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Retroviral *Lentivirus*: infectivity, transmission and diagnostic of the Equine Infectious Anemia and the challenges of retrovirology for the new millennium

Paulo Reis de Carvalho[1], Helvécio Mosetto[2], Eliana Monteforte Cassaro Villalobos[1], Luiz Florencio Franco Margatho[1]

[1]Veterinary Medical, Scientific Researcher, Laboratory of Animal Pathology APTA-Regional- Secretary of Agriculture and Supply (SAA/SP), Brazil.
[2]Veterinary Medical, Coordination of Livestock Defense-SAA/SP.

Abstract

Since the first citation in equids in 1843 in France until today, the *Lentivirus* (family *Retroviridae*), single-stranded RNA, continues to challenge the foundations of scientific knowledge in virology how prevention strategies and vaccine immunization. The AIDS of the horse, so-called due to its kinship with the *Lentivirus* in the same group of the Acquired Human Immunodeficiency Syndrome (HIV or AIDS human) once in contact with the cell, imposed its genetic mechanism and from the RNA if the replica expense of the host cell of the reticuloendothelial system through the synthesis of proteins for new virions. Opposite this scenario, in veterinary medicine the practice adopted in relation to serum-positive for Equine Infectious Anemia is the sacrifice. A total of 40.500 equine serums were processed in Laboratory Animal Pathology APTA-Bauru, originating from 225 municipalities and 21.608 different

establishments. This research was conducted in order to diagnostic, the sacrifice of positive animals and elimination of focus, studying if through of the proof of Coggins modified by Nakajima, being detecting 0.19% of total prevalence and 0.13% for males versus 0.21% for females over the period 1984 to 2011. The main changes were in relation to the immune and hematological system, seen that in the genesis of anemia there is intense erythrophagocytosis in diseased animals. The main anatomo-pathological findings were reticuloendotheliosis generalized in the organs. Beyond vertical transmission, horizontal through tools (objects perforating or cutting), vectors of blood-sucking Diptera, *Tabanus* sp and *Stomoxys calcitrans*, were mainly responsible for mechanical transmission and is very often found in the creations of horses, with intense proliferation in the warmer months and higher rainfall (September to March) of the year. How much the prevention, in this study reviewing the literature, there is mention of live attenuated vaccines against *Lentivirus*, which cited a vaccine of Chinese origin, used in outbreaks of disease in the China. However, no official recognition, as well as several authors has attested the absence of proof of your efficacy for use in other countries and confounding in serological diagnosis, precluding the distinction between animals healthy and diseased from the use of this.

Keywords: retrovirus, equids, *Tabanidae*, horsefly, mechanical transmission, IDGA

Retroviral *Lentivírus*: infectividade, transmissão, diagnóstico da Anemia Infecciosa Equina e os desafios da retrovirologia para o novo milênio

Resumo

Desde a primeira citação em eqüídeo em 1843 na França até os dias atuais, o *Lentivírus* (família *Retroviridae*), RNA fita simples continua desafiando os alicerces do conhecimento científico em virologia quanto às estratégias de prevenção ou imunização vacinal. A AIDS do cavalo, denominação esta em

face de seu parentesco com o *Lentivirus* do mesmo grupo da Imunodeficiência Adquirida Humana (HIV ou AIDS humana) uma vez em contato com a célula assume seu comando genético e a partir do RNA replica-se às custas da célula hospedeira do sistema reticulo endotelial através da síntese de proteína para novos virions. Frente este cenário, em medicina veterinária a prática adotada em relação aos equídeos sororeagentes para Anemia Infecciosa Equina é o sacrifício. Um total de 40.500 soros de equídeos foram processados no Laboratório de Patologia Animal da APTA-Bauru, originados de 225 municípios e 21.608 diferentes estabelecimentos. Nesta pesquisa, foi realizado o diagnóstico visando o sacrifício de animais positivos e eliminação de foco, estudando-se através da prova de Coggins modificada por Nakajima, detectando-se 0.19% de prevalência total e 0.13% para machos versus 0.21% para fêmeas no período abrangido de 1984 a 2011. As principais alterações observadas foram em relação aos sistemas imune e hematológico, visto que na gênese da anemia, há intensa eritrofagocitose em animais doentes. Os principais achados anatomopatológicos foram reticuloendoteliose e icterícia generalizada dos órgãos. Além da transmissão vertical, a horizontal através de utensílios (objetos perfurantes ou cortantes), os vetores Diptera sugadores de sangue *Tabanus* sp e *Stomoxys calcitrans*, foram os principais responsáveis pela transmissão mecânica, sendo freqüentemente encontrados nas criações de eqüídeos com intensa proliferação nos meses mais quentes e de maior precipitação pluviométrica (Setembro a Março) do ano. Quanto à prevenção, neste estudo, revisando a literatura há menção de vacinas vivas atenuadas contra *Lentivírus*, sendo citada uma vacina de procedência chinesa, usada em focos da doença na China. Entretanto, não tem reconhecimento oficial, bem como vários autores atestaram a inexistência de comprovação da sua eficácia para uso em outros países e confundimento no diagnóstico sorológico, impossibilitando a distinção entre animais sadios e doentes a partir do uso desta.

Palavras-chave: retrovírus, equídeos, *Tabanidae*, mosca do cavalo, transmissão mecânica, IDGA

INTRODUCTION

Among the many challenges in the practice of veterinary medicine such as the virus that infects horses of *Retroviridae* family and gender *Lentivirus* challenge the cell susceptibles and after replicate using the host cell genome can not be recognized and destroyed by the immune system of the infected animal. Animals company susceptibles retroviral infection has shown some weakness immune when in contact with the retrovirus which makes the disease be coated character priority among domestic animals and man.

According to Boulanger et al. (1972), the infectious nature of equine infectious anemia (EIA) was demonstrated in France in 1859 by Anginiard and cited in the monograph in 1968 by Goret et al. apud Boulanger et al. However, transmission of the virus through the filtered cells was demonstrated experimentally by Carré and Vallée in 1904 apud Boulanger et al. in the France and in the Brazil by Dupont (1967) in anatomo-pathological findings, although in São Paulo, Manente in 1952 apud Carvalho Jr. (1998) already had observed necropsy findings compatible with the incidence of this infirmity in equids. Wherefore, this *Lentivirus* constitutes a challenge to two centuries for humanity, that to example of the other viruses of the group *Retroviridae* which continue exterminating humans (AIDs) and animals (EIA) (LEROUX et al., 2004; WEISS, 2006).

All races and age groups of horses are susceptible. Blood and Henderson (1978) mentioned that the disease has been the occurrence in horses, however, has been described experimental transmission to pigs and sheep, besides a case has been described in humans, with the main manifestation to anemia.

The authors mention also the reproduction by inoculations in mice and chicken eggs. Regarding the transmission, the same authors also mentioned that the virus is present in all tissues, secretions and excretions and may persist for 18 years in the animal organism. In these cases, inapparent carriers (healthy carrier or chronic) are often responsible for introducing the disease into areas nonexistent for this viruses.

The considerations of Weiss (2006), in, study of, phylogeny of retroviruses, presented brilliant reasoning on Mendelian inheritance of retroviral genomes by their hosts (discovered in the late 1960s) and Darwin and the genetic inheritance, might be surprised to learn that humans are descended from viruses as well as from apes. Some 8% of human DNA represents fossil retroviral genomes, and that is not counting the line elements and other retrotransposons that are scattered so liberally across our genome (Figure 1). Three types of endogenous retroviruses were found around the same time: avian leukosis virus in the domestic fowl, and murine leukemia virus and murine mammary tumor virus in the laboratory mouse.

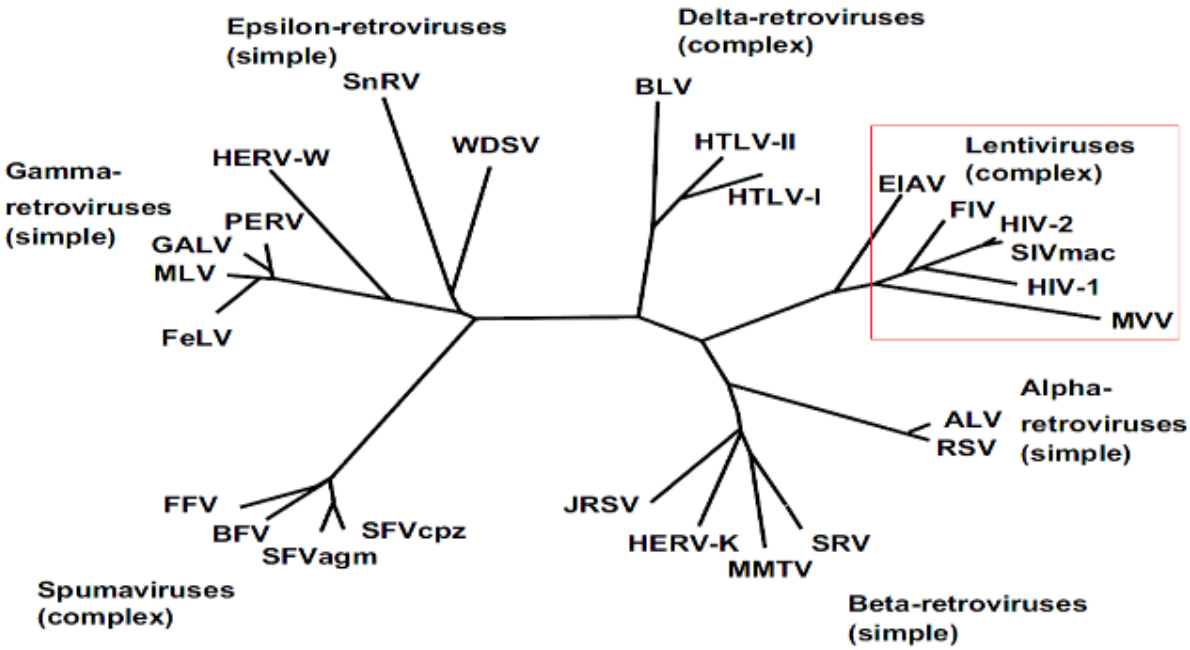

Figure 1- Phylogeny of retroviruses

Source: WEISS, R.A. (2006).

Equine infectious anemia virus (EIAV) is a *Lentivirus* of the *Retroviridae* family that infects and persists in the monocyte/macrophage populations of blood and tissues. During febrile periods, the virus replicates predominantly in

mature tissue macrophages of the spleen, liver, lymph nodes, lung, kidney and adrenal gland (SELLON et al. 1992).

The *Retroviridae* family have genomes composed of RNA, single stranded, positive sense, and that replicate the viral RNA through a process called reverse transcription, where double-stranded DNA molecules (dsDNA) are generated, starting from RNA by the action enzyme reverse transcriptase.

The DNA is then incorporated into the host's genome by an integrase enzyme. The virus thereafter replicates as part of the host cell's DNA. Retroviruses are enveloped viruses that belong to the viral family *Retroviridae* (BALTIMORE, 1971). A special variant of retroviruses are endogenous retroviruses, which are integrated into the genome of the host and inherited across generations. The virus itself stores its nucleic acid in the form of an +mRNA genome and serves as a means of delivery of that genome into cells it targets as an obligate parasite, and constitutes the infection. Once in the host's cell, the RNA strands undergo reverse transcription in the cytoplasm and are integrated into the host's genome, at which point the retroviral DNA is referred to as a provirus. It is difficult to detect the virus until it has infected the host. In most viruses, DNA is transcribed into RNA, and then RNA is translated into protein. However, retroviruses function differently their RNA is reverse-transcribed into DNA, which is integrated into the host cell's genome, when it becomes a provirus, and then undergoes the usual transcription and translational processes to express the genes carried by the virus. Therefore, the order of steps from a retroviral gene to a retroviral protein is: RNA → DNA → RNA → protein (Figure 2).

Figure 2- Mechanism of action of RNA reverse transcriptase to DNA synthesis and mRNA synthesis of viral protein for the virion.

Liu et al. (2010) considered that both EIAV and HIV are members of the *Lentivirus* genus of the Retroviridae family. Although the clinical manifestations of infections by EIAV and HIV are different, the underlying mechanisms of

persistence and pathogenesis are very similar. These similarities are based on the common genetic organization, the molecular mechanism of viral replication, and the conformational structures of the viral structural proteins. Most chronically infected horses survive the subclinical carrier phase after recurring cycles of fever, anemia, weight loss, and thrombocytopenia. Therefore, EIAV has been used as a model to study HIV-1 persistence, pathogenesis, and immune responses.

During the last two decades, the profusion of HIV research due to the urge to identify new therapeutic targets has led to a wealth of information on the retroviral replication cycle. However, while the late stages of the retrovirus life cycle, consisting of virus replication and egress, have been partly unraveled, the early steps remain largely enigmatic. These early steps consist of a long and perilous journey from the cell surface to the nucleus where the proviral DNA integrates into the host genome. Retroviral particles must bind specifically to their target cells, cross the plasma membrane, reverse-transcribe their RNA genome, while uncoating the cores, find their way to the nuclear membrane and penetrate into the nucleus to finally dock and integrate into the cellular genome. Along this journey, retroviruses hijack the cellular machinery, while at the same time counteracting cellular defenses. Elucidating these mechanisms and identifying which cellular factors are exploited by the retroviruses and which hinder their life cycle, will certainly lead to the discovery of new ways to inhibit viral replication and to improve retroviral vectors for gene transfer. Finally, as proven by many examples in the past, progresses in retrovirology Will undoubtedly also provide some priceless insights into cell biology. Understanding the precise interactions between cellular and viral partners occurring during the early steps of infection will certainly open new fields of research leading to the discovery of new antiretroviral drugs. The stepwise events allowing retroviruses to enter the target cell, to move within the cytoplasm, to penetrate into the nucleus and to integrate its genome into host chromosomes, are beginning to be unravelled, but many issues are still unanswered. This is particularly evident concerning the uncoating of incoming

viruses, a complex process involving cellular and viral proteins and which takes place all along this early journey (NISOLE and SAIB, 2004).

The retrovirus is an RNA virus that is replicated in a host cell via the enzyme reverse transcriptase to produce DNA from its RNA genome. The DNA is then incorporated into the host's genome by an integrase enzyme. The virus thereafter replicates as part of the host cell's DNA. Retroviruses are enveloped viruses that belong to the viral family *Retroviridae*. The virus itself stores its nucleic acid in the form of a +mRNA (including the 5'cap and 3'PolyA inside the virion) genome and serves as a means of delivery of that genome into cells it targets as an obligate parasite, and constitutes the infection.

Replication: When retroviruses have integrated their own genome into the germ line, their genome is passed on to a following generation. These endogenous retroviruses (ERVs), contrasted with exogenous ones, now make up 5-8% of the human genome. As insertions have no known function. However, many endogenous retroviruses play important roles in host biology, such as control of gene transcription, cell fusion during placental development in the course of the germination of an embryo, and resistance to exogenous retroviral infection. Endogenous retroviruses have also received special attention in the research of immunology-related pathologies, such as autoimmune diseases like multiple sclerosis, although endogenous retroviruses have not yet been proven to play any causal role in this class of disease (Figure 3).

While transcription was classically thought to only occur from DNA to RNA, reverse transcriptase transcribes RNA into DNA. Nominally, retro in retrovirus refers to this reversal, making DNA from RNA, of the central dogma of molecular biology, what deals with the detailed residue-by-residue transfer of sequential information.

While in human immunodeficiency virus type 1 and 2 [HIV-1 and HIV-2) there is retrovirulicide drugs, in the equine immunodeficiency (IEA) this is impractical from the standpoint of public health animal. A retrovirus infection is a serious problem in the area of veterinary medicine since ancient times.

Several animals and animals company are infected with the retrovirus. The receiver of the virus revealed that the mechanisms of pathogenesis are useful for developing drugs to prevent infection and the emergence and development of retroviral vectors to introduce genes exogenous utilities. Retroviruses are a problem in the, area, vet, equine infectious anemia is one of the windows to the difficulty of measuring the area of *Lentivirus*.

Equine infectious anemia virus (EIAV), a *Lentivirus* that infects equids, has been utilized as an animal model for the study of HIV. Furthermore, the disease associated with the equine *Lentivirus* poses a significant challenge to veterinary medicine around the world. As with all lentiviruses, EIAV has been shown to have a high propensity for genomic sequence and antigenic variation, especially in its envelope (Env) proteins. Recent studies have demonstrated Env variation to be a major determinant of vaccine efficacy, emphasizing the importance of defining natural variation among field isolates of EIAV. To date, however, published EIAV sequences have been reported only for cell-adapted strains of virus, predominantly derived from a single primary virus isolate, EIAVWyoming (EIAVWY). We present here the first characterization of the Env protein of a natural primary isolate from Pennsylvania (EIAVPA) since the widely utilized and referenced EIAVWY strain. The data demonstrated that the level of EIAVPA Env amino acid sequence variation, approximately 40% as compared to EIAVWY, is much greater than current perceptions or published reports of natural EIAV variation between field isolates. This variation did not appear to give rise to changes in the predicted secondary structure of the proteins. While the EIAVPA Env was serologically cross reactive with the Env proteins of the cell-adapted reference strain, EIAVPV (derivative of EIAVWY), the two variant Envs were shown to lack any cross neutralization by immune serum from horses infected with the respective virus strains.

Taking into account the significance of serum neutralization to universal vaccine efficacy, these findings are crucial considerations towards successful EIAV vaccine development and the potential inclusion of field isolate Envs in vaccine candidates (CRAIGO et al., 2009).

Equine infectious anemia (EIAV) is shown to have an associated RNA-instructed DNA polymerase similar in its cofactor requirements and reaction conditions to the RNA tumor virus DNA polymerases. Demonstrating this DNA polymerase activity requires a critical concentration of a nonionic detergent, all four deoxyribonucleoside triphosphates, and a divalent metal ion. The reaction is sensitive to RNase, and a substantial fraction of the FNA synthesized is complementary to viral RNA. The detection of a complex of tritium-labeled polymerase product DNA-template RNA, which sedimented at 60S to 70S, provided evidence that EIAV contains high-molecular-weight RNA. These results, obtained with both virus propagated in cell culture and virus from the serum of an experimentally infected horse, indicate that EIAV may properly be considered a member of the family Retroviridae. They may also be pertinent to the mechanism(s) of viral persistence and periodic recrudescence of disease in chronically infected horses (ARCHER et al., 1977).

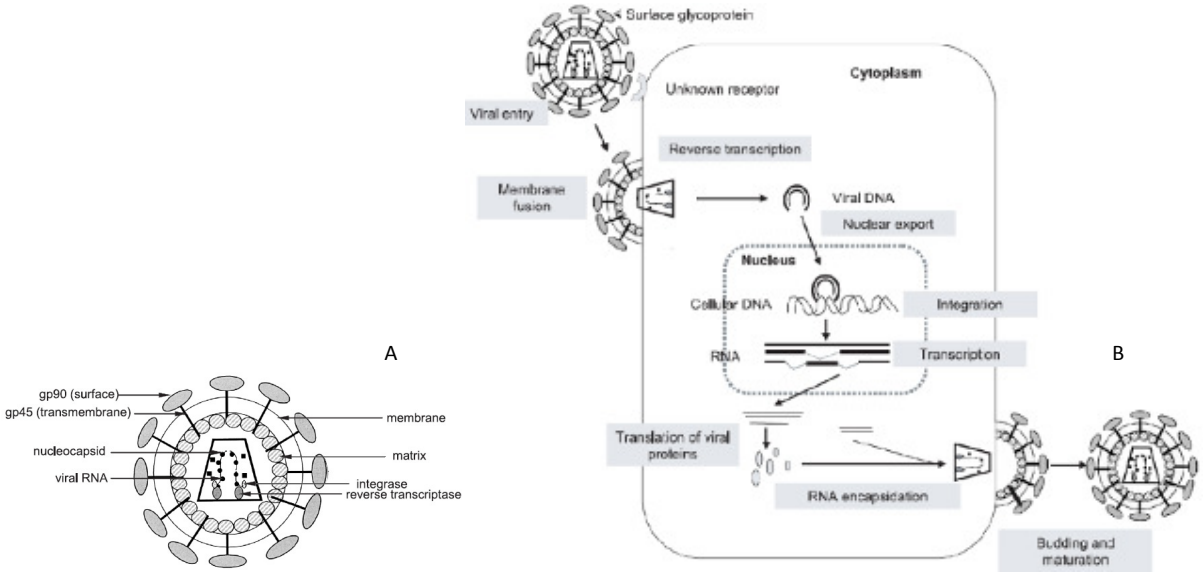

Figure 3- A: Structure of the EIAV virion; B: Schematic representation of the EIAV viral cycle (Retroviral cycle) into the host cells.
Source: LEROUX et al. (2004).

CTL epitopes have been mapped within Gag matrix (p15) and capsid (p26), surface glycoprotein (gp90). Although p26 is the most abundant protein in the virion, the humoral response against p26 is 10 to 100-fold lower than the reactivity against gp90 and gp45. Antibodies that recognize EIAV reverse transcriptase have also been described in experimentally and naturally EIA virus infected animals.

The p26 is an internal structural protein of the virus that is coded for by the *gag* gene. The p26 is more antigenically stable among EIAV strains than the virion glycoproteins gp45 and gp90. There is evidence of the strain variation in the p26 amino acid sequence; however there is no evidence to indicate that this variation influences any of the serological diagnostic tests (OIE, 2008).

Classification: *Retroviridae Lentivirinae* (exogenous): Visna/Maedi virus; Caprine arthritis-encephalitis virus; Human immunodeficiency virus; Equine infectious anemia virus; Feline immunodeficiency virus; Bovine immunodeficiency virus. *Oncornavirinae* (RNA tumor viruses): Type C oncornavirus group (most are exogenous): Feline leukemia virus; Feline sarcoma virus; Bovine leukemia virus; Ovine leukemia virus; Porcine leukemia virus; Gibbon ape leukemia virus (Simian sarcoma virus); Murine sarcoma/leukemia viruses; Human T cell leukemia virus (HTLV-1, HTLV-II); Simian T-cell leukemia virus (STLV-1); Tap type C oncovirus; Baboon type C oncovirus; Avian type C oncoviruses; Type B oncorivus group; Mouse mammary tumor virus (endogenous); Type D oncovirus group (exogenous); Jaagsiekte virus (pulmonary adenocarcinoma virus of sheep), mason-Pfizer monkey virus group (Simian retrovirus); Spumavirinae (foamy viruses, inapparent infections): Bovine syncytial virus; Feline syncytial virus; Hamster syncytial virus; Human foamy virus; Simian foamy virus (9 serotypes).

Exogenous retrovirus: The virus infects the cell from individual to individual are the retrovirus, transmissible. Moreover, in the endogenous Retroviruses, the germ cells infected with the retrovirus exogenous, the host cell will carry the retrovirus incorporated into its genome, and if transmitted by inheritance

from father to son. Are not considered infectious particles (WEISS, 2006; MIYAZAWA, 2009) - Figure 1.

When endogenous retroviruses (ERV) were discovered in the late 1960s. If Charles Darwin reappeared today, he might be surprised to learn that humans are descended from viruses as well as from apes. Some 8% of human DNA represents fossil retroviral genomes, and that is not counting the LINE elements and other retrotransposons that are scattered so liberally across our genome. Darwin might be reassured that we share most though not all of these insertions with chimpanzees. But how did endogenous viruses first come to light? (WEISS, 2006).

The research for receptors for animal retroviruses revealed that the absence of specific receptor on the host cell, it does that there is absence of specific antibody production and absence of immunity.

Although Cytotoxic T lymphocytes are important for control of lentiviruses, including equine infectious anemia virus, it is not known if generation of IL-2-dependent Cytotoxic T lymphocytes and neutralizing antibody in the control of equine infectious anemia virus can limit lentiviral replication in the absence of cytoplasmic domain of CD4 help and neutralizing antibody. CD4 is an important cell surface receptor for equine immunodeficiency virus.

Glycoproteins of the envelope protein are not constant for the amino acid sequence as in the synthesis and transduction of the mRNA, the constant mutation produces a series of CD4 mutants.

Equine infectious anemia virus shown to have an associated RNA-instructed DNA polymerase similar in its cofactor requirements and reaction conditions to the RNA tumor virus DNA polymerases. They may also be pertinent to the mechanism(s) of viral persistence and periodic recrudescence of disease in chronically infected horses Mealey et al. (2008).

The zoonotic introduction of an animal pathogen into the human population and the subsequent extension or alteration of its host range leading to the successful maintenance of the corresponding pathogen by human-to-human transmission pose a serious risk for world-wide health care. Such, a scenario

occurred for instance by the introduction of simian immunodeficiency viruses into the human population resulting in the human immunodeficiency viruses (HIV) and the subsequent AIDS pandemic or the proposed recent host range switch of the SARS coronavirus from a presently unknown animal species to humans. The occurrence of zoonotic transmissions of animal viruses to humans is a permanent threat to human health and is even increased by changes in the human lifestyle. In this study, the potential of the zoonotic transmission of bovine (BFV), feline (FFV) and equine foamy retroviruses (EFV) will be discussed in the light of well-documented cases of zoonotic transmissions of different simian foamy viruses (SFVs) to humans (BASTONE et al., 2003) – Figure 1.

After intense studies and a long-lasting debate, it is generally accepted that the prototypic HFV isolate is of chimpanzee origin and that all documented FV infections of men are most likely zoonotically derived from non-human primates (NHP). Whereas the zoonotic potential of SFVs from NHPs is under current investigation, little is presently known about the zoonotic potential of FVs from farm and livestock animals and pets. As especially in the northern hemisphere contact to the bovine, feline and equine FVs (BFV, FFV, EFV) – Figure 1 - is far more likely, the potential of corresponding zoonosis will be discussed after a brief summary of the biology of FVs. In summary, Leroux et al. (2004), Weiss (2006) and Miyazawa (2009) inferred that at present, no diagnostic and experimental data support the idea of intensive zoonotic transmission of FFV, BFV, or EFV into humans. However, recent zoonosis have significantly raised the public and health-system awareness for such scenarios with unpredictable and even fatal consequences. It is generally accepted that zoonosis will become a novel challenge for the public and international health systems and a scientific preparedness is a prerequisite to find appropriate solutions for these threats. The emerging insights into the zoonotic potential of different primate FVs, the substantial changes in lifestyle and medical technologies and the availability of data and reagents for the setup of novel

and sensitive detection systems make it possible to adequately address these issues of scientific and medical interest.

The discovery of endogenous retroviruses enabled new paradigms in retrovirology. Unlike exogenous retrovirus with a potential prompter of pathogenesis the susceptible, the endogenous retrovirus led to another interpretation of the retrovirus.

Diseases caused by animal retroviruses have been recognized since 19th century in veterinary field. Most livestock and companion animals have own retroviruses. To disclose the receptors for these retroviruses will be useful for understanding retroviral pathogenesis, developments of anti-retroviral drugs and vectors for human and animal gene therapies. Of retroviruses in veterinary field, receptors for the following viruses have been identified: equine infectious anemia virus, feline immunodeficiency virus, feline leukemia virus subgroups A, B, C, and T, Jaagsiekte sheep retrovirus, enzootic nasal tumor virus, avian leukosis virus subgroups A, B, C, D, E, and J, reticuloendotheliosis virus, RD-114 virus (a feline endogenous retrovirus), and porcine endogenous retrovirus subgroup A. Primate lentiviruses require two molecules (CD4 and chemokine receptors such as CXCR4) as receptors. Likewise, feline immunodeficiency virus also requires two molecules, i.e., CD134 (an activation marker of CD4 T cells) and CXCR4 in infection. Gammaretroviruses utilize multi-spanning transmembrane proteins, most of which are transporters of amino acids, vitamins and inorganic ions. Betaretroviruses and alpharetroviruses utilize transmembrane and/or GPI-anchored proteins as receptors (MIYAZAWA, 2009).

The ability of EIA *Lentivirus* to persistently infect horses in the face of a profound immune response by the host makes it a potentially devastating disease for the horse population of the United States. Its ability to evade host immune defenses by lying dormant in apparently healthy animals and by rapidly changing its antigenic determinants is proving to be a major obstacle to vaccine development. Because most infected horses appear clinically normal and a large proportion of horses in this country remain untested, the virus is

not likely to be eradicated in the near future. Yet, for the same reason, because most horses infected with EIAV appear clinically normal, there is a tendency for the horse industry to become complacent in its efforts to control the virus. The cooperation of horse owners, veterinarians, and regulatory officials is necessary to keep the threat of EIA in check in the United States (SELLON, 1993).

Equine Infectious Anemia Virus: What has this *Lentivirus* of parentage with HIV? Belongs to the same group. The New Millennium search if the engine improviment of attenuated vaccines like the Chinese vaccine, which according to Craigo et al. (2005) seems to be a valid option however must be improved. Thus, the equine infectious anemia (EIA) vaccine development of an efficacious vaccine against lentiviral infections remains a high priority in human and veterinary medicine.

Despite international efforts and interesting results, no vaccine is actually available against AIDs infection. Most naturally or experimentally EIAV-infected animals successfully control viral replication and disease within a few months. This innate ability to control *Lentivirus* replication suggests that a vaccine against EIA may be effective in controlling disease development. This made EIAV a unique model to test various vaccine strategies. Unfortunately, EIA-vaccine development has only been moderately successful and classical vaccines based on inactivated or attenuated whole virus and on viral recombinant protein failed to elicit a broadly protective immune response. Chinese scientists reported a successful vaccine trial using a live attenuated EIA strain produced by serial passages on donkey leukocyte cells, since twenty years ago. But no independent investigations have been possible on this very promising vaccine trial and the results are not easily accessible to the international scientific community.

Retroviruses are responsible for many kinds of medically important pathologies including immunodeficiencies and cancers. Hence, there is a large publication demand and a significant research interest for an open access journal, which emphasizes basic retrovirus research. The open access format is

ideally suited for communicating cutting edge information to a large audience quickly Leroux et al. (2004).

In the study of pathology equine virus should be to analyze the family *Retroviridae* contains a diverse group of RNA viruses that are divided into subfamilies: oncornaviruses (onco=tumor); lentiviruses (lente=slow); and spumaviruses (spuma=foam). Important members of each group are listed in Figure 1.

The family derives its name from the presence of the enzyme reverse transcriptase (RNA-dependent DNA-polymerase), which is carried in the virion of all member viruses (retro=reverse). The genome of these viruses consists of two single-stranded positive-sense molecules of RNA (i.e., diploid). In contrast to other RNA viruses, the information stored in the virion nucleic acid is not directly translated; instead, a double-stranded DNA replica of viral RNA is generated by way of reverse transcription. This multistep process is dependent on and catalyzed by virally encoded reverse transcriptase. The virally generated DNA is synthesized um the cytoplasm, but subsequently may be integrated into host-cell DNA and termed "provirus integrated DNA." Expression of provirus DNA can occur whether or not the viral DNA is integrated, but perpetuation of the vírus is clearly most efficient IF integration has occurred. The proviral DNA of certain retroviruses is integrated in germ cells and transmitted vertically (genetic transmission) with every cell thus containing proviral DNA. These viruses are known as endogenous and generally remain quiescent, not being expressed and of low or no pathogenicity. Vertical transmission can also occur by virion passage across the placenta (congenital transmission). Viruses transmitted by the horizontal route termed exogenous and are the retroviruses of most importance as causes of disease. Expression of provirus DNA can occur whether or not the viral DNA is integrated, but perpetuation of the vírus is clearly most efficient IF integration has occurred. The proviral DNA of certain retroviruses is integrated in germ cells and transmitted vertically (genetic transmission) with every cell thus containing proviral DNA. These viruses are known as endogenous and

generally remain quiescent, not being expressed and of low or no pathogenicity. Vertical transmission can also occur by virion passage across the placenta (congenital transmission). Viruses transmitted by the horizontal route termed exogenous and are the retroviruses of most importance as causes of disease. Retroviruses characteristically cause a chronic cellular infection that does not lead to early cell lysis; in fact, with the oncoviruses, infection leads to uncontrolled cellular proliferation. This is in contrast to most other RNA viruses, for which infection and replication lead to cell death. All retroviruses contain three or four genes in common. Each bears genes for reverse transcriptase referred to as pool genes (polymerase), a gene for core proteins called the gag gene (group specific antigenic), and a gene encoding for virion peplomer proteins called the env gene (envelope). Members of the oncovirus subfamily may contain a fourth gene responsible for cellular transformation. This is called the viral oncogene, or v-onc (BOULANGER et al., 1972; CORDES and ISSEL, 1996; LEROX et al., 2004; WEISS, 2006; MIYAZAWA, 2009).

All retroviruses share a similar morphology, consisting of an inner core or nucleoid of RNA surrounded by an icosahedral capsid, which in turn is surrounded by a lipid-containing envelope bearing glycoprotein projections. Virions are 80 nm to 130 nm in diameter. There are differences between groups of retroviruses with respect to the shape of the nucleoid, the prominence of envelop projections, and the processo f budding from cell membranes to produce mature virions. All lentiviruses, however, bud from cell membranes with a characteristic crescent at the cell membrane. Replication of oncornaviruses only occurs in dividing cell, whereas lentiviruses can replicate in non-dividing cells. The use of pig tissues for xenotransplantation in humans may be a source of endogenous retrovirus infection (MIYAZAWA, 2009).

Diseases caused by *Lentivirinae*: lentiviruses have come from general obscurity into the forefront of viral pathology with the Discovery that the cause of acquired immunodeficiency syndrome (AIDS) of humans is a lentivirus, human immunodeficiency vírus (HIV). Lentiviruses affecting animals include

the viruses of visna and maedi (which are nearly identical), caprine arthritis-encephalitis virus, equine infectious anemia vírus, feline immunodeficiency vírus, bovine immunodeficiency vírus, and simian immunodeficiency vírus. Lentiviruses establishment chronic persistent infections with a protracted incubation period. Lentiviral infections persist even though they initiate humoral and cellular immune responses. A number of hypotheses have been put forward to explain persistence, but the mechanism these viruses use to escape elimination is largely unclear. They share the characteristics described above with other retroviruses, but differ antigenically from one another and the members of the oncornavirus and spumavirus subfamilies. They also differ in their morphogenesis and have more genes. They do not require dividing cells for replications, and transcription and translation occurs from nonintegrated viral DNA. The established for human immunodeficiency vírus. The lesions associated with the different lentiviruses may vary, but most causes some combination of the following: 1- lymphadenopathy with marked follicular hyperplasia, which may progress to lymphoid depletion; 2- lymphocytic infiltrates, often nodular in any tissue; 3- interstitial pneumonia; 4-encephalomyelitis; or 4- arthritis. In severely immnodeficient animals, opportunistic infections may develop (especially true for HIV) and may dominate the pathologic findings and be the immediate cause of presenting signs or death (COGGINS, 1975).

EIA: a lentiviral disease with worldwide distribuition, first recognized and described in France in 1843 (HENSON and McGUIRE, 1974), is not only a serious economic problem but also a model useful for the study of mechanisms involved in prolonged persistence of vírus in the host and its pathogenic effects. Once the vírus gains Access to a susceptible animal, it can be demonstrated in the circulating blood as long as the animal lives; despite the immune response, the vírus persists as with other lentiviral infections. The infection may be almost subclinical, or it may be acute with febrile manifestations and a rapidly fatal outcome. Infected horses have lived as long as 18 years with few signs; yet, at any time, minute amounts of their blood

injected into normal horses induces acute infectious anemia. Horses that apparently have recovered from the acute disease may suddenly exhibit severe symptoms and die after exposure to some deleterious influence (hard work, for example, or injection of an additional, though minute, amount of infectious anemia virus). It would seem that host and parasite, under some conditions maintain a delicate balance (HENSON and McGUIRE, 1971).

Nevertheless, analogies have been drawn between the clinic-pathologic course of EIA infection and others lentiviral infections.

Clinical manifestations: for purposes of description, the clinical disease is usually divided into three types: acute, subacute, and chronic. Cases of the disease, however, often fall simultaneously into two or more of these groups and, in the various stages, may pass through all three. The pathologic manifestation within each clinical type are similar to those in the other two; nonetheless, this classification is convenient, and even though it is arbitrary, it Will be followed in the discussion of clinical and pathologic features. Acute equine infectious anemia is characterized by rapid onset and high fever (42.22°C) after an incubation period of 1 to 3 weeks. The fever is accompanied by extreme weakness, excessive thirst, inappetence, depression, edema of the lower abdomen, and sublingual or nasal hemorrhages. Such attack leads to death (in less than a month) or survival, with the disease assuming the subacute or chronic form. Anemia is not a prominent feature at the onset, but there is a gradual reduction in circulating red blood cells. The normal count of 8 millin/mm^3 drops to about 4 million/mm^3 in most cases. In the subacute form, the disease is manifested by relapsing fever and recurrence of other symptoms at irregular intervals. The symptoms during these exacerbations are similar to these of the acute type, but generally less severe. The attacks may increase in severity, with gradual weight loss, weakness, edema of dependent parts, and unsteady gait becoming increasingly evident. Death may supervene during any of these recurrent attacks. Pallor of mucous membranes usually indicates the loss of circulating erythrocytes, which may fall as low as 1.5 million/mm3, and as a rule, the sedimentation rate is greatly increased.

Accentuation of symptoms may be brought about by hard work, starvation, "blood letting", or other unfavorable factors (COGGINS, 1984).

The chronic form of the disease may develop after the animal has passed through an acute infection, or it may occur in the absence of an obvious attack. Experimental injection of minute amounts of the vírus often results in chronic infectious anemia. Some animals appear to be in good health except for transient febrile manifestations at intervals of a month or two. Others remain thin despite a good diet, occasionally show edema under the thorax and abdomen, and may become weak or uncoordinated. The red cell count is usually 2 or 3 million/mm3 below normal, but evidence of severe anemia is seldom observed (HENSON and McGUIRE, 1974).

Pathogenesis: the anemia that appears intermittently in this disease seems to be caused principally by destruction of red blood cells by means of an immunologically mediated mechanism. Erythrocytes of infected horses are coated with antiviral antibodies and complement 3 (McGUIRE et al., 1969a, b). This binding to the cell surface results in increased osmotic fragility, shortened half-life, and erythrophagocytosis. Plasma hemoglobin level increases animals and serum haptoglobin level decreases in infected animals. In the erythrophagocytosis these findings point toward hemolysis as a key factor in genesis of the anemia. Another, probably less important factor is depression of the bone marrow during acute episodes, as indicated by a decrease in both plasma iron turnover and utilization of radioactive iron. Renal glomeruli (glomerulitis) are affected in horses with active disease. Lesions: the nature of the lesions found during necropsy of horses that died of infections anemia or were sacrificed during its course depends to a great extent on the clinical type of disease and the duration of illness. In other words, an animal that dies during an attack after several exacerbations characteristic of the chronic disease exhibits different tissue changes than one that dies after a single acute attack. It is convenient, therefore, to describe the lesions in relation to the clinical type of the disease. General lesions: acute disease: Icterus, edema, and hemorrhage are the principal Gross findings at necropsy. Edema is most

prominent in the subcutis of the ventral wall of the abdomen, at the base of the heart, and in the perirenal and sublumbar fat. The hemorrages are petechial or, less often, ecchymotic and are found in the edematous areas or in serous membranes, particularly the pleura and peritoneum. Swelling of the parenchymatous organs is common and is more fully described in connection with each system.

Histopathologic changes indicative of EIA were noted in all test-positive recipients. The most consistent lesion was paracortical lymphoid hyperplasia in the splenic lymph node (ISSEL et al., 1982).

Transmission: Like HIV, EIA can be transmitted through blood, saliva, milk, maternal body secretions, urine, semen, feces, sweat, and tears can transmit the virus but are not the most important pathways, and may occasionally occur. The main means of transmission of the disease are application of medicaments or bloodletting without the proper care disinfection, or through bite blood-sucking insects, mainly horseflies, hematophagous. It is possible to direct contagion horizontal (needles, bridles, spurs, and others) via by venereal (genital mucosal microdamage) route, or vertical - transplacental (intrauterine) route. The intrauterine infection may not be lethal leading to a carrier congenital.

The penetration of parenteral virus inoculation can be given by the mechanics of biting insects or instrumental. The lympho-hematogenous dissemination after inoculation of virus libre along with hemagglutinins and macrophages. In the host the virus is captured by the macrophages and virion integrates with the cell forming provirus in the genome of the defense cells (lymphocytes). However, the transmission of the virus from EIA (EIAV) is generally related to the transfer of blood from an infected horse to a healthy receiver. 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% (KEMEN and COGGINS, 1971; HENSON and McGUIRE, 1974).

Altogether, several species of hematophagous Diptera (flies, horse flies, mosquitoes) are implicated in mechanical transmission (without multiplication of the agent in the vector) pathogenic agents. The diptera contaminate if during their feeding on infected animals and transmission occurs when after interruption of feeding on an infected animal, restart his repast on another animal (healthy). The horseflies have been associated with transmission of more than 35 pathogenic agents, including the EIAV (KRINSKY, 1976).

Once a horse is infected with EIA virus (EIAV), its blood remains infectious for the remainder of its life. This means that the horse is a viraemic carrier and can potentially transmit the infection to other horses. Transmission occurs by transfer of blood from an infected horse. In nature, spread of the virus is most likely via interrupted feeding of bloodsucking horseflies on a clinically ill horse and then on susceptible horses.

Transmission can also occur by the iatrogenic transfer of blood through the use of contaminated needles. In utero infection of the fetus may occur. The virus titre is higher in horses with clinical signs and the risk of transmission is higher from these animals than the carrier animals with a lower virus titre (SILVA et al., 2001).

The virus of Equine Infectious Anemia (EIA) is classified in the *Retroviridae* family, of what make party several sub-family of viruses that have in common the ability to cause immunodeficiency or tumors.

There are several factors involved in the mechanical transmission of equine infectious anemia (EIA) virus by insects. Large hematophagous insects, especially tabanids, which feed from extravascular sites (ie, pool feeding) appear to be the most efficient vectors. The biology of the host-seeking and blood-feeding behavior of the vectors are important variables that have been overlooked in the mechanical transmission of pathogens like EIA virus. The biology, population levels, and diversity of the vectors, in addition to the clinical status and proximity of EIA virus-infected horses maintained with susceptible animals are all important variables that contribute to EIA virus transmission in nature (HAWKINS et al., 1973; ISSEL and FOIL, 1984).

Equine infectious anemia has been managed in most countries by the imposition of testing and quarantine regulations. In the United States, about 700,000 of the more than 7,000,000 horses are tested annually. As long as the status of greater than 90% of the horse population remains unknown and horses are transported and congregate in a relatively unrestricted manner, EIA will continue to exact its toll. Therefore, it is incumbent on the scientific community to continue to develop and refine practical and sensitive diagnostic tests for EIA, which will be used in an expanding market to reduce the number of untested horses and to increase the accuracy of test results. Under ideal conditions, EIA can spread rapidly in a localized population with potentially devastating results. Although strict adherence to sanitary regulations will minimize the likelihood of epizootics, the existence of a large reservoir of untested horses with occasional contact with uninfected test-negative horses will ensure the continued transmission of EIAV. The change of this transmission occurring as a result of human intervention can be eliminated but it is not possible to eliminate the threat posed by blood feeding insects. If these "chance encounters" between an untested EIAV infected horse and a test-negative horse occur under field conditions where horse flies are abundant and the proximate distance between the horses is minimal, transmission is efficient if the quantity of EIAV in the blood of the donor horse is high (FOIL et al., 1983; ISSEL et al., 1982; ISSEL et al., 1990).

Equines (horses, mules, asses) are the only species susceptible to natural or experimental exposure. The virus is transmitted mechanically by the bite of the mosquitoes (*Anopheles psorophora* or *Culex pipiens quinquefasciatus*) or biting fly (*Stomoxys calcitrans* or *Tabanus fuscicostatus*) or by the transfer of a minute amount of blood from an infected horse to a normal one by the use of un-sterilized hypodermic needles, tattoo needles, curry combs, or items of equipment, such as harness, bit, or saddle. The disease is not spread by ordinary contact. Humoral antibody response is not quantitatively deficient in infected animals: thus, loss of antibody response cannot be accepted as explanation of the life-long persistence of the vírus in the equine host.

Circulating virus-antibody complexes are demonstrable and may lead to immune complex discase. An Agar-gel immunodiffusion test, developed by Coggins, has proved effective in the detecting antibodies and the presence of vírus in horses (Coggins and NORCROSS, 1970; COGGINS et al., 1972; HENSON and McGUIRE, 1974).

Standards for Prevention and Control of Equine Infectious Anemia - EIA, in Brazil, are contained in Instruction Normative Nº. 45, June 15 of 2004, of the Ministry of Agriculture, Livestock and Supply – MAPA (BRAZIL, 2004).

Standards for laboratory diagnosis of equine infectious anemia by agar gel immunodiffusion, are regulated by, Ordinance 84/ 1992 of MAPA (Brazil, 1992).

Vaccines: On the protective effect of the vaccine Chinese, Liu et al. (2010) had considered that to induce a potent and cross-reactive neutralizing antibody (nAb), an effective envelope immunogen is crucial for many viral vaccines, including the vaccine for the human immunodeficiency virus (HIV). The Chinese equine infectious anemia virus (EIAV) attenuated vaccine has controlled the epidemic of this virus after its vaccination in over 70 million equine animals during the last 3 decades in China. Data from our past studies demonstrate that the envelope (Env) protein of this vaccine plays a pivotal role in protecting horses from both homologous and heterogeneous EIAV challenges. Therefore, the amino acid sequence information from the Chinese EIAV attenuated vaccine, in comparison with the parental wild-type EIAV strains, was applied to modify the corresponding region of the envelope glycoprotein of HIV-1 CN54. The direction of the mutations was made towards the amino acids conserved in the two EIAV vaccine strains, distinguishing them from the two wild-type strains. The purpose of the modification was to enhance the immunogenicity of the HIV Env (LIU et al., 2010).

Disinfection:

Sodium hypochlorite (NaOCl) is the most widely used disinfectant in the food industry despite the increasing availability of other disinfectants. Sodium hypochlorite fulfills many requirements as the ideal disinfectant and

furthermore it has an excellent cleaning action. The effectiveness of sodium hypochlorite in the cleaning and disinfection processes depends on the concentration of available chlorine and the pH of the solution. Hypochlorous acid (HOCl) is a weak acid and dissociates to the hypochlorite ion (−OCl) and proton (H+) depending on the solution pH. It is generally believed that HOCl is the active species in the germicidal action, whereas the concentration of −OCl is a key factor determining the cleaning efficiency. This implies that the optimal pH region of the germicidal activity of sodium hypochlorite differs from that of its cleaning activity. This paper describes the theory and practice of the cleaning and disinfecting operations based on the use of sodium hypochlorite solution (FUKUZAKI, 2006).

To assess the virucidal activity of three disinfectants (sodium hypochlorite, a phenolic, and a quaternary ammonium compound) in the presence and absence of blood. Disinfectants at varying concentrations (hypochlorite: 5,000, 500, or 50 ppm; phenolic: 1:10 or 1:128 dilution; hypochlorite at a final concentration of 5,000 ppm (1:10 dilution) should be used to decontaminate blood spills, but even after decontamination care should be used to avoid sharps injuries (WEBER et al., 1999).

Materials and methods

A total of 40,500 serums equine were processed in Laboratory Animal Pathology APTA-Bauru/Secretary of Agriculture of the of São Paulo State (LPA-UPDB), originating from 225 municipalities and 21,608 different establishments of equine breeding classified into jockey club, equestrian society, haras, breeding equine farm and military unit in the course of the years 1984 to 2011. During this period, the platform for reception of the LPA, accredited by the Ministry of Agriculture, Livestock and Supply (MAPA), attended the flow of samples coming from different units of creation, receiving daily as routine analysis, samples of blood serum for examination of Equine Infectious Anemia

coming from different localities of the São Paulo of State. Regularly, the purpose of the diagnostic request was for attend the formal requirements of MAPA on the transport of equids destined to reproduction, work and leisure. We used the Immunodiffusion in agar gel on glass blades (COGGINS and NORCROSS, 1970; NAKAGIMA and USHIMA, 1971; NAKAGIMA et al., 1972; COGGINS, 1975) to identify positive for equine infectious anemia virus and subsequent sacrifice (BRAZIL, 1992; BRAZIL, 2004).

Sampling of serums from the equids, should bedone after prior disinfection of the region of neck in order to venipuncture from the jugular vein. After obtaining whole blood without separating anticoagulant serum fraction to be aliguotada in two samples of 2 mL each and stored in glass tube with lid. One of the samples should be sealed as an against-proof to the test and maintained environment under refrigeration at −20 °C. The horses were bled from the jugular vein using a vacuum system.

The methodology of agar gel immunodiffusion for the diagnosis of equine infectious anemia is based on double radial migration of antigen (Ag) and antibody (Ac) through, the agar gel. The meeting of the reactants in equivalent proportions (volume/volume), leads to the formation of Ag-Ac complex insoluble which if precipitate becoming visible in the form of a line or band of precipitation. An extreme variation in concentration of Ag and Ac can change the location or inhibit the formation of this precipitation line.

This reaction may be also influenced by a variety of physicochemical conditions, among them, electrolyte concentration, buffer pH and temperature. Sudden changes in temperature during incubation, leading to formation of technical artifacts, undesirable for testing. High levels of lipids and proteins in the reactants can affect the formation and observation of the precipitation line.

Preparation of reagents:

In continuation of the analytical procedures with the following test procedure: Immunodiffusion reactions are carried out in a layer of agar in blade glass. For blade glass that has 75mm in length x 25mm in width, 4.5-5.0 mL of 1% Noble agar in 0.145 M borate buffer (9 g H3BO3, plus 2 g NaOH per

liter), pH 8.6 (± 0.2) is used. For the preparation of 2% agar to be used in the basal layer of the blade, use the same criteria with 2 g of Agar Noble. For fusion of the agar gel, heat in water bath or steam-flowing until the complete fusion of the agar.

Fractionate the solution of agar gel buffered in aliquots according to the need for laboratory use. This avoids the successive mergers, leading to oxidation and modification of physico-chemical properties of agar; In the preparing the glass slides with agar gel, microscope slides are used, measuring 25mm x 75mm, transferred to 4.5 mL of agar gel 1% for the glass slides previously prepared by the solidification of the basal layer with Agar 2%.

After solidification of the second layer of agar gel 1%, carry out the holes in the gel at 1% with the cutter pattern, with 7 holes, measuring 4 mm in diameter and 3 mm equidistant between them, being a central hole (Ag) and 6 peripheral [to control serum standard (SP) alternated with equine serum under test (SE)].

Previous to the inoculation procedure, observe if the wells drilled are free of moisture cavities and if there exists proceed to evaporation; Proof of Coggins follows with the inoculation of the serum to be tested with the following distribution of reagents: Inoculate 25 microliters of serum to be tested (serum equid) alternating lateral cavities. Then inoculate 25 microliters of standard serum (positive control), alternately in the lateral cavities.

Continuing, inoculate 25 microliters of standard antigen (Ag) in the central cavity. The inoculated slides should be incubated in moist chamber at room temperature between 20 °C to 25 °C. The readings should be performed at 24 hours and 48 hours after inoculation. In the daily readings should be used light source of variable intensity with narrow beam center, being the built environment and prepared with special tint to obtain the black background (COGGINS and NORCROSS, 1970; COGGINS et al., 1972; CARVALHO Jr. et al., 1976; CARVALHO Jr., 1981).

Preparation of standard antiserum: A known positive antiserum may be collected from a horse previously infected with EIAV. This serum should yield a single dense precipitation line that is specific for EIA, as demonstrated by a reaction of identity with a known standard serum. It is essential to balance the antigen and antibody concentrations in order to ensure the optimal sensitivity of the test. Reagent concentrations should be adjusted to form a narrow precipitation line approximately equidistant between the two wells containing antigen and serum (OIE, 2008).

Necropsies: eleven sero-positives animals, the Coggins proof for equine infectious anemia, were sacrificed to study macroscopically of the lesions, and the fragments of various tissues were collected with fixation in 10% formalin for study anatomo-pathological (microscopic) of lesions.

Blood-sucking insects: the genera most common flies stables and farms studied were observed how much as to morphology, the difference between genders and the habit of different blood-sucking insects of equids.

RESULTS

In the interpretation of proof of agar gel immunodiffusion (IDGA) should consider the following reactions, which may vary according to the sample to be tested: The precipitation line formed with standard serum control (SP) is the reference for the reading and interpretation of proof IDGA the sample to be tested of the equid (SE). Wherefore, have to provide distinctness, which reflects the quality and adequate preparation of the reagents used in analytical procedures.

The reaction between Ag and SP should show sharp line of precipitation, and be mildly, equidistant between the cavities, thereby highlighting the quality of proof. Under these conditions, the sample examined is considered negative if the precipitation lines formed between Ag and SP continue until the cavities of the samples under test (SE) without bending if where if found the samples tested (Figure 4-A), signifying the absence of identity between the serum SP and SE. Likewise, the present sample is considered positive if the lines of

precipitation formed between Ag and SP, extending up to the cavities of the samples under test (SE), curving if in the direction of the samples in test. In this situation, the line of precipitation between the Ag and SP if fused with those formed by the samples in test (SE) and form a continuous line of total identity (Figure 4 - B). In weak positive reaction, the precipitation line of a sample weakly positive tends to form more next to the cavity where the sample being tested. In cases of low titers of Ac can if viewing only a convergence of two lines of control, in the direction of the cavity where if found the serum which is being tested (Figure 4 - C). In strong reaction positive, the line of precipitation of a sample with high titer of Ac shall if as a band diffuse between the two control lines. In extreme cases, there may be inhibiting the formation of this band and alone you will see the two control lines interrupted equidistant from the tested serum was not observed precipitation between these lines. The samples that showed strong positive reactions should be diluted in borate buffer and retested in dilutions, 1/4 and 1/8 (Figure 4 - D). In reactions with nonspecific presentation of lines, no specific reaction to form a continuous line with those of SP. They are formed by Ag-Ac reactions other than those specific for EIA. A sample can produce a specific reaction to the IEA (positive) and a line of nonspecific precipitation (Figure 4 - E).

The proof will be considered positive when there is reaction of identity between the serum and control sample. The readings of the test are performed from 24 h and 48 h reading is conclusive for the issuance of the result, positive or negative (Figure 4).

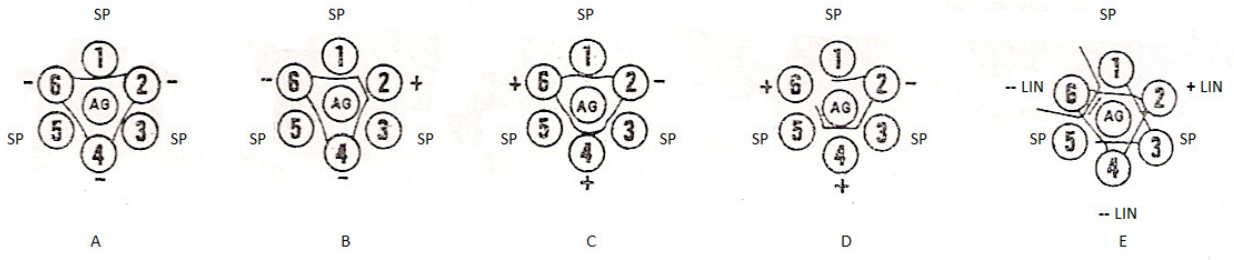

Figure 4: A: Negative, B: Positive, C: Weak positive, D: Strong positive, and E: Lines nonspecific, and positive reaction. SP: Standard serum positive control; --LIN: Negative line of precipitation; +LIN: Positive line of precipitation.

Table 1- Results of the test, the agar gel immunodiffusion for the population of animals examined for equine infectious anemia and reagents according to the sex of the equids examined

	Male	**Female**	**Total**
Examined total	11259	29241	40500
% by sex	27.8	72.2	100
Reagents (%) *	15 (0.13)	62 (0.21)	77 (0.345)
% of reagents (sex)	19.48	80.52	100

*P<0.05

The total of 77 horses were diagnosed positive to the agar gel immunodiffusion for Equine Infectious Anemia, representing a relatively low prevalence of 0.19% of total population tested in the period studied. Among the horses positive IDGA, considering separately the populations of each sex, the higher prevalence of 0.21% for females was observable versus 0.13% for males (Table 1).

The scientific classification of bloodsucking flies observed parasitizing horses can be summarized in: [*Stomoxys*: Kingdom: Animalia, Phylum: Arthropoda, Class: Insecta, Order: Diptera, Suborder: Brachycera, Family: Muscidae, Gender: Stomoxys, Specie: calcitrans (LINNAEUS, 1758)]; and [*Tabanus*: Superfamily: Tabanoidea, Family: *Tabanidae*, Subfamily: *Tabanidae*, Genus: *Tabanus* (LINNAEUS, 1758) and species *Tabanus importunus* (WIEDEMANN, 1828). The blood-sucking flies most frequent parasite of horses were *Tabanus* sp and *Stomoxys calcitrans* (Figure 5- A, B). Insects in the order Diptera, family *Tabanidae*, are commonly called horse flies. Often considered pests for

the bites that many inflict, they are among the world's largest true flies. They are known to be extremely noisy during flight. Tabanids occur worldwide, being absent only at extreme northern and southern latitudes. *Tabanus* is a genus of biting horseflies and the females of family *Tabanidae* have scissor-like mouthparts that aim to cut the skin. The horsefly can then lap up the blood. Horseflies of this genus are known to be potential vectors of anthrax, worms and trypanosomes. Some species, such as *Tabanus bovinus*, prefer bovine animals and are less harmful to humans.

B

A: *Stomoxys calcitrans* (Linneaus, 1758) B: *Tabanus* sp

Figure 5 - A: *Stomoxys calcitrans* (Linneaus, 1758) and B: *Tabanus* sp.

Lesions macroscopically observed the in Necropsy:

The main lesions generally found during necropsy of equine sacrificied with EIA sacrificied were: lymph nodes increased in volume, icterus, serosal hemorrhage and edema of the subcutaneous. Edema is most prominent in the subcutis of the ventral wall of the abdomen, at the base of the heart, and in the perirenal and sublumbar fat. The hemorrhages are petechial or less often ecchymotic and are found in the edematous areas or in serous membranes, particularly the pleura and peritoneum. Swelling of the parenchymatous organs is common and is more fully described in connection with each system. Swelling and pigmentation of the liver; and hyperplasia of the bone marrow. Hypertrophy of the spleen and bone marrow may be the only pathologic changes in a sacrificed animal (Figure 6). Anatomo-pathologic study: Overall, reticuloendotheliosis widespread in organs was observed.

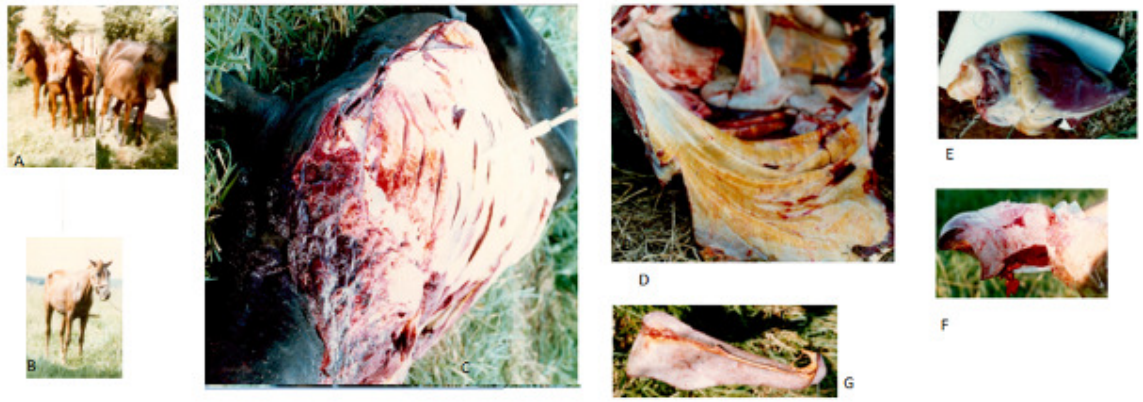

Figure 6 - Equine Infectious anemia. A: inapparent carriers; B: symptomatic carrier; C: hemosiderosis, bleeding from the subcutaneous tissue; D: icterus generalized to deposit pigment in subcutaneous; E: heart with increasing in volume; F: liver with an increase in volume and edema of the serosa and capsule; G: spleen with increasing volume and deposit pigment showing mild jaundice of the capsule coating.

Liver: the liver may be grossly enlarged, red to dark brown, and may occasionally exhibit hemorrhages. Microscopic examination usually reveals lymphocytic infiltration of Glisson's capsule; congestion and dilation of sinusoids; swelling of kupffer's cells (some containing hemosiderin); macrophages containing hemosiderin in the sinusoids; and sometimes, loss of liver cells at the Center of lobules. The reticuloendothelial cells often form small nodules of cells within the sinusoids and occasionally in portal areas. The Kupffer's cells are enlarged and often filled with hemosiderosis. Glisson's capsule usually contains many lymphocytes, plasma cells, and some histiocytes. Myeloid cells have been described in these portal areas in cases chronic duration.

Spleen: is involved in the sequestration of erythrocytes and cells of imunologic system, infected by the virus.involved in this, sequestration of erythrocytes, and cells of imunologic system infected by the virus. The spleen may be the only visceral organ in which gross or microscopic evidence of this smouldering viral infection can be seen. The spleen is enlarged and its cut surface is light red or reddish brown and somewhat turgid and does not exude fluid. The trabeculae are usually widely separated, as are the splenic corpuscles, which are enlarged and clearly visible in some gross specimens. In older affected

horses, the enlarged, soft, nonfibrous organ has more nearly the appearance of the spleen of colts. Microscopic evidence of reticuloendothelial hyperplasia, similar to that described for the acute form is the basis for its gross appearance. Hemosiderin is usually present in macrophages.

Lymph nodes: The changes in lymph nodes, which may be enlarged, mimic those described in the spleen. If acude disease is prolonged, the lesions are proliferative, characterized by replacement of the normal cytoarchitecture by diffuse sheets of mononuclear cells considered to be reticuloendothelial cells or immature lymphocytes. There may be cellular infiltration of the trabeculae, capsule, and perinodal fat. The proliferative response with replacement of normal structures by reticuloendothelial and lymphoid cells is generally more pronounced than in the acute type. The most consistent lesion was paracortical lymphoid hyperplasia in the splenic lymph node.

Bone Morrow in cases chronic disease: the marrow of the long bones, even in aged horses is predominantly red rather than fatty. This hyperplasia of the hematopoietic marrow is more obvious in the Gross specimen than in microscopic sections, since the effect is quantitative. The microscopic picture is one of hematopoietic marrow with myeloid and erythroid elements in approximately normal proportion. It is obvious from these findings that hematopoiesis is not depressed but rather stimulated, probably as the results of destruction of erythrocytes. Large numbers of reticuloendothelial and lymphoid cells are interposed between the erythroid and myeloid elements.

Kidney: The kidneys are usually involved in the edema that affects the perirenal tissues, particularly in the region of the pelvis. Intense infiltration of immature lymphoid cells into the interstitial stroma of both cortex and medulla is especially prominent around blood vessels and occasionally around renal glomerular. This lymphoid infiltration takes on a nodular distribution in some cases. There is generally an increased cellularity of the glomeruli. Immunoglobulins and complement have been demonstrated with immunofluorescent techniques.

Others organs: it is possible for all organs and tissues of the body to show evidence of the reticuloendothelial hyperplasia described in spleen, lymph nodes, liver, and kidney, but these changes are not constant nor particularly distinctive. In the adrenal gland, lymphoid cell masses may separate parenchyma cells in much the same manner as in the liver. Endothelial cells of the adrenal have been reported to contain hemosiderin. Rarely, neurologic signs and granulomatous encephalomyelitis have been described. The frequency of this lesion in horses is not know, but equine infectious anemia should be suspected when granulomatous encephalitis is encountered, considering the frequency of encephalitis in other lentiviral disease.

Hematologic picture: The anemia is characteristically normocytic and normochromic. Reticulocytosis or other evidence of increased erythropoiesis is not evident. The anemia appears to result from a combination of hemolysis, erythrophagocytosis, and a decreased production of erythrocytes. The response of the bone marrow has received litle attention; although usually described as hyperplastic, there is little evidence of increased erythropoiesis. Following and immediate lymphopenia, the disease is characterized by lymphocytosis and the appearance of iron-laden monocytes or siderocytes in the peripheral blood. Thrombocytopenia is a usual finding. Serum levels of gamma globulin are usually increased in the acute stages

Diagnosis: A presumptive diagnosis can be made in the living animal during acute exacerbations of the disease or by recognizing characteristic cross and microscopic lesions at necropsy. Definitive diagnosis, however, depends on specific identification of the virus, either in the affected animal or after isolation in horse leukocyte cultures. The gel-diffusion test is particularly useful in detecting humoral antibodies in the serum of infected horses. The presence of these antibodies is consistently associated with virus, hence the test is a useful method to detect the virus. Fluorescein-labeled immunoglobulins are useful to demonstrate viral antigens in infected tissues.

DISCUSSION

The reaction of agar gel immunodiffusion (IDGA) has shown satisfactory results for the diagnosis and prevention of retroviral – *Lentivirus* of the equine infectious anemia, sometimes also so-called "AIDS of the horse" face their genetic similarity with the genus *Lentivirus* causes Acquired Human Immunodeficiency Virus (AIDS), also of the family *Retroviridae*. The IDGA proof was the first commercially available test, and the only officially prescribed for transit by the World Organization for Animal Health in 1970, and in Brazil starting in 1974. The IDGA established by Coggins and Norcross (1970) also known as proof of Coggins to be prepared in a petri dish and modified by Nakajima and Ushima (1971) with glass blade in preparation. The proof presented if easy and practical implementation, high specificity and high sensitivity around 99% (Carvalho Jr., 1998). The proof is based on that Ag and Ac specific react to form a clear precipitin line. Being the serum researched carrier antibodies for EIA, should form the identity line with the standard serum, merging if both.

The low rate of 0.19% prevalence in the state of Sao Paulo evidenced in the present research, has been maintained through a rigorous program of sanitary control with application of diagnostic measures required for animals to be transported and membership of the owners, the examination of EIA when the reproductive programs of interest to breeders, and breeders' associations (Table above). Prevalence results were obtained in the diagnosis of the situation of equine infectious anemia in the state of São Paulo (CATI, 1980) to report that in 2,493 serums sampled statistically from the total number of animals equivalent to 301,457 equids, distributed in 69,073 properties, the rate showed prevalence in the range of 0.18% to 0.21% for equids and 0.56% to 0.63% for the sampling of properties examined.

Likewise, in serological survey of EIA conducted by Carvalho Jr. (1976) in 126 municipalities of Sao Paulo State resulted in 0.79% (14 horses and mules 8 positives) as well as in the survey realized by Carvalho Jr. (1998) resulted in 0.46% positive in the southeastern Brazil.

In another study, covering the period 1974 to 1993 with the processing of 3,553,626 (of a total of 9,615,845 existing equids) serum sampling from all regions of Brazil, the percentage of positivity for the regions were: North: 11.51%; Northeast: 3.36% Midwest: 8%; Southeast: 0.43% and South: 0.32% and overall average of 2.64% were positive. The author mentions also that the Southeast and South health programs, were more easy, to bring to completion, with sacrifice of the positives, and focus control.

However, in some regions of Brazil this reality is well different, especially when it comes to some areas of Brazil specifically of Pantanal Matogrossense where the prevalence of so-called "swamp fever" may be greater than 30% in animals of labor according with Silva et al. (1999a). These authors mentioned that of 3.285 examined horses resulted in a mean prevalence of 24.8% of EIA in horses studied. The prevalences were 18.2% for service animals (living next to man), 1.0% in wild animals (non tame), 4.0% for animal breeding, and 0.2% for animals in dressage.

Silva et al. (1999b) investigated a group of 700 horses available in specific micro-region of Pantanal, being that of these, 268 animals were examined, of which resulted in 34.1% of service animals and 5.6% of animals (wild/non tame) positives. The authors also infer that seropositivity found in animals will showed the prevalence of infection in the population maintained under natural conditions, probably determined only by the mechanical transmission by vectors of the virus of the EIA, without any human interference.

How the quality of Coggins proof, Nogueira et al. (2009) compared two techniques for diagnosis of EIA, for a population of 197 horses (173 equines and 24 mules: *equus caballus* x *Equus asinus*) of origination of the micro-region of the Pantanal and Paraguai, resulting in a prevalence of 58.4% of horses seropositive by IDGA and ELISA, while 2.9% of these were negative by ELISA rgp90. For mules, 45.8% by IDGA and 37.5% by ELISA rgp90 were positives. 5.2% of samples from horses (9 animals) and 4.2% of mule (an animal) had indeterminate results by ELISA rgp90, being all ten positive in

IDGA. These results confirm the high sensitivity of ELISA, however, there is the possibility of false negative by this test.

The similarity of equine infectious anemia virus and other immunodeficiency virus, such as the human immunodeficiency virus (HIV) his "cousin"group thus recognized by Leroux et al. (2004), has been the main reason of the advances, scientific research, in the areas of genetics and immunogenetics retrovirus family. The retroviral - *Lentivirus* that affects horses has wide distribution and almost universal (Figure 1). Presents the persistent infection infection characterized by recurring febrile episodes associating viremia, fever, thrombocytopenia, and wasting symptoms. Among lentiviruses, EIA virus is unique in that, despite a rapid virus replication and antigenic variation (Figure 2), most animals progress from a chronic stage characterized by recurring peaks of viremia and fever to an asymptomatic stage of infection. The inapparent carriers remain infective for life, as demonstrated by experimental transfer of blood to naive animals. The understanding of the correlates of this immune control is of great interest in defining vaccine strategies. Over the last decades has produced some interesting results on natural immunological control of *Lentivirus* replication and disease and on the nature and role of virus variation in persistence and pathogenesis (Figure 3). These studies are of interest in the context of HIV and efforts to develop a vaccine. This review will focus on some of the most recent results (COGGINS, 1984; LEROUX et al., 2004).

This proof received the name "Coggins Test". It is qualitative, ie, identifies the pet carrier and not carrying the *Lentivirus*, is recognized worldwide as the most important laboratory method for diagnosis of EIA, for its high specificity, due to the fact that the majority the nonspecific reactions could be identified the formation of lines of "no identity", easiness of implementation and high degree of sensitivity – around 95% (SELLON, 1993), or 99% (CARVALHO Jr., 1998).

The antigen in the immunodiffusion test is the main protein of the viral core, the p26. This protein was shown to be highly conserved in different

variants isolated since the virus undergoes antigenic variation high, resulting in mutants with different antigens. The test, Agar Gel Immunodiffusion (IDGA) is simple. In a petri dish containing agar solidified or blade are made cavities 7 (1 central and 6 peripheral) with a capacity volume of 25 microliters. In the central cavity is placed antigen (p26), while the others were to alternate positive control serum and serum testing. During incubation (48h), the diffusion occurs from the central cavity of the antigen and antibody if present from external cavities. If the serum test exists antibodies against the antigen p26 is formed a line of precipitation in the meeting of these antibodies with p26, which is the continuous line formed (total identity) between the antigen, serum positive pattern and serum test (Figure 4).

In some situations it may be difficult to determine if the test is negative or positive. In the absence of sufficient antibodies to form a visible line, there may be a false negative result. In doubt, repeat the serum of the animal collection and repeat the test. Another alternative test for the diagnosis, the ELISA has been used to survey the population, however, can result in false negatives, and therefore must be confirmed by IDGA (NOGUEIRA et al., 2009).

In this study, the main lesions triggered by anemia or destruction of red blood cells with deposit of excess hematic pigments in tissues appearing icterus, revealed that the system of phagocytosis by splenic sequestration of parasitized red blood cells exceeds physiological limits the agencies involved in cleaning. Consequently, the evidence mainly of lymph nodes, spleen capsules, liver and saturated, and bleeding. Similar description was presented by Blood and Henderson (1978) to enumerate the lesions macroscopically, with the subcutaneous tissue showing icterus, hemorrhages and focal petechiae and ecchymoses in the mucous membranes and visceral serosa, hypertrophy of the bone marrow, anemia, jaundice, and atrophy adipose tissue, subcapsular hemorrhages in all organs, hepatomegaly and splenomegaly, serous atrophy of bone marrow, and widespread hemorrhagic infarction;

Microscopic: intravascular hemolysis, lymphocytic infiltration and immune deposits in renal glomeruli and vascular walls, erythrophagocytosis by

macrophages, especially in the bone marrow, with, proliferation associated with plasma cells, hemosiderin deposits in the liver, spleen, and ganglion (Figure 6).

Hematologic picture: The anemia appears to result from a combination of hemolysis, erythrophagocytosis, and a decreased production of erythrocytes. The response of the bone marrow has received little attention; although usually described as hyperplastic, there is little evidence of increased erythropoiesis. Differently, research of McGUIRE et al. (1969) indicate both a decrease in erythrocyte life span and production. Histopathologic changes indicative of EIA were noted in all test-positive recipients. The most consistent lesion was paracortical lymphoid hyperplasia in the splenic lymph node (ISSEL et al., 1982).

Confirming pathogenicity virus IEA reported in this present research, Cheevers and McGuire (1985) equine infectious anemia (EIA) is a chronic, relapsing infectious disease of horses caused by a nononcogenic retrovirus. Virus persists in infected animals for life and can be reliably detected by serologic tests that measure levels of antibody to the major structural protein of the virus. Periodic virus replication in macrophages leads to an immunologically mediated acute disease characterized primarily by severe anemia. Recrudescence of acute EIA is the result of antigenic variation of the surface glycoprotein of EIA virus. The frequency and severity of clinical episodes of EIA decrease in most horses, leading to an unapparent carrier state. This cessation of clinical illness is probably brought about by the ability of the infected animals to eventually achieve a threshold efficiency of the immune response against antigenic epitopes common to all EIA virus strains. This condition described above was confirmed by McGuire et al. (1971) when analyzing the tissues from 24 horses infected with the virus of EIA were examined by the direct FA technic. Virus-infected cells were found in 18 horses examined 6-40 days after inoculation. Horses infected for 98, 218 and 915 days had no demonstrable fluorescence. No fluorescence was noted in 3 horses without clinical evidence of disease and infected for only 4-6 days. Virus was

found in the spleen of all 18 horses, the splenic lymph node of 14 horses, liver of 12 horses, and kidney of 9 horses. Other organs in which viral fluorescence was detected included the hepatic, renal, mesenteric, mandibular, cervical, bronchial, prefemoral and prescapular lymph nodes, and cerebrum, cerebellum, pituitary, lung, heart, stomach, bone marrow, intestine, pancreas, adrenal, and thymus. Kupffer cells were infected in the liver and macrophages appeared to be infected in all other organs. Mononuclear cells in blood vessels of many organs contained ETA virus. The viral antigen was located in the cytoplasm of the infected cells. The cell type infected and the distribution of infected cells are similar to that observed in certain other persistent virus infections. Cells containing viral antigen occurred in the sinusoids and appeared to be in Kupffer cells. The kidney had fluorescent cells in the glomeruli and in the interstitial areas of the cortex. In a kidney taken from a horse, being the Distribution of EIA virus in the Tissues of Experimentally Infected Horses (positives/total): Spleen: 18/24; Splenic lymph node: 14/24; Liver: 12/24; Kidney: 9/24; Lung: 9/13; Bone marrow: 8/13.

Important aspects of the transmission: In acute cases, lymph nodes, spleen and liver are hyperemic and 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. Differential diagnoses include equine viral arteritis and other causes of edema, fever, or anemia. Once a horse is infected with EIA virus, its blood remains infectious for the remainder of its life. This means that the horse is a viraemic carrier and can potentially transmit the infection to other horses. Transmission occurs by transfer of blood from an infected horse. In nature, spread of the virus is most likely via interrupted feeding of blood-sucking horseflies on a clinically ill horse and then on susceptible horses (Figure 5). Transmission can also occur by the iatrogenic transfer of blood through the use of contaminated needles. In uterus infection of the fetus may occur. The virus titre is higher in horses with clinical signs and

the risk of transmission is higher from these animals than the carrier animals with a lower virus titre (OIE, 2008).

The Tabanids (horseflies, deerflies, clegs) are large telmophagous insects that inflict painful bites and are strong fliers. They have been associated with the transmission of over 35 pathogenic agents.

Craigo and Montelaro (2010) considered that unlike other lentiviruses, EIA virus possesses a unique and dynamic disease presentation that enables consummate analyses of the interactions between a virus, host immune system, and the effects of viral evolution on vaccine efficacy. Hence, EIA virus provides a novel animal *Lentivirus* system with which to dissect the viral and immune correlates of vaccine efficacy and a system with which to examine vaccine candidates for the ability to elicit broadly protective vaccine immunity. The current review summarizes the key findings that have thus far provided a fundamental understanding of the role of the viral Env in immune control of infection, disease, and vaccine efficacy.

Considerations on the Chinese vaccine: Regarding the attenuated vaccine, Craigo et al. (2005) mentioned that, due to the lack of systematic monitoring during the immunization program, however, reliable data on the current rate of EIA infections or the incidence of vaccine reversions appear to be unavailable. Although offering an important and informative *Lentivirus* vaccine model for discerning immune correlates of protection, attenuated EIAV vaccines pose a number of practical problems for application. First, there are the theoretical concerns about potential reversion of the attenuated vaccines strain to virulence, a possibility made more likely by the persistent nature of the infection and the propensity for relentless genomic mutation during EIAV replication. To minimize the potential for reversion, attenuated vaccines could be engineered with multiple gene mutations, but the experiments described here highlight the delicate balance between the extent of attenuation and the degree of vaccine protection. Second, the persistent infection by an attenuated EIAV poses an undefined risk for in uterus transmission and abortion and for virulence in immune compromised horses or foals with developing immune

systems. Finally, attenuated EIAV vaccines elicit antibody responses to viral core and envelope proteins, resulting in seropositivity in current diagnostic assays used to control EIA in the United States, Europe, and other countries. In developing countries where EIAV infection is common and government regulatory policies are not defined, it is possible that the risk to benefit calculation could favor use of an attenuated EIAV vaccine for several years to break the cycle of infection and disease, as reportedly done in the China.

The Chinese vaccine was not approved in the challenge test: However, experimental challenge of the experimentally immunized horses by our standard virulent EIAV(PV) strain by using a low-dose multiple exposure protocol (three inoculations with 10 median horse infective doses, administered intravenously) revealed a marked difference in the protective efficacy of the various attenuated proviral vaccine strains that was evidently associated with the extent of vaccine virus attenuation, time of viral challenge, and the apparent maturation of virus-specific immunity.

Tabanus sp: Barros (2001) collected 3,442 tabanids, identifying 21 *Tabanidae* species belonging to 12 genera and three subfamilies in the Pantanal region. Found the percentage of 56% de *Tabanus importunus*, being the most abundant species. The same author studying the ecology of tabanids in the region observed that the vector season occurs in the first half of the rainy season, from September/October to December/January. Blood from persistently infected horses is the major source of EIA virus (EIAV) transmission occurring mechanically either because of man or blood-feeding vectors.

Research of Silva et al. (1995b) revealed that the main vector of the protozoan *Trypanosoma evansi* in the Pantanal of Mato Grosso is the *Tabanus importunus* (horsefly). Thus being, this co-infection is a predisposing factor for incidence of mortality or exacerbation of symptoms for EIA positive, since that this protozoan is a frequent cause of outbreak of trypanosomosis in horses of Pantanal (SILVA et al. 2004).

CONCLUSIONS

The low prevalence of equine infectious anemia in the São Paulo State observed in the present research, in contrast to other states of the federation, is mainly due to the rigorous system of diagnosis and implementation of measures to protect animal health with immediate sacrifice of positive to proof of Coggins.

Contribute significantly to the difficulty of eradicating of this infirmity, especially the mechanical transmission of retroviral - *Lentivirus* by blood-sucking flies of the genus *Tabanus* sp and *Stomoxys calcitrans*, vectors that to interrupt the biting in an equid reservoir carrier or chronic carrier inapparent of *Lentivirus*, in following change the repast for a healthy equid.

The application of research to further deepen the understanding of eco-epidemiology of sucking flies equids, will certainly contribute to prevent the high incidence in the months of higher incidence of mechanical transmission vectors in areas where the epidemiology promote the transmission mainly in the month hot and with high rainfall during the year, coupled with the adoption of strategies to control of the spread of this infirmity.

The attenuated vaccine of Chinese origin mentioned in the literature, according to several authors offer no security biological how to aspects of immunization. How much the aspect of diagnosis, the use of these can cause confusion when serological interpretation test. Therefore, vaccines existing need to be improved in the aspects questioned before being used commercially.

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