Equine Infectious Anemia in 2014: Live with It or Eradicate It?

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KEYWORDS

- Equine infectious anemia
- Serology
- Host immune response
- Agar gel immunodiffusion test
- Transmission by insects and man

KEY POINTS

- Equine infectious anemia (EIA) control programs in the United States have been effective, although they are now at a crossroads in that the mobile and tested population is mostly segregated and, therefore, at low risk from untested equids that constitute the remaining reservoir for EIA virus (EIAV).
- Consequently, the goals of testing should be reexamined, “smarter” testing conducted by increasing the intervals between tests for frequently tested equid populations, and strategies developed to increase testing of the untested reservoir population.
- In many areas, required testing at change of ownership has proved invaluable in the identification of new cases.
- In areas of the world where working equids are still important agricultural animals and EIA is endemic, strategies other than destruction without compensation must be developed if control of EIA in more than localized situations is the goal.
- Additional research is required to improve direct detection techniques such as polymerase chain reaction–based methods for amplification of EIAV genetic material.
- The most important recommendation is to assume that all equid contacts are infected with EIAV.

INTRODUCTION

Clinical signs of swamp fever or equine infectious anemia were first described in 1843, with research published in 1904 proving it was caused by a filterable agent or virus (EIAV) that persisted in its equid hosts and was transmitted in blood.\textsuperscript{1} Mechanical transmission by large hematophagus insect vectors (horse flies and deer flies) was demonstrated by the 1940s.\textsuperscript{2} Despite these early advances, there were no practical control measures for EIAV until 1972 when Leroy Coggins and coworkers

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demonstrated that antibodies to the virus were detectable in an agar gel immunodiffusion test (AGIDT) using antigen extracted from spleens of acutely infected horses. Furthermore, these antibodies were correlated with the presence of EIAV by horse inoculation tests. This test received rapid international acceptance, and is known as the Coggins test by the veterinary community and horse owners alike.

What have we learned since the early 1970s when testing was adopted on a wide scale? During the first 10 years of widespread official AGIDT use, more than 57,000 positive results were reported to the US Department of Agriculture (USDA) with most of the infected equids being removed from the population by nonmandatory slaughter. However, from 2007 to 2012 more than 8 million samples were tested in the United States, with 485 identified as positive for EIAV, meaning that it costs about $650,000 to find each positive animal. Are we using the investment the horse industry makes for this passive EIA surveillance wisely?

In the absence of an effective vaccine, the success of the test and removal approach for the control of EIA cannot be overstated, at least in those areas where testing has been traditionally routine. This article addresses 4 main questions:

1. What have we learned about EIAV, host control of its replication, and inapparent carriers?
2. Where do we stand internationally in the control of EIA?
3. Are we doing our best at diagnosing the infection when samples are submitted to the laboratory?
4. How can veterinarians reduce the chance that they are spreading blood-borne infections?

This article attempts to put these issues into practical contemporary perspectives for the equine practitioner. The reader is referred to recent reviews for more detailed information on EIA and EIAV.

**ETIOLOGY**

**Taxonomy**

EIAV is classified within the *Lentivirus* genus of the subfamily Orthoretrovirinae in the family Retroviridae, and as such has been designated the “country cousin” of human immunodeficiency virus (HIV).

**Morphology**

Each circular or oval 115-nm EIAV particle contains 2 copies of a single-stranded positive-sense genomic RNA enclosed within a conical-shaped core. The shape of the core is one of the defining features of lentiviruses, and provided some of the first evidence that HIV or “lymphadenopathy-associated virus,” as it was originally labeled, is a member of the lentiviral genus. Surrounding the core component is a proteinaceous matrix that in turn is bounded by a host-cell–derived lipid membrane containing numerous 6- to 8-nm projections.

**Equine Infectious Anemia Virus and Its Relationship with Other Lentiviruses**

At approximately 8.2 kbp, EIAV (see Fig. 1B) has the smallest genome of all known living lentiviruses, yet is a highly successful pathogen despite possessing just 84% of the coding capacity of HIV. The proviral DNA of all retroviruses consists of 3 major structural genes, *gag*, *pol* and *env*, bounded at each end by long terminal repeats. Lentiviruses are known as complex retroviruses because they possess several open reading frames (ORFs) in addition to the 3 major structural genes. These ORFs encode molecules termed accessory proteins; they perform regulatory
functions, counter host defenses, and/or enhance pathogenicity. EIAV is again unusual in that it contains the least number of ORFs (3 compared with, eg, 4 in bovine immunodeficiency virus and 6 in HIV), giving it the simplest genome organization of any extant lentivirus (see Fig. 1B). Although 2 of the accessory proteins (Tat, Rev) are common to all lentiviruses, the third (S2) is unique to EIAV.11–13 Furthermore, the equine lentivirus is the only surviving member of the genus that does not possess an additional ORF encoding a Vif orthologue. This protein is directed against important host retroviral defense proteins of the apolipoprotein β editing complex 3 family.

Fig. 1. (A) The equine infectious anemia virion structure showing location and identity of structural proteins. (B) The 8 kbp equine infectious anemia virus (EIAV) provirus is shown, with long terminal repeats (LTR) and protein-coding regions (gag, pol, env, tat, S2, and rev), with protein names added. (C) Major protein antigens of EIAV used in current commercial test kits (all p26 based; one commercial ELISA kit includes p26 and a determinant of gp45, but no discrimination is made). The immunoblot test is mainly a research test today, but can detect immune responses against all 3 major proteins of EIAV. AGID, agar gel immunodiffusion; ELISA, enzyme-linked immunosorbent assay.
(APOβEC3), and its absence in EIAV is somewhat unexpected because horses possess more APOβEC3 genes than any other nonprimate species.  

**Proteins Encoded by Equine Infectious Anemia Virus**

**Gag/Pol**
Gag and Pol proteins are produced from a genomic full-length viral mRNA. Gag proteins (see Fig. 1B) are produced following cleavage of a polyprotein precursor (PR55\textsuperscript{gag}) by the virally encoded protease (Pr) to yield p15 matrix (MA), p26 capsid antigen (CA), p11 nucleocapsid (NC), and the p9 “late domain” protein. These proteins form the viral core, with p11 binding to the viral RNA genome, p26 comprising the conical core structure, and p15 forming the matrix that surrounds the core (see Fig. 1A). In addition, p15 and p26 are essential for the formation of viral particles while p9 is critical for the release of progeny virus particles from the host cell. The Pol proteins are also produced by proteolytic cleavage (again by the viral Pr) of a polyprotein precursor (PR180\textsuperscript{gag/pol}), and consist of Pr, reverse transcriptase (RT), Integrase (IN), and other enzymes important in viral replication.

**Env**
The EIAV env gene products are also produced as a polyprotein that is cleaved by cellular endoproteinases to yield the surface unit (SU) or gp90 and transmembrane (TM) or gp45 envelope glycoproteins. These proteins are involved in binding to the cellular receptor (recently identified as equine lentivirus receptor-1 [ELR-1], a member of the tumor necrosis factor receptor family\textsuperscript{15}) and subsequent infection of host cells.

**Accessory proteins**
Tat recruits host cell proteins that are essential for the elongation of nascent viral RNA transcripts by RNA Pol II while Rev promotes the nuclear export of full-length genomic and singly spliced viral RNAs. Although the mode of action of S2 is not completely understood, deletion of this accessory protein results in attenuation of EIAV, with peak viral replication rates in vivo reduced by several orders of magnitude. It is possible that S2 may enhance inflammatory cytokine production.\textsuperscript{16–21}

**Host Cells**
In common with all other members of the genus, EIAV infects cells of the monocyte/macrophage lineage, although viral protein expression and progeny virus production only occur in mature tissue macrophages or dendritic cells. However, in contrast to HIV, EIAV cannot infect CD4\textsuperscript{+} T-helper lymphocytes and so does not cause long-term immunodeficiency in equids. In addition, some strains may infect endothelial cells,\textsuperscript{22} and a recent report has demonstrated EIAV antigen expression in lung epithelial cells from infected horses in Romania.\textsuperscript{23} The significance of this finding in terms of the potential for aerosol transmission remains to be determined, although field strains of EIAV can be adapted under laboratory conditions to replicate this process in equine or canine fibroblastic cells, resulting in attenuation in vivo.

**Mechanisms of Persistence**
EIAV can resist immunologic and other forms of host defense, including retroviral restriction factors (RRF), to persist for life. Several strategies have evolved to achieve this goal:

- Integration of proviral DNA into host cell chromatin.
- EIAV infects monocytes but is not transcriptionally active, which permits a period of latency whereby it can be transported around the body without being subjected to immunologic surveillance.
Innate resistance to RRFs. A recent report suggests that EIAV Env proteins are resistant to the equine orthologue of tetherin, an RRF that anchors progeny viral particles to the host cell membrane, thereby preventing them from continuing the infection. It has also been suggested EIAV is resistant to equine APOβEC3 proteins. However, another study has questioned this finding, instead presenting evidence that the most effective members of the APOβEC3 family against EIAV are simply not expressed in horse macrophages.

Structural resistance to neutralizing antibodies. EIAV SU is the only protein known to contain epitopes recognized by neutralizing antibodies. The fact that SU is innately resistant to this class of antibody is suggested by the finding that neutralization titers of postinfection serum samples are increased 1000- to 10,000-fold by introduction of specific amino acid substitutions within this glycoprotein.

Genetic and antigenic variation. The RT of retroviruses is error prone, and as the enzyme has no proofreading ability it is unable to correct mistakes. In the case of EIAV the error rate is such that 1 nucleotide substitution is likely to occur during each replication cycle. As mutations are also introduced by frequent recombination events between the 2 copies of genomic RNA within each virus particle, the viral population rapidly becomes a “swarm” or quasispecies consisting of many different but related genotypes. This process enables EIAV to respond quickly to selection pressure such as that from the immune system.

EPIDEMIOLOGY

EIAV has a worldwide distribution. The virus appears to infect all equids, but clinical responses depend on viral, individual host, and host species factors. For example, donkeys infected with horse-adapted EIAV strains have lower viral loads, mild or no clinical responses, and mount slower, lower antibody responses than horses infected under identical conditions. Viral factors are suggested by the fact that virulence can be increased by sequential passages in vivo.

EIAV is a blood-borne virus that is naturally transmitted mechanically (no insect viral replication step) by blood-feeding insects, especially members of the Tabanidae (horse flies and deer flies). For transmission to occur feeding must be interrupted, with the fly seeking and finding a second host to feed to repletion. Whether a fly returns to the original host or seeks a new host is determined by proximity. Experimentally it has been determined that 99% of horse flies will return to the original host if an alternative is more than 50 m away. Factors controlling insect-mediated EIAV transmission include the volume of blood retained on insect mouthparts, and the time taken to reach a second host and blood-associated viral loads, with optimal conditions for transfer being high virus titers, high vector populations, and a high density of susceptible hosts. Infectious microorganisms that employ mosquitoes as vectors generally have the ability to replicate in Culicidae cells and migrate though the gut to reach the salivary gland so that they can be injected into a new host when the insect feeds. Lentiviruses do not possess these abilities, so when mosquitoes feed on EIAV-infected equids, virus particles that are ingested will be destroyed by contact with digestive enzymes. Furthermore, the volume of blood retained by mosquitoes is significantly lower than the small volumes (10 ± 5 μL) of bloodmeal residue that have been measured on the mouthparts of horse flies such as Tabanus fuscicostatus, thereby making it unlikely the Culicidae are major contributors to the mechanical transmission of EIAV. However, another extremely important mode of transmission is that mediated through the actions of humans. For comparison of risks from insects and humans, see Box 1.
Intraisolate variation in equine infectious anemia virus

In infected equids, gag and pol are relatively conserved over time while extensive genetic variation occurs in env and the ORF encoding Rev.\(^{31–34}\) It has been demonstrated that EIAV undergoes antigenic drift,\(^{35,36}\) in that each febrile episode in an infected animal is associated with a different antigenic variant or “immunologic escape mutant.” This conclusion is supported by nucleotide sequence analysis of SU isolated during sequential clinical episodes from individual horses or ponies,\(^{34,37}\) and shows that most amino acid substitutions are within 8 hypervariable domains.

Interisolate variation in equine infectious anemia virus

To date only 4 complete genomic sequences from EIAV field isolates have been published (EIAV\(_{LIA}\) [China], EIAV\(_{IRE}\) [Ireland], EIAV\(_{MIY}\) [Japan], EIAV\(_{WY}\) [United States]). These sequences share 80% or more nucleotide sequence identity, with each comprising a separate clade, suggesting they all evolved independently since diverging from a common ancestor.\(^{11–13}\) However, phylogenetic analysis based on more numerous available gag sequences (13 clades from just 23 samples) suggest the molecular epidemiology of EIAV is likely to be far more complex, posing considerable difficulties for future vaccine development programs.
Variation between EIAV isolates is not distributed evenly throughout the genome, with Gag p26 and pol gene products being relatively conserved (80%–89% amino acid identity), whereas amino acid identity between Gag p9 and S2 is less than 50%. Conservation of p26 is somewhat fortuitous because this antigen forms the basis for almost all commercially available serologic tests for the detection of EIAV-infected equids. Although there is significant genetic variation between isolates, most of the previously identified structural/functional motifs within viral proteins are either maintained or contain highly conservative amino acid substitutions. For example, despite significant variation, the late domain in Gag p9 (tyrosine [Y], proline [P], aspartic acid [D], leucine [L]) that is implicated in the release of progeny virions from the host cell is present in all strains analyzed to date. Analysis of the immunologically important SU glycoprotein demonstrates that amino acid substitutions between strains are distributed throughout the molecule with the exception of the amino terminus. The only other conserved feature is the presence of cysteine residues, suggesting that disulfide bridges are essential to the structural and functional integrity of SU.

PATHOGENESIS

Clinical disease is initially caused by proinflammatory cytokines that include tumor necrosis factor α (TNFα), interleukin-1α and -β (IL-1α, IL-1β), interleukin 6 (IL-6), and transforming growth factor β (TGFβ). These cytokines are released when viral loads reach a critical threshold level that equates experimentally with plasma-associated EIAV RNA burdens (Fig. 2) of $5 \times 10^7$ to $1 \times 10^8$ copies/mL (or viremia exceeding $10^5$ median horse infective doses per milliliter of plasma). IL-6 and TNFα induce febrile responses by activating the arachidonic pathway to increase production of prostaglandin E$_2$, while TNFα/TGFβ contribute to thrombocytopenia by suppressing...

Fig. 2. Kinetics of events following intravenous inoculation of a horse with $10^3$ median horse infective doses of a horse-pathogenic laboratory strain of EIAV. Virus load (plasma viremia in copies of viral RNA/mL) is designated by cartoons of virus particles. The first positive test for antibody against EIAV is shown as a + on the timeline shortly after the first febrile episode.
equine megakaryocyte growth and TNFα promotes anemia by downregulating erythropoiesis. Furthermore, in some species TNFα induces severe thrombocytopenia by stimulating release of platelet agonists including thrombin, plasmin, and serotonin. During later stages of the disease adaptive immune responses may also contribute to pathogenesis (Box 2) by immune-mediated destruction of antibody-coated platelets and phagocytosis of complement C3–coated erythrocytes, resulting in the presence of hemosiderin granules in macrophages and thickened glomerular tufts within the kidney caused by excess levels of C3. Induction of oxidative stress produced by changes in glutathione peroxidase and uric acid levels may also play a role in the pathogenesis of EIAV infections by escalating inflammatory responses while simultaneously decreasing immune cell proliferation.

**CLINICAL SIGNS**

That EIA can have 3 distinct clinical phases designated acute (first disease episode), chronic (multiple sequential disease episodes), and inapparent (see Fig. 2; Table 1) was first described in 1904. Depending on the virulence and inoculum size of the infecting EIAV strain, acute disease can occur following a 1- to 4-week incubation period. However, signs of disease may be completely absent, or limited to mild febrile episodes that go undetected. When they do occur (see Fig. 2), the most common acute clinical signs include fever, thrombocytopenia, lethargy, and inappetance. In severe cases, petechiation, hemolytic anemia, and epistaxis can occur. Classical chronic disease signs include anemia, thrombocytopenia, weight loss, dependent edema, and occasionally neurologic signs (ataxia and/or encephalitis) (see earlier discussion for pathologic lesions that may be associated with EIA).

**HOST IMMUNE RESPONSES**

Studies in horses with severe combined immunodeficiency (SCID), which have intact innate immunity but lack functional B and T lymphocytes, have demonstrated that adaptive immune responses (which include cytotoxic T lymphocytes [CTL] and neutralizing antibodies) are required to control viremia and clinical disease. In experimental infections in horses or ponies, antibodies against EIAV are detectable in sensitive immunoblot assays or enzyme-linked immunosorbent assays (ELISA) 14 to 28 days postinfection (pi). However, these early antibodies lack significant viral-neutralizing activity, a property that is usually not observed until 38 to 87 days pi and may not reach maximal levels until 90 to 148 days pi, which is usually long after resolution of the acute disease episode. As virus-specific CTL can be detected at 14 days pi, it is currently believed that cell-mediated and not humoral immune responses are responsible for the initial control of EIAV replication, along with alleviating clinical signs.

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<th>Box 2</th>
<th>Pathology associated with EIA</th>
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<td>Hemosiderin granules in macrophages of liver, spleen, and lymph nodes</td>
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<td>Splenomegaly</td>
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<td>Hepatomegaly (nonsupportive hepatitis with periportal lymphocytic/monocytic infiltration)</td>
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<td>Glomerulonephritis (immune complex deposition)</td>
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<td>Interstitial lung lesions</td>
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Initial mammalian immune responses are limited by the phenomenon of immunodominance to just a few of the many potential epitopes that are present within microbial organisms. Although such restricted host immune responses seem to be sufficient against most infectious agents, they may constitute a weakness that is exploited by highly mutable pathogens such as EIAV, because only a relatively few genetic substitutions are required in the viral genome for escape from immunologic surveillance. As mentioned earlier, viruses associated with each new febrile episode are not neutralized by antibodies generated against previous virus isolates from the same infected equid. Furthermore, not all immune responses are created equal, in that control of viral loads and clinical disease is often associated with CTL that bind to their respective epitopes with high avidity, whereas those that have weaker binding are significantly less effective. It has been observed that epitopes recognized by high-avidity CTL are subject to rapid mutational changes in EIAV-infected individuals, whereas those bound by their less avid counterparts may persist for years.

The ability of EIAV to evade initial immunodominant-restricted responses forces the equid host into a cycle of “catch-up” whereby its immune system must respond to the emergence of each new antigenic viral variant. However, this cycle can be broken by maturation and broadening of the immune response. There is increased recognition in terms of the number of epitopes associated with cell-mediated immune responses while humoral immune responses evolve from low-avidity interactions with linear epitopes, to high-avidity binding, to more conformational epitopes. Furthermore, transition from the chronic to the inapparent clinical phase of EIA is associated with a switch from strain-specific to cross-reactive neutralizing antibodies. If present at high enough titer before infection, these cross reactive or broadly neutralizing antibodies have the potential to protect against genetically diverse EIAV strains. Overall, the fact active immune responses are required for maintenance of the inapparent carrier state is demonstrated by the fact immunosuppression with corticosteroids results in a significant increase in plasma-associated viral loads and, very often, recrudescence of clinical disease.

**DIAGNOSIS**

As no pathognomonic signs or lesions exist for EIA, reliance on laboratory tests is imperative. Traditional virus isolation techniques using equine monocyte–derived macrophage cultures are not practical because of viability coupled with sensitivity...
issues, and no laboratories offer this service. Several systems based on the polymerase chain reaction (PCR) technique have been developed to detect EIAV genetic material (directly in the case of proviral DNA or coupled with an RT step with viral RNA), and some have been used successfully with field isolates.\textsuperscript{38,65–68} However, before these techniques can be adopted routinely it must be shown: (1) that the primers (and probes) used in these assays are located in highly conserved regions of the viral genome, as variation in these sequences can either prevent or significantly reduce the sensitivity of detection; and (2) that PCR-based techniques are sensitive enough to detect the extremely low levels of EIAV-specific nucleic acids present in some inapparent carrier animals. Unfortunately there is no conclusive evidence demonstrating that any of the PCR-based assays described to date meets these criteria. Therefore, current diagnostic techniques for EIA are reliant on serologic detection (see \textit{Fig. 1C}). Although this is an indirect approach and is unable to detect recent infections before the development of antibodies, it is the only viable option at present.

In many countries the only officially recognized test for diagnosis of EIAV infections is the AGIDT. However, in some countries such as the United States, several commercial ELISA-based tests are also approved for use. Furthermore, the United States permits the immunoblot (or Western blot) assay to be used as a supplemental test at specific reference laboratories (National Veterinary Services Laboratories and University of Kentucky) to reach consensus when other diagnostic tests have yielded contradictory results. The immunoblot test has proved to be highly sensitive and capable of detecting antibodies directed against 3 major EIAV antigens (p26, gp90, gp45; see \textit{Fig. 1C}).

Although the AGIDT is highly specific, it is relatively insensitive. Recent studies conducted in Italy demonstrated that the number of EIA positive cases identified increased by 17% when serum samples were screened by ELISA instead of AGIDT.\textsuperscript{69,70} Results from these studies have prompted the development of a 3-tiered testing scheme for EIA. In this scheme, all samples are screened by ELISA tests with those found positive for EIA are confirmed by AGIDT. In the few cases where these results do not agree, additional immunoblot tests are performed. Thus, the sensitivity of ELISA tests is combined with the specificity of the AGIDT and the power of the immunoblot test for the accurate diagnosis of EIA.

\textbf{THERAPEUTIC STRATEGIES}

Supportive therapy may be administered to aid in recovery from febrile episodes and associated signs. Treatment with corticosteroids is contraindicated because of the resultant increase in viral load and clinical disease. Because supportive therapy has no effect on the virus itself and the virus persists for life, most bodies including the American Association of Equine Practitioners (AAEP) recommend the humane destruction of EIAV test–positive equids.

\textbf{CONTROL STRATEGIES}

There are currently no vaccines against EIAV in clinical use. Major impediments to developing a uniformly effective EIAV vaccine include tremendous strain diversity worldwide, antigenic variation, neutralization resistance, periodic viral latency, and proviral integration into the host genome. Although experimental inactivated, subunit, and recombinant vaccines have had variable or disappointing results, an experimental live attenuated vaccine has shown efficacy against challenge.\textsuperscript{71–73} A live attenuated vaccine was developed in China in the 1970s by serial passage of a virulent virus strain through donkey leukocyte cultures.\textsuperscript{74} This vaccine was used in millions of Chinese horses between 1975 and 1990 and was credited with controlling EIA in China, after
which the vaccine program was discontinued.\textsuperscript{74–77} The protective immunity elicited by these live vaccines most likely involves broadly active CTL, helper T-cell, and neutralizing antibody responses. Although attenuated EIAV vaccines can protect against viremia and clinical disease, they do not guarantee sterile protection in every vaccinated, and vaccine strains can persist in vivo. Because of the interference with diagnostic testing, in addition to the potential for modified live viruses to revert to virulence (a concern for lentiviruses given their ability to mutate and recombine), vaccination is not a component of current EIA control strategies. It is possible that continued research will lead to a future vaccine, perhaps a novel viral vectored vaccine that overcomes the aforementioned obstacles. This vaccine might play a role in future EIA control programs, especially in endemic countries.

In the absence of effective immune-prophylactic approaches, breaking the cycle of transmission depends on detection and removal (euthanasia or lifelong segregation) of infected equids. Segregation generally entails application of a permanent form of visible identification coupled with keeping the infected animal 200 m from all other equids, as at this distance it is generally accepted that blood-feeding insect vectors will invariably return to the original and will not seek an alternative host should feeding be interrupted. As these required constraints cannot usually be met, most owners opt for humane destruction as recommended by the AAEP and other regulatory bodies. In some jurisdictions, mandatory destruction is required.

Insect-mediated mechanical transmission of EIAV is not efficient, as blood contamination of insect mouthparts is generally below one hundred thousandth of a milliliter of blood. This figure is especially relevant in the case of inapparent carriers where viremia levels may be less than one 50\% horse-infective dose per milliliter (HID\textsubscript{50}/mL). In limited studies under field conditions whereby human involvement was strictly controlled, transmission between adult reproductively active inapparent carriers and sentinels was minimal and progeny were raised free of the infection at a high rate, even in areas of high vector pressures. However, intervention by man can radically alter this situation, especially in areas of the world where education about transmission of infectious diseases is lacking. That being said, even the best education can be ignored, as most cases in well-publicized outbreaks in 2006 appeared to be mediated by man, including veterinarians.

If control of EIA in highly mobile populations (where testing is required in most areas) is the goal, the test and segregate/remove strategy has been proved to be very successful (witness the statistics in the United States available in the USDA video\textsuperscript{78}). If eradication of EIA is the goal, different approaches must be taken. This statement is made because in many countries of the world where EIA is endemic, working equid inapparent carriers may be required as agricultural animals, and their testing and removal without compensation may not be possible or practical. In these circumstances, other strategies must be used.

\textbf{SUMMARY}

Four questions are posed in the introduction of this review. It is hoped that it will be apparent from the foregoing discussion that almost all new cases of EIA are inapparent carriers and are associated with host-mediated, long-term control of viral replication. Although the mechanisms associated with this control are not fully understood, they depend on active immune responses, as shown by higher viral loads and frequent recrudescence of disease following corticosteroid induced immunosuppression.

EIA control programs in the United States have been effective, although they are now at a crossroads in that the mobile and tested population is mostly segregated.
and, therefore, at a low risk from untested equids that constitute the remaining reservoir for EIAV. Consequently, the goals of testing should be reexamined; “smarter” testing should be conducted by increasing the intervals between tests for frequently tested equid populations and the development of strategies for increased testing of the untested reservoir population. In many areas, required testing at change of ownership has proved invaluable in the identification of new cases. In areas of the world where working equids are still important agricultural animals and EIA is endemic, strategies other than destruction without compensation must be developed if control of EIA in more than localized situations is the goal.

Recent evidence, particularly from the Italian National Surveillance Program, demonstrates that EIA diagnostic approaches based on AGIDT alone can generate up to 17% false-negative results. In this situation veterinarians are clearly not doing their best to detect EIA when samples are submitted to the laboratory. Although our armamentarium of laboratory tools for EIA diagnostics is currently limited to serologic tests, accuracy can be dramatically improved by adoption of a 3-tier system in which samples are initially screened by EIA-ELISA, with test-positives confirmed by AGIDT and those producing discordant results analyzed using the immunoblot test. However, even the best serologic diagnostic tests cannot detect very recent infections before humoral responses have developed. Therefore, additional research is required to improve direct detection techniques such as PCR-based methods for amplification of EIAV genetic material.

Finally, what can veterinarians do in their practices and hospitals to reduce the chance that they are contributing to the spread of blood-borne infections? The most important recommendation is to assume that all equid contacts are infected with EIAV. Although this seems to be out of proportion to the risk, it is the authors’ belief that making this assumption is the only way to effectively adopt and faithfully follow methods designed to minimize and eliminate the risk. This point was brought into sharp focus during the outbreak of EIA in Ireland in 2006. Unfortunately the virus was not on the radar, as there had not been a serologic test–positive case in the country since the 1970s. As a result, several horses became infected through iatrogenic means. The equine community would be wise to develop and adopt standard or universal precautions similar to those adopted after the advent of AIDS, especially in reference to transmission of EIA, piroplasmosis, Theiler disease, and other blood-borne conditions.

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