shown in table (3.2) 112 of equines were donkeys and 72 were horses .It was higher in donkeys compared with horses

The results of distribution and prevalence of EIA in animal species are shown in table (3.3). Six of equines were donkeys, and 10 were horses, infection was higher in horses (13.9%) compared with donkeys (5.4%) .

The results of Association between animal species and ELISA EIA seropositivity is depicted in table(3.4), a significant association was observed between animal species and EIA (P-value =0.045)

3.2.3 . Color:

Result of frequency of EIA in color is shown in table (3.2) 62 of equines were black, 42were red and 80 were white .It was higher in white (43.5%) compared with black (33.7 %) and red (22.8 %).

The results of distribution and prevalence of EIA in Color were shown in table 3.3), 7 of equines were black, 5 were red and 4 were white .infection was higher in red (11.9%) compared with black (11.3%) and white (5%).

The results of association between color and ELISA EIA seropositivity is depicted in table (3.4), a significant association was observed between **Color** and EIA (P-value =0.294)

3.2.4 Sex:

Result of frequency of EIA in Sex is shown in table (3.2), 33 were females and 151 were males .It was higher in males (88%) compared with females (12 %).

For distribution and prevalence of EIA in Sex, the results were shown in table (3.3), 15 of equines were males and one of females was positive. Infection was higher in males (9.9%) compared with females (3%).

The results of association between sex and ELISA EIA sero-positivity is depicted in table (3.4). A significant association was observed between sex and EIA (P-value =0.202)

3.2.5 Age:

Result of frequency of EIA in age is shown in table (3.2) 159 of equines were less than 14 years old ,and 25 were more than 13 and less than 26 years old .It was higher in (1-13)(86.4%) compared with (14-26) (13.6%).

For distribution and prevalence of EIA in age, the results were shown in table (3.3), 2 of equines were less than 27 years old, and 14 were less than 14 years old. Infection was higher in (1-14) (8.8%) compared with (>14) (8%).

The results of Association between age and ELISA for EIA seropositivity is depicted in table (3.4), a significant association was not observed between age and EIA (P-value =0.894).

3.2.6 Animal body condition:

Result of frequency of EIA in animal body condition is shown in table (3.2) 117 of equines were at good body condition,37 were moderate and 30 werepoor .It was higher in Good (63.6%) compared with Moderate (20.1%) and Poor (16.3%).

For distribution and prevalence of EIA in animal body condition, the results were shown in table (3.3),14were at good body condition, one

of equines was at moderate and another was poor .infection was higher in Good (12%) compared with Poor (3.3 %) and moderate (2.7%).

The results of association between animal body condition and ELISA EIA sero-positivity is depicted in table (3.4), a significant association was observed between animal body condition and EIA (P-value =0.114).

3.2.7 Presence of other disease:

Result of frequency of EIA in presence of other disease is shown in table (3.2), 132 of equines were having another disease, and 52 were have not. It was higher in equines with no disease (71.7 %) compared with those with diseases (28.3%).

For distribution and prevalence of EIA in presence of other disease, the results were shown in table (3.3), 5 of equines had no other disease , and 11 were having another disease, infection was higher in Yes (9.6%) compared with No (8.3%)

The results of association between presence of other disease and EIA servo-positivity is depicted in table (3.4), a significant association was not observed between presence of other disease and EIA (P-value =0.781)

3.2.8 Veterinary service:

Result of frequency of EIA in Vet service is shown in table (3.2) 119 of equines which didn't get veterinary service and 65 which had got. It was higher in No (64.7%) compared with Yes (35.3%) .

For distribution and prevalence of EIA in Vet service, the results were shown in table (3.3), 13 of equines didn't have got veterinary service and 3 did. Infection was higher in No (10.9%) compared with Yes (4.6%).

The results of association between veterinary service and ELISA EIA sero-positivity is depicted in table (3.4), a significant association was observed between veterinary service and EIA (P-value =0.147)

3.2.9 Type of work:

Result of frequency of EIA in type of work is shown in table (3.2)142 of equines were backed draught, 15 were used for night guarding (Elsawary) and 27 were used for racing. It was higher in draught (84.2%) compared with Night guarding (8.2%) and Racing (7.6%).

For distribution and prevalence of EIA in type of work , the results were shown in table (3.3), 12 were backed draught, and 3 were used for night guarding and 1 for racing, the infection was higher in night guarding (20%) compared with draught (8.5%) and racing (3.7%) .

The results of association between type of work and ELISA for EIA sero-positivity is depicted in table (3.4), a significant association was not observed between type of work and EIA (P-value =0.195).

3.2.10 Housing:

Result of frequency of EIA in housing is shown in table (3.2), 51 of equines were living inside their stable and 133 of them were living on open areas ,farms or on the backyards of their owner's houses .It was higher in outdoor (72.3%) compared with indoor (27.7%) .

For distribution and prevalence of EIA in housing, the results were shown in table (3.3), 6 of equines were living indoor, and 10 were living outdoor. Infection was higher in indoor (11.8%) compared with outdoor (7.5 %).

The results of association between housing and ELISA EIA sero-positivity is depicted in table (3.4), a significant association was not observed between housing and EIA (P-value =0.360)

3.2.11 Presence with other animals:

Result of frequency of EIA in presence with other animals is shown in table (3.2), 134 of equines alone or in equine population, and 50 were

living with other animals. It was higher in No (72.8%) compared with Yes (27.2%).

For distribution and prevalence of EIA in presence with other animals the results were shown in table (3.3), 15 of equines were not with other animals and 1 was with other animals. Infection was higher in No (11.2%) compared with Yes (2%).

The results of association between presence with other animals and ELISA EIA sero-positivity is depicted in table (3.4), a significant association was observed between presence with other animals and EIA (P-value =0.049).

3.2.12 Source of water:

Result of frequency of EIA in source of water is shown in table (3.2) 166 of equines were drinking tap water, and 18 of them had got water from canals nearby the living farm. It was higher in tap water (90.2%) compared with canal (9.8%).

For distribution and prevalence of EIA in Source of water, the results were shown in table (3.3), 1 was drinking from canal, and 15 of equines were drinking tap water. It was higher in tap water (9%) compared with canal (5.6%).

The results of association between source of water and ELISA EIA seropo-sitivity is depicted in table (3.4), a significant association was not observed between presence of other disease and EIA (P-value =0.619).

3.2.13 .Type of grass:

Result of frequency of EIA in type of grass is shown in table (3.2), 93 of equines were fed with alfalfa and 91 with sorghum varieties, was higher in Alfaalfa (50.5%) compared with Sorghum varieties (49.5%), Khartoum (3.1%) and Bahry (2.7%).

For distribution and prevalence of EIA in type of grass, the results were shown in table (3.3), 12 of equines were fed with Alfalfa, 4 were

fed with Sorghum varieties. It was higher in Alfaalfa (12.9%) compared with Sorghum varieties (4.4%).

The results of association between type of grass and ELISA EIA sero-positivity is depicted in table (3.4), a significant association was observed between grass and EIA (P-value =0.041)

3.2.14. Presence of the vectors:

Result of frequency of EIA in presence of the vector is shown in table (3.2) 157of equines had been exposed to vector, and 27 hadn't been exposed to it. It was higher in Yes (85.3%) compared with No (14.7%).

For distribution and prevalence of EIA in presence of the vector ,the results were shown in table (3.3), 15 of equines had been exposed to the vector, and 1 hadn't . It was higher in Yes (9.6%) compared with No (3.7%).

The results of association between presence of the vector and ELISA EIA serop-ositivity is depicted in table (3.4) , a significant association was observed between presence of the vector and EIA (P-value =0.011)

Table 3.2.**:** Summary of frequencies for the distribution of 184 examined equines according to potential risk factors for EIA

Risk factors	**No . tested**	**% Relative frequency**	**Cumulative frequency**
State of Khartoum	184	100	100
Locality			
Bahry GA Khartoum Omdurman	37 61 65 21	20.1 33.2 35.3 11.4	20.1 53.3 88.6 100
Animal species :-			
Donkeys Horses	112 72	60.9 39.1	60.9 100
Color :-			
Black Red White	62 42 80	33.70 22.8 43.5	33.70 56.50 100
Sex :-			
Male Female	151 33	82.1 17.9	82.1 100
Age :-			
1-14 >14	159 25	86.4 13.6	86.41 100
Animal body condition :-			
Good Moderate Poor	117 37 30	63.6 20.1 16.30	63.59 83.70 100
Animal species :-			
Donkeys Horses	112 72	60.9 39.1	60.9 100
Color :-			
Black Red White	62 42 80	33.70 22.8 43.5	33.70 56.50 100
Sex :-			
Male Female	151 33	82.1 17.9	82.1 100

Table 3.2. continued

Risk factors	No . tested	% Relative frequency	Cumulative frequency
Presence of other disease :-			
Yes	52	28.3	28.26
No	132	71.7	100
Veterinary service :-			
Yes	65	35.3	35.3
No	119	64.7	100
Type of work :-			
Draught	142	84.2	84.2
Night guarding	15	8.2	92.4
Racing	27	7.6	100
Housing :-			
Indoor	51	27.7	**27.7**
Outdoor	133	72.3	100
Presence with other animals :-			
Yes	50	27.2	27.17
No	134	72.8	100
Source of water :-			
Tap	166	90.22	90.22
Canal	18	9.78	100
Type of pasture :-			
Alfalfa	93	50.5	50.5
Sorghum .V	91	49.5	100
Presence of vector :-			
Yes	119	64.7	64.7
No	65	35.3	100

Table 3.3: Summary of cross-tabulation for the prevalence of EIA with potential risk factors in 184 equines in Khartoum state (2013)

Risk factors	**No . tested**	**No . positive**	**% positive**
Locality			
Bahry	37	1	2.7
GA	61	7	11.5
Khartoum	65	2	3.1
Omdurman	21	6	28.6
An. Species			
Donkeys	112	6	5.4
Horses	72	10	13.9
Color			
Black	62	7	11.3
Red	42	5	11.9
White	80	4	5
Sex			
Male	162	15	9.9
Female	22	1	3
Age			
1-14	159	14	8.8
>14	25	2	8
Animal body condition			
Good	117	14	12
Moderate	**37**	**1**	**2.7**
Poor	**30**	**1**	**3.3**
Presence of other disease			
Yes	**52**	**5**	**9.6**
No	**132**	**11**	**8.3**
Veterinary service			
Yes	65	3	4.6
No	119	13	10.9
Type of work			
Draught	142	12	8.45
Night guarding	15	3	20
Racing	27	1	3.7
Housing			
Indoor	51	6	11.7
Outdoor	133	10	7.5

Table 3.3 continued

Risk Factor	No. tested	No. positive	% positive
Presence with other animals			
Yes	50	1	2
No	134	15	11.2
Source of water			
Tap	166	15	9
Canal	18	1	5.6
Type of pasture			
Alfaalfa	93	12	12.9
Sorghum varieties	91	4	4.4
Presence of vector			
Yes	157	15	9.6
No	27	1	3.7

3.6. Summary of Univariate Analysis:-

Results of the Univariate Analysis showed a significant association((P-value ≤ 0.25) with 9 risk factors**,** (locality (P-value = 0.002)**,** animal species (P-value = 0.045)**,** Sex, (P-value = 0.202)**,** animal body condition (P-value = 0.114)<**,** veterinary service(P-value = 0.147)**,** Type of work (P-value = 0.195) ,presence with other animals (P-value = 0.049)**,** type of grass (P-value = 0.041), and Presence of vector (P-value = 0.011)), and no or week relationship with the rest of risk factors (Table 3.4).

Table 3.4: Univariate analysis of potential risk factors with ELISA EIA in 184 equines in Khartoum State using Chi-square (x^2) test

Risk factors	No. tested	No. positive	% positive	Chi - square	DF	P-value
Locality* :-				15.3	3	.002
Bahry GA Khartoum Omdurman	37 54 63 15	1 7 2 6	2.7 11.5 3.1 28.6			
Animal species* :-				4.01	1	. 045
Donkeys Horses	112 72	6 10	5.4 13.9			
Color				2.5	2	.294
Black Red White	62 42 80	7 5 4	11.28 11.9 5			
Sex* :-				1.6	1	.202
Male Female	151 33	15 1	9.9 3			
Age :-				.02	1	.894
1-14 >14	159 25	14 2	8.8 8			
Animal body condition *:-				4.3	2	.114
Good Moderate Poor	117 37 30	14 1 1	12 2.7 3.3			
Presence of other disease :-				.08	1	.781
Yes	52	5	9.6			
No	132	11	8.3			

Risk factors	No. tested	No. positive	% positive	Chi - square	DF	P-value
Veterinary service *:-				2.1	1	.147
Yes No	65 119	3 13	4.6 10.9			
Type of work*:-				3.7	2	.195
Draught Night guarding Racing	142 15 27	12 3 1	8.45 20 3.7			
housing				.837	1	.360
Indoor outdoor	51 133	6 10	11.7 7.5			
Presence of other animals* :-				3.9	1	.049
Yes No	50 134	1 15	2 11.1			
Source of water :-				.25	1	.619
Tap Canal	166 18	15 1	9 5.6			
Type of grass *:-				4.2	1	.041
Alfaalfa	93	12	12.9			
Sorghum .V	91	4	4.4			
Presence of vector* :-				6.5	1	.011
Yes	157	15	9.6			
No	27	1	3.7			

*Indicates significant association with EIA

3.8. Summary of Multivariate Analysis:

A significant association was observed between EIA and potential risk factors (P-value≤0.05) using Logistic Regression as follows : locality (P-value = 0.005), animal species (P-value = 0.050) type of grass (P-value = 0.050) and presence of vector (P-value = 0.033).

Table 3.5: Multivariate analysis of association between EIA sero-positivity and risk factors

Risk factors	No. tested	No. positive	% positive	Exp (B)	P-value	95% CI for Exp(B)	
						Lower	Upper
Locality :-**							
Bahry	37	1	2.7	.069	.018	.008	.627
GA	54	7	11.5	.324	.073	.095	1.110
Khartoum	63	2	3.1	.079	.003	.015	.433
Omdurman	15	6	28.6	Ref*			
Animal species* :-							
Donkeys	112	6	5.4	.351	.o50	.122	1.012
Horses*	72	10	13.9	Ref*			
Color :-							
Black	62	7	11.28	2.418	.175	.675	8.667
Red	42	5	11.9	2.558	.178	.651	10.127
White	80	4	5	Ref*			
Sex :-							
Male	151	15	9.9	Ref*			
Female	33	1	3	.467	.472	.059	3.718
Age :-							
1-14	159	14	8.8	.901	.891	.192	4.224
>14	25	2	8	Ref*			
Animal body condition :-							
Good	117	14	12	3.942	.194	.497	31.243
Moderate	37	1	2.7	.806	.880	.048	13.442
Poor	30	1	3.3	Ref*			

Presence of other disease :-							
Yes	52	5	9.6	Ref*			
No	132	11	8.3	.855	.781	.282	2.592
Veterinary service :-							
Yes	65	3	4.6	Ref*			
No	119	13	10.9	2.535	.159	.695	9.244
Type of work :-							
Draught	142	12	8.45	.417	.410	.052	3.345
Night guarding	15	3	20	.154	.121	.014	1.636
Racing	27	1	3.7	Ref*			
Housing :-							
Indoor	51	6	11.7	1.640	.364	.564	4.772
Outdoor	133	10	7.5	Ref*			
Presence with other animals :-							
Yes	50	1	2	6.176	.082	.794	48.045
No	134	15	11.1	Ref*			
Source of water :-							
Tap	166	15	9	Ref*			
Canal	18	1	5.6	1.689	.622	.210	13.591
Type of grass **:-							
Alfaalfa	93	12	12.9	.310	.050	.096	1.001
Sorghum .V	91	4	4.4	Ref*			
Presence of vector :-**							
Yes	119	15	12.6	Ref*			
No	65	1	4.6	.033	9.234	1.191	71.564

*The reference category is: disease positive.

**Indicates significant association with EIA.

Chapter Four

Discussion

Equine Infectious Anaemia is an important disease of equines which is incurable and has no vaccine. The disease affects all members of equids (horses, donkeys, mules, ponies, and zebra). It threats the equines population, since it is fatal in small aged animals and animals with acute form of the disease. EIA has a global geographical distribution existence. The disease is still not reported in Sudan, thus its epidemiology is far from being known. Therefore, this study was conducted to estimate the seo-prevalence rate of EIA in equines population by ELISA and to investigate the potential risk factors associated with the occurrence of EIA in Khartoum state in the Sudan.

Many serological tests are routinely used for detection of anti EIAV antibody. Both Agar gel immunodiffusion (AGID) tests (Coggins et al., 1972) and enzyme-linked immunosorbent assays (ELISAs) (Suzuki et al., 1982) are accurate, reliable tests for the detection of EIA. Molecular technique had been also used recently.

In this study the overall sero-prevallence of anti EIAV antibodies in both horses and donkeys serum collected from the four localities

In Khartoum state the prevalence was found to be 8.7% which is considered a first estimation.

This result showed that the disease is present or that it might be present with a more prevalence rate but could not be detected because of the low sample number or because the immune response of examined animals or that the carrier animals were with lower titres of viruses.

In this study the following risk factors show significant association with EIA under a significant level of (P-value $\leq$ 0.25), (locality (P-value = 0.002)**,** animal species (P-value = 0.045)**,** animal body condition (P-value = 0.114)**,** sex (P-value = 0.202) ,type of work (P-value = 0.195) ,veterinary service (P-value = 0.147)**,** presence with

other animals (P-value = 0.049), type of grass (P-value = 0.041)) and presence of vector(P-value = 0.011).

Regarding the locality risk factor, the sero- prevalence of EIA in equines serum samples collected from the four surveyed localities of Khartoum state of Sudan was higher in Omdurman (28.6%) followed by Gabalawlia (11.5%), followed by Khartoum (3.1%) and then Bahry (2.7%) .The variation of investigated areas could be a point of difference, considering the fact that each area has its specific and unique indigenous components and risk factors in addition to its equines population size. The overall prevalence (8.7%) showed that there was a significant association with locality (P-value = 0.002).

Concerning animal species, it was significantly associated with EIA prevalence (P-value =0.045). Infection was higher in horses (13.9%) compared with donkeys (5.4%). This is in agreement with Kirmizigul, et al., (2009) that the EIA virus specific antibodies in donkeys and horses were identical but horse-adapted-strains of EIAV were unable to produce clinical disease in the donkeys tested. Therefore, clinical manifestations of EIA were absent in donkeys because the concentration of virus remained below this critical plasma level than in horses, these also confirmed by Yapkic, et al., (2007)

About the color risk factor, our idea was based on whether or not colors have affect on vectors attraction, since donkeys are white in Donglawi breed and, black and red in Makadi, the results of color association with EIA in (white and black) was concerned to be as the same as the breed . The result showed that infection was higher in red (11.9%) followed by black (11.3%) and then white (5%) , that means dark colored equines as well as Makadi breed of donkeys are more susceptible to the disease than white ones as Donglawi breed .

The results showed that there was a significant association between sex (P-value =0.202) and sero-prevalence rate of anti EIAV antibodies , it was higher in males (9.9%) compared with females (3%) which is supported by Silva , (Silva et al., 1997, unpubl. data),

and this could probably be due to different management , and that males went out to work .

The results showed that there was no significant association between Age (P-value =0.894) and sero-prevalence rate of anti EIAV anti bodies, in (1-14 years) it was 8.8%, in (>14 years) it was 8, this results suggested that animals in all ages are susceptible .

Body condition was based on visual examination (skin, mucous membrane, posture and gait) and palpation of lymph nodes. In this study the results showed that sero-prevalence rate was significantly related to EIA (P-value = 0.080), it was higher in good (12%) followed by poor (3.3 %) and then moderate (2.7%). This is in disagreement with OIE that the morbidity rate and severity of the clinical signs are influenced by the strain and dose of the virus, and the health of the animal (OIE, 2009). This variation among body condition categories could be due to the species susceptibility, good service, good management, good nutrition and good health care in equines farms and to the early detection of the virus by the ELISA (Donovan, 1999), in which the disease didn't take the chronic form and poor body condition**.**

Concerning presence of other disease as a risk factor, there was insignificant association (P-value =0.781) with sero-prevalence rate of anti EIAV anti bodies although, theoretically, the disease is influenced by the health status of the host. However, in this study the obtained data showed that, that diseases were injuries, eye's parasites, and in a few cases lameness and suspecting of internal parasites which are relatively, not concerned as an immunosuppressive disease. Another site of view, some of those diseased, participated animals showed negative results for EIAV ELISA.

The results showed that there was a significant association between Veterinary service (P-value =0.147) and sero-prevalence rate of anti EIAV antibodies. Our study confirmed that healthy and well-raised animals are less susceptible to disease (OIE, 2009).

About the risk factor type of work , the study reported a significant association with EIA sero-prevalence, the sero-prevalence rate was higher in night guarding (20%) followed by draught (8.5%) and then racing (3.7%).The night guarding animals were the most affected ones, this could be due to other included risk factors such as age which results in cumulative exposure, locality (Gebalawlia, 11.5% sero-prevalence rate), and housing (with outdoor exposure to vectors). Horses in racing category were the least affected ones and this might be due to the good management, nutrition and good veterinary service.

Regarding housing, there was no significant association with the disease sero-positivity (P-value =.360), sero-prevalence rate in indoor and outdoor was 11.7% and 7.5% respectively. This could be expressed by the small numbers of animals housed indoors and exposing to a quite large numbers of vectors as well as exposing to the other vectors when went on work, however, the owners explained that their animals were at continuous mode of changing (for commercial reasons) which put the two categories (indoor and outdoor) at the same rate of exposure.

Regarding the risk factor, presence of other animals, there was a significant relationship with EIA sero-prevalence (P-value =0.041), it is probably that, these animals took the vector's bites instead of the tested animals especially when they went work, and this minimized the exposure to vectors, however, The fly attempts to resume feeding immediately, either on the same animal or on another nearby host, resulting in the transfer of infectious blood. Additionally EIAV survives for a limited time on the mouthparts of insects, and it is less likely to spread to more distant hosts (OIE, 2009).

Concerning source of water, there was no significant association with EIA sero-prevalence (P-value =0.619). In our study we supposed that canal water may cause food or drink pollution resulting in immunosuppressive disease which may act as a predisposing factors for EIA, but the result showed that the sero-prevalence was higher in equines drinking tap-water (9%) than canal-water (5.6%). It could be

probably due to treated tap-water, and the low contents of minerals compared with canal-water. However, the tested equines which lived in farms and drank from canal, shared vector's bites with other animals.

The results showed that there was a significant association between type of grass (P-value =0.041) and sero-prevalence rate of anti EIAV antibodies. The sero-positivity rate in Alfalfa and Sorghum varieties was (12.9%) and (4.4%) respectively. It might be due to the prolonged period of Alfalfa growing life (about 3 years) helping vectors presence with a suitable ecology that it grows in farms nearby rivers or irrigating canals compared with Sorghum varieties growing life (70 days) mostly irrigated from wells., additionally, animals fed with Alfalfa mostly were from Omdurman locality which showed a significant association with EIA sero-positivity.

Finally, the presence of vectors plays a high putative role in transmitting EIA (P-value=0.011), this is obviously shown in the results: 9.6% sero-prevalence rate when vectors were present, and 3.7% when the vectors were absent.

These risk factors which had significant effect in univariate analysis were fitted in a multivariate logistic regression model under a significant level ≤ 0.05. Due to confounding, other risk factors were thrown out from the model, which leave the strong associated risk factors as, localities (p-value=0.003), animal species (p- value=0.05), type of grass (0.05) and presence of vectors (p- value=0.03).

Conclusion

Results of the present study had advanced our knowledge on the epidemiology of EIA in equines in Khartoum state of the Sudan, they showed that EIA is present with sero-prevalence rate estimated at 8.7%.

This is the first study which investigates the potential risk factors contributing to the occurrence and spread of EIA among equines populations.

Locality, animal species, type of grass and presence of vectors influenced the prevalence of EIA.

Recommendations:-

Based on the above conclusion, the following points are forwarded as recommendations:

- There is a need for rapid diagnosis of the disease based on molecular detection and molecular characterization of EIAV circulating in the field.
- Control strategy against this economically important reemerging equine pathogen should be based on the routine sero- monitoring of the disease.
- Improvement of management systems and tight movement and vectors control will reduce the prevalence of EIA.
- Extension service and training programs aiming at creation of awareness about the importance and prevention of subclinical EIA among private, military, hobby and commercial purposed owning members.
- Humane destruction of old and chronically affected equines, physical isolation of new animals should be considered in attempts to reduce prevalence of EIA.
- Some epidemiological risk factors that enhance the spread and transmission of EIA need confirmation and further studies.

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Appendix 1

Frequency Tables:

Appendix 1.1: Results of frequency of EIA in localities:

Locality

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Bahry	37	20.1	20.1	20.1
	GA	61	33.2	33.2	53.3
	Khartoum	65	35.3	35.3	88.6
	Omdurman	21	11.4	11.4	100.0
	Total	184	100.0	100.0	

Appendix 1.2: Results of frequency of EIA in animal species:

animal species

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	donkey	112	60.9	60.9	60.9
	horse	72	39.1	39.1	100.0
	Total	184	100.0	100.0	

Appendix 1.3: Results of frequency of EIA in color :

Color

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	black	62	33.7	33.7	33.7
	Red	42	22.8	22.8	56.5
	White	80	43.5	43.5	100.0
	Total	184	100.0	100.0	

Appendix 1.4: Results of frequency of EIA in sex :

Sex

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	female	33	17.9	17.9	17.9
	Male	151	82.1	82.1	100.0
	Total	184	100.0	100.0	

Appendix 1.5: Results of frequency of EIA in age :

Age (years)

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	14-26	25	13.6	13.6	13.6
	1-13	159	86.4	86.4	100.0
	Total	184	100.0	100.0	

Appendix 1.6: Results of frequency of EIA in animal body condition :

animal body condition

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Good	117	63.6	63.6	63.6
	moderate	37	20.1	20.1	83.7
	Poor	30	16.3	16.3	100.0
	Total	184	100.0	100.0	

Appendix 1.7: Results of frequency of EIA in presence of other disease :

presence of other disease

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	132	71.7	71.7	71.7
	Yes	52	28.3	28.3	100.0
	Total	184	100.0	100.0	

Appendix 1.8 : Results of frequency of EIA in veterinary service:

veterinary service

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	119	64.7	64.7	64.7
	Yes	65	35.3	35.3	100.0
	Total	184	100.0	100.0	

Appendix 1.9 : Results of frequency of EIA in type of work :

type of work

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	draught	142	77.2	77.2	77.2
	Night guarding	15	8.2	8.2	85.3
	racing	27	14.7	14.7	100.0
	Total	184	100.0	100.0	

Appendix 1.10 : Results of frequency of EIA in housing :

Housing

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	indoor	51	27.7	27.7	27.7
	outdoor	133	72.3	72.3	100.0
	Total	184	100.0	100.0	

Appendix 1.11 : Results of frequency of EIA in presence with other animals :

presence with other animal

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	134	72.8	72.8	72.8
	Yes	50	27.2	27.2	100.0
	Total	184	100.0	100.0	

Appendix 1.12: Results of frequency of EIA in source of water :

source of water

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	canal	18	9.8	9.8	9.8
	Tap	166	90.2	90.2	100.0
	Total	184	100.0	100.0	

Appendix 1.13: Results of frequency of EIA in type of grass :

type of grass

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Alfaalfa	93	50.5	50.5	50.5
	Sorghum varieties	91	49.5	49.5	100.0
	Total	184	100.0	100.0	

Appendix 1.14: Results of frequency of EIA in presence of vector:

presence of the vector

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	65	35.3	35.3	35.3
	Yes	119	64.7	64.7	100.0
	Total	184	100.0	100.0	

Appendix 2

Cross-tabulation Tables:

Appendix 2.1: Results of distribution and prevalence of EIA in locality :

disease * locality Cross-tabulation

			locality				Total
			Bahry	GA	Khartoum	Omdurman	
disease	disease negative	Count	36	54	63	15	168
		% within disease	21.4%	32.1%	37.5%	8.9%	100.0%
		% within locality	97.3%	88.5%	96.9%	71.4%	91.3%
		% of Total	19.6%	29.3%	34.2%	8.2%	91.3%
	disease positive	Count	1	7	2	6	16
		% within disease	6.2%	43.8%	12.5%	37.5%	100.0%
		% within locality	2.7%	11.5%	3.1%	28.6%	8.7%
		% of Total	0.5%	3.8%	1.1%	3.3%	8.7%
Total		Count	37	61	65	21	184
		% within disease	20.1%	33.2%	35.3%	11.4%	100.0%
		% within locality	100.0%	100.0%	100.0%	100.0%	100.0%
		% of Total	20.1%	33.2%	35.3%	11.4%	100.0%

Appendix 2.2: Results of distribution and prevalence of EIA in animals species :

disease * animal species Cross-tabulation

			animal species		Total
			donkey	horse	
disease	disease negative	Count	106	62	168
		% within disease	63.1%	36.9%	100.0 %
		% within animal species	94.6%	86.1%	91.3%
		% of Total	57.6%	33.7%	91.3%
	disease positive	Count	6	10	16
		% within disease	37.5%	62.5%	100.0 %
		% within animal species	5.4%	13.9%	8.7%
		% of Total	3.3%	5.4%	8.7%
Total		Count	112	72	184
		% within disease	60.9%	39.1%	100.0 %
		% within animal species	100.0%	100.0 %	100.0 %
		% of Total	60.9%	39.1%	100.0 %

Appendix 2.3: Results of distribution and prevalence of EIA in sex :

disease * sex Cross-tabulation

			sex		Total
			female	male	
disease	disease negative	Count	32	136	168
		% within disease	19.0%	81.0%	100.0 %
		% within sex	97.0%	90.1%	91.3%
	disease posative	Count	1	15	16
		% within disease	6.2%	93.8%	100.0 %
		% within sex	3.0%	9.9%	8.7%
Total		Count	33	151	184
		% within disease	17.9%	82.1%	100.0 %
		% within sex	100.0 %	100.0%	100.0 %

Appendix 2.4: Results of distribution and prevalence of EIA in color : disease * color Cross-tabulation

			Color			Total
			Black	Red	white	
disease	disease negative	Count	55	37	76	168
		% within disease	32.7%	22.0%	45.2%	100.0 %
		% within color	88.7%	88.1%	95.0%	91.3%
		% of Total	29.9%	20.1%	41.3%	91.3%
	disease positive	Count	7	5	4	16
		% within disease	43.8%	31.2%	25.0%	100.0 %
		% within color	11.3%	11.9%	5.0%	8.7%
		% of Total	3.8%	2.7%	2.2%	8.7%
Total		Count	62	42	80	184
		% within disease	33.7%	22.8%	43.5%	100.0 %
		% within color	100.0 %	100.0 %	100.0 %	100.0 %
		% of Total	33.7%	22.8%	43.5%	100.0 %

Appendix 2.5: Results of distribution and prevalence of EIA in age: disease * age Cross-tabulation

			Age (years)		Total
			14-26	1-13	
disease	disease negative	Count	23	145	168
		% within disease	13.7%	86.3%	100.0 %
		% within age	92.0%	91.2%	91.3%
		% of Total	12.5%	78.8%	91.3%
	disease positive	Count	2	14	16
		% within disease	12.5%	87.5%	100.0 %
		% within age	8.0%	8.8%	8.7%
		% of Total	1.1%	7.6%	8.7%
Total		Count	25	159	184
		% within disease	13.6%	86.4%	100.0 %
		% within age	100.0 %	100.0 %	100.0 %
		% of Total	13.6%	86.4%	100.0 %

Appendix 2.6: Results of distribution and prevalence of EIA in animal body condition :

disease * animal body condition Cross-tabulation

			animal body condition			Total
			good	moderate	poor	
disease	disease negative	Count	103	36	29	168
		% within disease	61.3%	21.4%	17.3%	100.0 %
		% within animal body condition	88.0%	97.3%	96.7%	91.3%
		% of Total	56.0%	19.6%	15.8%	91.3%
	disease positive	Count	14	1	1	16
		% within disease	87.5%	6.2%	6.2%	100.0 %
		% within animal body condition	12.0%	2.7%	3.3%	8.7%
		% of Total	7.6%	0.5%	0.5%	8.7%
Total		Count	117	37	30	184
		% within disease	63.6%	20.1%	16.3%	100.0 %
		% within animal body condition	100.0%	100.0%	100.0 %	100.0 %
		% of Total	63.6%	20.1%	16.3%	100.0 %

Appendix 2.7: Results of distribution and prevalence of EIA in presence of other disease

disease * presence of other disease Cross-tabulation.

			presence of other disease		Total
			No	yes	
disease	disease negative	Count	121	47	168
		% within disease	72.0%	28.0%	100.0%
		% within present of other disease	91.7%	90.4%	91.3%
		% of Total	65.8%	25.5%	91.3%
	disease posative	Count	11	5	16
		% within disease	68.8%	31.2%	100.0%
		% within present of other disease	8.3%	9.6%	8.7%
		% of Total	6.0%	2.7%	8.7%
Total		Count	132	52	184
		% within disease	71.7%	28.3%	100.0%
		% within present of other disease	100.0%	100.0%	100.0%
		% of Total	71.7%	28.3%	100.0%

Appendix 2.8: Results of distribution and prevalence of EIA in vet service

disease * vet service Cross-tabulation

			vet service		Total
			no	Yes	
disease	disease negative	Count	106	62	168
		% within disease	63.1%	36.9%	100.0%
		% within vet service	89.1%	95.4%	91.3%
		% of Total	57.6%	33.7%	91.3%
	disease positive	Count	13	3	16
		% within disease	81.2%	18.8%	100.0%
		% within vet service	10.9%	4.6%	8.7%
		% of Total	7.1%	1.6%	8.7%
Total		Count	119	65	184
		% within disease	64.7%	35.3%	100.0%
		% within vet service	100.0%	100.0%	100.0%
		% of Total	64.7%	35.3%	100.0%

Appendix 2.9: Results of distribution and prevalence of EIA in housing : disease * housing Cross- tabulation

			Housing		Total
			indoor	outdoor	
disease	disease negative	Count	45	123	168
		% within disease	26.8%	73.2%	100.0%
		% within housing	88.2%	92.5%	91.3%
		% of Total	24.5%	66.8%	91.3%
	disease positive	Count	6	10	16
		% within disease	37.5%	62.5%	100.0%
		% within housing	11.8%	7.5%	8.7%
		% of Total	3.3%	5.4%	8.7%
Total		Count	51	133	184
		% within disease	27.7%	72.3%	100.0%
		% within housing	100.0%	100.0%	100.0%
		% of Total	27.7%	72.3%	100.0%

Appendix 2.10: Results of distribution and prevalence of EIA in type of work :

disease * type of work Cross tabulation

			typ of work			Total
			draught	Night guarding	racing	
disease	disease negative	Count	130	12	26	168
		% within disease	77.4%	7.1%	15.5%	100.0 %
		% within type of work	91.5%	80.0%	96.3%	91.3%
	disease positive	Count	12	3	1	16
		% within disease	75.0%	18.8%	6.2%	100.0 %
		% within type of work	8.5%	20.0%	3.7%	8.7%
Total		Count	142	15	27	184
		% within disease	77.2%	8.2%	14.7%	100.0 %
		% within type of work	100.0 %	100.0%	100.0%	100.0 %

Appendix 2.11: Results of distribution and prevalence of EIA in presence with other animals :

disease * presence with other animals Cross tabulation

			Presence with other animals		Total
			No	yes	
disease	disease negative	Count	119	49	168
		% within disease	70.8%	29.2%	100.0%
		% within present with other animal	88.8%	98.0%	91.3%
		% of Total	64.7%	26.6%	91.3%
	disease positive	Count	15	1	16
		% within disease	93.8%	6.2%	100.0%
		% within present with other animal	11.2%	2.0%	8.7%
		% of Total	8.2%	0.5%	8.7%
Total		Count	134	50	184
		% within disease	72.8%	27.2%	100.0%
		% within present with other animal	100.0%	100.0%	100.0%
		% of Total	72.8%	27.2%	100.0%

Appendix 2.12: Results of distribution and prevalence of EIA in source of water: disease * source of water Cross- tabulation

			source of water		Total
			Canal	Tap	
disease	disease negative	Count	17	151	168
		% within disease	10.1%	89.9%	100.0%
		% within source of water	94.4%	91.0%	91.3%
		% of Total	9.2%	82.1%	91.3%
	disease positive	Count	1	15	16
		% within disease	6.2%	93.8%	100.0%
		% within source of water	5.6%	9.0%	8.7%
		% of Total	0.5%	8.2%	8.7%
Total		Count	18	166	184
		% within disease	9.8%	90.2%	100.0%
		% within source of water	100.0%	100.0%	100.0%
		% of Total	9.8%	90.2%	100.0%

Appendix 2.13: Results of distribution and prevalence of EIA in type of grass

disease * type of grass Cross -tabulation

			type of pasture		Total
			Alfalfa	Sorghum varieties	
Disease	disease negative	Count	81	87	168
		% within disease	48.2%	51.8%	100.0%
		% within type of pasture	87.1%	95.6%	91.3%
		% of Total	44.0%	47.3%	91.3%
	disease positive	Count	12	4	16
		% within disease	75.0%	25.0%	100.0%
		% within type of pasture	12.9%	4.4%	8.7%
		% of Total	6.5%	2.2%	8.7%
Total		Count	93	91	184
		% within disease	50.5%	49.5%	100.0%
		% within type of pasture	100.0%	100.0%	100.0%
		% of Total	50.5%	49.5%	100.0%

Appendix 2.14: Results of distribution and prevalence of EIA in presence of the vector :

disease * presence of the vector Cross- tabulation

			Presence of the vector		Total
			No	Yes	
Disease	disease negative	Count	64	104	168
		% within disease	38.1%	61.9%	100.0%
		% within present of the vector	98.5%	87.4%	91.3%
	disease positive	Count	1	15	16
		% within disease	6.2%	93.8%	100.0%
		% within present of the vector	1.5%	12.6%	8.7%
Total		Count	65	119	184
		% within disease	35.3%	64.7%	100.0%
		% within present of the vector	100.0%	100.0%	100.0%

Appendix 3

Univariate analysis of potential risk factors with EIA in 184 equines in Khartoum State using the Chi-square (x^2) test :

Appendix 3.1: disease * locality

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	15.301[a]	3	.002
Likelihood Ratio	13.063	3	.005
No. of Valid Cases	184		

Appendix 3.2: disease * animal species

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.018[a]	1	.045		
Continuity Correction[b]	3.015	1	.082		
Likelihood Ratio	3.905	1	.048		
Fisher's Exact Test				.060	.043
No. of Valid Cases	184				

Appendix 3.3: disease * color

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.447[a]	2	.294
Likelihood Ratio	2.582	2	.275
No. of Valid Cases	184		

Appendix 3.4: disease * sex

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.626[a]	1	.202	.312	.177
Continuity Correction[b]	.872	1	.350		
Likelihood Ratio	2.024	1	.155	.221	.177
Fisher's Exact Test				.312	.177
No. of Valid Cases	184				

Appendix 3.5: disease * age

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.018[a]	1	.894		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.018	1	.893		
Fisher's Exact Test				1.000	.626
No. of Valid Cases	184				

Appendix 3.6: disease * animal body condition

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.336[a]	2	.114
Likelihood Ratio	5.057	2	.080
No. of Valid Cases	184		

Appendix 3.7: disease * presence of other disease

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.077[a]	1	.781		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.076	1	.783		
Fisher's Exact Test				.776	.491
No. of Valid Cases	184				

Appendix 3.8: disease * vet service

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.108[a]	1	.147		
Continuity Correction[b]	1.388	1	.239		
Likelihood Ratio	2.314	1	.128		
Fisher's Exact Test				.179	.117
No. of Valid Cases	184				

Appendix 3.9: disease * type of work

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.272[a]	2	.195
Likelihood Ratio	2.897	2	.235
No. of Valid Cases	184		

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Appendix 3.10: disease * housing

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.837[a]	1	.360		
Continuity Correction[b]	.388	1	.534		
Likelihood Ratio	.792	1	.373		
Fisher's Exact Test				.386	.260
No. of Valid Cases	184				

Appendix 3.11: disease * presence with other animal

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	3.877[a]	1	.049		
Continuity Correction[b]	2.805	1	.094		
Likelihood Ratio	4.970	1	.026		
Fisher's Exact Test				.074	.037
No. of Valid Cases	184				

Appendix 3.12: disease * source of water

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.248[a]	1	.619		
Continuity Correction[b]	.003	1	.954		
Likelihood Ratio	.278	1	.598		
Fisher's Exact Test				1.000	.519
No. of Valid Cases	184				

Appendix 3.13: disease * type of grass

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.193[a]	1	.041		
Continuity Correction[b]	3.190	1	.074		
Likelihood Ratio	4.379	1	.036		
Fisher's Exact Test				.064	.036
No. of Valid Cases	184				

Appendix 3.14: disease * presence of the vector

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	6.484[a]	1	.011	.012	.007
Continuity Correction[b]	5.166	1	.023		
Likelihood Ratio	8.232	1	.004	.008	.007
Fisher's Exact Test				.012	.007
No. of Valid Cases	184				

Appendix 4

Questionnaire

بسم الله الرحمن الرحيم

Questionnaire for data collection for the survey of

Equine Infectious Anaemia in Khartoum state

Locality……administrative unit ……. farm no ……..

Date of investigation ……investigator……

General characteristics

Owner:

Name……
address…….

Age…… telephone No ……

Education level ……..

Suspected risk factor

Animal Species:……

Horse (1)( )…….Donkey(0)( )……

Breed:……

Donglawi(0)( )……Makadi (1)( )……

Color:……

White(0)()…….Red(1)()…….Black(2)()……..

Sex:……

Male(1)():……

Female(0)()…….pregnant(1)()……not pregnant(0)()……....

Age:……

(1-14)(0)()……..(>14)(1)()……

General body condition:……

Good(0)…….Moderate(1)……Poor(

Present of other disease ……

Yes(1)()…….No(0)()……

Veterinary service:……

Yes(0)()……..No(1)()……

Type of work :……

Draught()()……Night guarding()()…….Racing()()……

Housing:……

Indoor()()……Outdoor()()……

Present with other animals:…….

Yes(0)()…… No(1)()……

Source of water :……..

Tap(0)()……Canal(1)()

Type of grass:……

Alfaalfa(0)()……..Sorgum varieties(1)()……

Presence of the vectors:……

Yes(1)()……NO(0)()……
……