United States Department of Agriculture

Animal and Plant Health Inspection Service

Program Aid No. 1707

Equine Infectious Anemia: 2001 Update

Cover photo: What is the chance that these free-ranging equids are carriers of the equine infectious anemia (EIA) virus? This photo is used to emphasize that control of EIA is dependent on finding carriers of the EIA virus through testing of privately and publicly owned equids and restricting the movement of those found to be test-positive. *(APHIS photo by Ann Czapiewski.)*

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Note: The July 2004 reissuance of this publication is somewhat shorter than the 2001 original and contains new photographs. Web site references and internal dates have also been revised.

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Equine Infectious Anemia: 2001 Update

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Introduction

Equine infectious anemia (EIA) has been recognized as a major infectious disease of equines for more than 150 years. Since 1970, tools have been available to identify persistently infected carriers of the equine infectious anemia virus (EIAV). Testing of serum for antibodies to EIAV makes it possible to accurately monitor equines for the infection. The tests commonly used are the agar gel immunodiffusion (AGID or Coggins) test and several enzyme-linked immunosorbent assay (ELISA) tests. In many parts of the world, testing and removal of carriers have become routine and/or required and have provided a measure of protection against exposure to the virus because equines are the only known reservoir of infection.

In 1996, the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) published and distributed more than 3,000 copies of a video brochure package entitled "Equine Infectious Anemia: How To Protect Your Horse." The brochure in that package, "EIA: a Status Report on Its Control," contained much basic information on the lentivirus that causes EIA and on its transmission and control. The 1996 brochure is
available on the Internet at <http://www.aphis.usda.gov/vs/nahps/equine/eia/eia-1996.pdf>.

In this brochure, we discuss what has been learned about EIA since 1996, what effect that information has had on determining the best approaches to controlling EIA in the field, and what additional actions are needed to further the control of EIA nationally. We have also updated the 1996 bibliography here. We also reiterate and debunk some of the popular myths about EIA that have led to confusion and overreaction by those forced to deal with this disease for the first time.

At the Federal level, the requirements for testing horses for interstate movement are explained at Title 9, Code of Federal Regulations (9 CFR), section 75.4. In addition, 9 CFR 75.4 outlines the procedures for the recognition of laboratories and personnel as qualified to conduct EIA testing. The physical facilities of the laboratory must be inspected, and personnel who perform EIA tests must complete an approved training course and demonstrate individual proficiency in conducting EIA tests.

Within-State regulations concerning EIA are the jurisdiction of each State, and control of its spread remains a high priority for most State regulatory agencies. Control is predicated on finding the persistently infected equine carriers of EIAV and controlling their movement. In many jurisdictions, destruction of the carrier is recommended or mandated. As most of these carriers are without clinical signs suggestive of EIA at the time the horses are tested, some owners resist testing and/or acting on this recommendation.

Here we attempt to put these issues in perspective and offer several proposals to better control the spread of EIA.

Ten Useful Facts About EIA

1. Equine species (horses, donkeys, mules, etc.) are the only ones in which EIAV replicates.
2. When equines become infected, they produce antibodies against EIAV.
3. Once infected with EIAV, equines remain infected for life.
4. Some strains of EIAV kill rapidly, and some induce severe chronic disease, but many field strains present today appear to induce few or no overt clinical signs of disease in equines.
5. EIAV is a lentivirus and mutates at a high rate.
6. All strains of EIAV are believed to have the genetic potential to induce disease in equines.
7. EIA is a bloodborne infection: transmission occurs by transfer of blood between equines.
8. Blood-feeding insects (especially horse flies and deer flies) are important natural vectors of EIAV.
9. Separating infected from uninfected horses by at least 200 yards is an effective way to break mechanical transmission of EIAV by insects.
10. Serological testing to identify EIAV carriers is an important tool in controlling EIA.

Figure 2.

Popular Myths and Facts About EIA

Myth #1. EIA is a contagious disease.

Facts: EIA is an infectious disease (it is caused by the invasion and multiplication of the EIAV in tissues of the equine), but it is not thought to be contagious. That is, the infection is not directly transmitted from one equine to another; it can spread only with the intervention of vectors, e.g., insects or humans. Many publications incorrectly refer to EIA as a contagious disease. Perpetuation of this myth, or imprecise use of the terms “contagious” and “infectious,” undoubtedly leads to increased fear of the infection and disease. EIA is most accurately described as a bloodborne infection.

Myth #2. EIAV induces disease and death in a high percentage of infected equines.

Facts: Although EIAV can induce severe disease with a high mortality rate, most field strains of EIAV found in test-positive carriers today are not associated with overt clinical disease. EIAV-infected horses usually look healthy. In part, the current low rate of severe clinical EIA is related to the testing and removal of reactors with clinical disease over the past 25 years. The EIAV strains with the greatest potential to induce severe disease have been selected against over the years.

Myth #3. EIAV spreads rapidly through a population.

Facts: Transmission of EIAV is predictable only in the sense that infected equines persistently carry virus in their blood. If enough blood is transferred to a second equine, the virus will initiate an infection. In the absence of humans, transmission of EIAV requires the transfer of blood (or possibly other virus-rich secretions) between equines in close proximity, generally via blood-feeding insects. The probability and rate of transmission of EIAV in equine populations are multifactorial but highest when these three conditions are present: (1) the level of EIAV in the blood is high (i.e., during clinical disease), (2) blood-feeding vectors are abundant, and (3) equines are crowded. In some cases, the spread of EIAV from carrier horses has been explosive. In others, there has been no transmission over periods of years. Transmission of EIAV is a chance phenomenon. As EIAV mutates at a high rate, for purposes of disease control every virus strain is assumed to have the potential to initiate explosive epidemics. Why take chances if horse owners can test and avoid reservoirs of the infection?

Myth #4. Quarantine farms for EIA reactors are dangerous and should not be permitted.

Facts: There is no scientific basis for the fear of acquiring EIA from known test-positive equines in safe quarantine (>200 yards from other equines). The chance of acquiring the virus by commingling with untested equines in an area where only 1 in 10,000 equines is infected is significantly greater than the chance of acquiring it from the quarantined equine directly, maybe more than a millionfold greater. When fear subsides and logic prevails, quarantine farms might become acceptable alternatives to mandated destruction.

EIA continues to challenge horse owners because they cannot tell if the equines they encounter have ever been tested for EIA or—if they have been tested—with what other horses theirs have commingled since the test. If infected equines commingle with healthy ones, nobody knows for sure how likely it is that the infection will be transmitted.

The following are suggestions on how to reduce significantly the risk of acquiring EIA in the United States. The remainder of this brochure is devoted mainly to discussing these actions.

1. Promote and implement the adoption of effective, permanent methods to identify individual equines.
2. Encourage State programs to enhance the public understanding of EIA.
3. Develop novel cooperative programs that promote areawide testing of equines.
4. Consider the establishment of quarantine farms to permit the safe containment of EIAV-infected equines.
5. Obtain a better understanding of the biological risks of EIAV transmission, and then apply this knowledge to develop quantitative risk-assessment models on which to base regulatory decisions.

Implementing these five suggestions will help the horse industry find solutions to problems such as:

How can EIA control methods be modified to maintain or increase surveillance for EIA and give a higher benefit-to-cost ratio to the industry? Can science do a better job of finding reservoirs of EIAV that have not been tested? How can veterinarians and government regulations better serve the equine community in relation to EIA?

EIA Literature

EIAV as a virus is better known today, and the genesis of some of the clinical signs is better understood, but control strategies are essentially unchanged since 1972.

The vast majority of articles published on EIA and EIAV during the past 8 years (found by searching the National Center for Biotechnology Information's "PubMed" Web site at http://www.ncbi.nlm.nih.gov) focus on understanding the basic molecular aspects and control of viral replication, generally in cell cultures in the laboratory. Much of the research was conducted because EIAV is genetically related to the human immunodeficiency virus (HIV), not because EIAV causes infections in horses.

There still are modestly funded efforts to define how EIAV causes disease in horses, to identify the immune effectors that help horses control viral replication, and to produce safe vaccines that would protect horses against EIAV infection if exposed. Developing a vaccine is tricky because some of the disease signs in infected horses are related to immune responses (immunopathology) shown to be stimulated by some experimental vaccines.

In the past 8 years, several groups have demonstrated that the genetic material of EIAV can be found persistently and sometimes in relatively high levels in test-positive horses without clinical signs of EIA. Such horses are called inapparent carriers. These data provide clear evidence that these inapparent carriers should be isolated or quarantined once their test status is verified.

Realities and Surprises

Despite our collective efforts over 25 years, EIA is still an unknown or an enigma to many owners. To most, it represents a problem only when it knocks at their door.

Since 1980, on average, 1 million equine samples have been tested for EIA each year in a major surveillance effort by industry, veterinarians, and regulatory agencies. In spite of this, the National Animal Health Monitoring Systems' survey of the equine industry reports that 58 percent of owners in the Midwestern States have never heard of EIA, and only 12 percent of equines in the West are tested for it. The same survey also reports that EIA is perceived as the most important viral infectious disease of equines nationally.

EIA appears to be of greatest concern in areas of the country where the infection has occurred historically at highest frequency (States in the Southeast) or where regulations have helped to inculcate a sense of urgency about EIA control (States in the Northeast). For example, a review of the surveillance statistics reported for fiscal year 1998 reveals the significance of State regulations. In that period, more than 179,000 equine samples were tested in the Northeastern States, and only 3 test-positive horses were found. Extensive testing, comprehensive regulations, and aggressive followup on new infections for the past 25 years have significantly reduced the risk of contacting a test-positive equine in the Northeast. State regulations are available at the Web site http://www.aphis.usda.gov/vs/sregs.

Regulations, Laws, and Challenges

Once an accurate test to identify carriers of EIAV became available, the Infectious Diseases of Horses Committee (IDoHC) of the United States Animal Health Association (USAHA) formulated recommendations for the control of EIA. Those recommendations have been used by most authorities interested in promulgating regulations or laws to control the infection. In 1997, a set of guidelines for EIA control was drawn up by a working group from the IDoHC. These guidelines were adopted by USDA and used in the brochure "Equine Infectious Anemia, Uniform Methods and Rules, Effective January 1, 1998" to help standardize control recommendations in different jurisdictions. A revised version of the publication, under the same title but showing an effective date of March 1, 2002, was published in March 2002. It is available for download from the Web in .pdf format at <http://www.aphis.usda.gov/oa/pubs/eiaumr.pdf>.

In 1993, Louisiana made regulatory history by taking the bold step of requiring permanent identification and annual EIA testing of all equines. Although Louisiana authorities estimate that fewer than 40 percent of equines are actually tested every year, the public has responded favorably to the increased emphasis on EIA control. Finding methods to increase compliance beyond 40 percent remains a formidable challenge.

During the past 5 years, Arkansas and Texas have enacted dramatic changes in State regulations following Louisiana's lead. Besides identification and testing, Louisiana law also requires the destruction of test-positive equines (or removal to a research facility) and the quarantine and testing of equines that have been within 200 yards of the reactor.

Legislation passed in Arkansas in 1997 requires that every equine have an EIA test annually and whenever there is a change of ownership. The law also mandates

destruction of each reactor (or removal to a research facility) and requires State authorities to quarantine and test all equines that have been within 440 yards of the reactor.

In 1999, and after appropriate public debate, the legislation was modified further to broaden a good-neighbor provision. In Arkansas, any horse owner can now request that the State authorities verify that any other owner had tested their equines during the past year. If no evidence of a current test is produced, then the State is mandated to test the animals. This provision is novel, and time will tell if it increases compliance and goodwill among owners or has the opposite effect.

The regulations in Texas are similar in that they require testing for congregation and for change of ownership, as well as requiring State authorities to perform traceback testing on all equines within 200 yards of reactors. The number of samples tested for EIA increased dramatically after these regulations were adopted.

In summary, three States where EIA has occurred frequently in the past have instituted comprehensive regulations or laws that require testing each year and when there is a change of ownership. Regulations for EIA and other equine diseases change frequently, so we advise a careful review of individual State requirements before moving equines to another State.

Distribution of EIA

Since testing for EIA was initiated, the vast majority of new cases have been found each year in the area we have designated the Hot Zone. In the past 5 years, some anomalous case distributions have been noted, mostly in groups of horses on specific premises or in specific regions being tested for EIA for the first time. For example, the increased rate of positive tests in 1998 in Indiana was traced to 32 new cases on 1 farm; in Arizona to 15 new cases on 1 premises; and in Utah to 127 new cases found in free-roaming horses.

Results from two free-roaming populations of horses in diverse geographic areas deserve further discussion. In North Carolina in 1996, EIA was discovered in horses on Shackleford Banks, a barrier island in the Cape Lookout National Seashore, managed by the National Park Service. On the first testing of this isolated, insular, free-roaming population, 41 percent (76/184) of the horses were reactors. If the 10 test-positive foals of reactor mares are subtracted, the reactor rate of the population drops to 38 percent.

In Utah in 1998, reactor rates in one hot area were similar to those seen in North Carolina. There the rate was 49.5 percent (53/107) and 44 percent if the test-positive foals of reactor mares are subtracted.

In both of these cases, it appears that EIA had been present in the population for years and had stabilized, as the rate of infection increased with age. In both cases, the EIA test-positive rate was exceptionally high in mature stallions: 88 percent (16 of the 18 dominant herd stallions) on Shackleford Banks and 89 percent (17 of 19 stallions >3 years of age) in the index area in Utah.

Figure 3—Horses on the Outer Banks of North Carolina represent a unique national resource. EIA was found on Shackleford Banks for the first time in 1996. Removal of test-positive horses and repeated annual testing since 1998 suggest that EIA has been eradicated from this population. The Shackleford horse population remains at risk of acquiring EIA, however, mainly from untested horses on the mainland or other barrier islands that could swim to Shackleford during storms. The risk can be eliminated only by testing and maintaining segregation from all known carriers.

In order to find EIA, horses must be tested for it. Undertested areas may have carriers, and transmission may be slow or epidemic. In two free-roaming horse populations, about 40 percent of animals tested positive for EIA.

These data suggest that stallion behavior plays a role in increasing the risk of EIAV transmission between horses. The most obvious behavior to be investigated is the combative behavior between stallions associated with establishing and defending harems.

In both of these populations, most test-positive horses were inapparent carriers. In Utah, however, two stallions appeared to have signs of the chronic form of EIA, and one died during its first day of captivity. Also, EIA appeared to decrease reproductive success in the Utah group. The rate of foaling in the test-positive group of mature mares was 52 percent (12/23) compared to 87 percent (13 of 15) in test-negative mares from the same area.

Foals and EIA: the Possibilities and the Realities

In many jurisdictions, young foals are exempted from testing if the mare has tested negative for EIA. If mares test positive, their foals will generally be test-positive as well. In these cases, the foals could be infected or merely carrying passive antibodies to EIAV obtained in colostrum. The risk of transmission to the foal is assumed to be higher if the mare has recently experienced clinical signs of EIA. When the mare is a stable inapparent carrier of EIAV, field studies have shown that a high percentage (>90 percent) of foals can be raised uninfected, even when weaned at 5 to 8 months of age in areas with high populations of insect vectors. Recent studies in Oklahoma have extended scientific knowledge on this subject. Over 3 years, more than 97 percent of the foals of test-positive Choctaw or Cherokee bloodlines have been raised free of the infection.

In foals of test-positive mares, declining levels of antibody to EIAV and sensitive polymerase chain reaction tests showing no viral RNA are good indicators that the foals are not infected. These foals should be in isolation or quarantine from all sources of EIAV for at least 60 days *before release.*

Accuracy of Testing

In order to control EIA effectively, the most accurate tests available must be used. The agar gel immunodiffusion (AGID), or Coggins, test has been approved since 1972. Are better tests available today?

The answer is a qualified no. It is true that, to give a positive result, the AGID assay requires more antibody than an ELISA test. The AGID test for EIA, though, is the only serologic test whose results correlate positively with results of the horse inoculation test, which tests for the virus itself. Together, the AGID and ELISA tests for EIA give veterinarians and owners better options. By using more than one type of test, owners can essentially maximize the advantages and minimize the disadvantages of each individual test. The next several paragraphs cover the advantages and disadvantages of the AGID test and the ELISA test.

Advantages of the AGID Test

1. The AGID test is specific, and nonspecific reactions can be distinguished from EIAV-specific reactions.
2. The AGID test correlates with the virus content measured by the horse inoculation test.
3. The AGID test has international acceptance.
4. There has been nearly 30 years of experience with the AGID test.

Disadvantages of the AGID Test

1. The AGID test requires skilled, subjective interpretation of results.
2. Results are not available for at least 24 hours.
3. The end-user must make up plates with agar, and errors can lead to decreased accuracy of test results.

Advantages of ELISA Tests

1. All ELISA test kits available today incorporate the same virus-specific antigen as in the AGID test; one test kit also includes an additional virus-specific antigen.
2. ELISA tests are easier to interpret than the AGID test.
3. Results can be calculated objectively if assay color development is measured with a spectrophotometer.
4. ELISA tests are more rapid than the AGID test; results are available within minutes.

Disadvantages of ELISA Tests

1. The ELISA method can be less specific than the AGID test in that false-positive results may occur. Positive ELISA results MUST be confirmed with an AGID test.
2. ELISA tests are more expensive per sample.
3. ELISA results are not accepted in some States or for international travel [as of July 2004].

Advantages of Having More Than One Type of Test for Diagnosis of EIA

1. Increased accuracy and power are obtained by using several antigens (similar to the confirmatory tests for HIV).
2. The impact of the majority of human errors is minimized.

The AGID test remains the test of choice for EIA, and because it has been correlated with viral presence, it will remain the standard against which all other tests are compared. Thus, if a positive reaction is noted in an ELISA assay, it must be confirmed by an AGID test before a positive result is released and acted upon. In the vast majority of cases, there is agreement between test results from all available kits.

Sometimes discordant test results occur. Discordants are results that differ from test to test, e.g., between ELISA and AGID, from laboratory to laboratory, from test run to test run with the same test method in the same laboratory, or between two samples from the same animal. When discordant results are seen, which test or tests should be used as the definitive test? And most importantly, are differences in test results related to biological phenomena or to differences in human performance?

All licensed kits are standardized to an equivalent sensitivity, but when comparing any two tests, interpreters sometimes come to different conclusions. These occur most often when samples have reactions that are near the cutoff point in ELISA or at the limit of detection in AGID.

A frequent biological reason for divergent test results is that the horse in question has very low levels of antibody against EIAV. The horse may have recently been exposed and is just beginning to produce antibodies. Rarely, inapparent carriers have consistently low antibody levels against EIAV, suggesting a low level of viral replication and low stimulation. In both cases, the end result is the same: the antibody level is so low that it escapes detection in some routine tests. The next most common reason for divergent test results is that the horse has been exposed to a related agent that cross-reacts with antigens of EIAV. Low levels of specific antibodies would result primarily in false-negative AGID reactions, while nonspecific antibodies would result primarily in false-positive ELISA results. Fortunately these types of reactions have been observed at a very low rate.

In order to minimize human errors in testing, USDA–APHIS–Veterinary Services (VS) mandates that, prior to receiving approval to conduct EIA tests, a technician must have specific training and must demonstrate individual competence. This governmental oversight is outlined in 9 CFR 75.4. In addition, APHIS monitors laboratory performance through annual proficiency tests which must be completed with accuracy by all approved laboratories. Nonetheless, human errors in testing for EIA and reporting the results can occur at multiple points. First, a technical error could have been made in testing the sample. For example, errors in preparing agar for the AGID test or in washing ELISA test wells may lead to incorrect results. Second, the technician may be uncomfortable or unwilling to interpret and report as positive a sample with a very weak

AGID test reaction. Third, loss of sample integrity could occur by cross contamination or mislabeling. When laboratories are testing large numbers of samples, consistent attention to detail is required to ensure that all tests are properly conducted and reported.

For samples with positive reactions, many laboratories confirm the initial reaction by testing the sample a second time as originally run and also in other approved types of tests before issuing the positive test report. We recommend collecting a second sample from each reactor to confirm the accuracy and reproducibility of test results. This is important to ensure the integrity of the first report, especially to minimize the impact of human error.

Biologically false-negative reactions in EIA testing are extremely rare. The number of EIAV-infected horses estimated to be reported falsely as negative is less than 1 percent of the number of reactor horses found each year. The number of false reactions can be decreased if diagnosticians capitalize on the strengths of the available tests for EIA. The impact of these false-negative equines is thought to be significantly lower than that of the millions of equines that remain untested.

Checks and cross-checks are used to minimize the impact of inaccurate reports. Repeat confirmation testing of positives should be mandatory. Continued education of diagnosticians should be required; this should include the routine submission of challenging samples to be tested blindly and reported.

Risks of Acquiring EIA (from highest to lowest)

- **Never testing for EIA**
- **Acquiring a new animal**
- **Adding a new animal to the facility or band without a test**
- **Commingling with animals with unknown test status**
- **Being within 200 yards of animals with unknown test status**
- **Adding a new animal with an old negative test but whose contacts were untested**
- **Being near safely quarantined reactors but at a distance greater than 200 yards**

Most new cases of EIA found in the United States each year are in or have originated from equines that have never been tested for EIA. Eventually, through attrition, the untested reservoirs of EIA will be discovered. The period of time required to reach that goal can be reduced significantly through the design and application of industrywide standards and cooperative programs.

Because of the low risk of acquiring EIA in closed herds where all horses have tested negative for it, testing at frequent intervals may seem unnecessary but is arguably prudent.

The horse industry should require a negative test for all changes of ownership, have the States inform the public of the rule, and enforce penalties on those sellers who ignore it.

Figure 4—

Final Word: Federal Involvement in EIA Control

This brochure covers what the authors have learned about EIA since 1996 and what might be done to further control EIA at the State level. However, involvement at the Federal level merits discussion. USDA–APHIS–VS remains committed to the national EIA control program and proposes to improve the program as follows:

1. Provide the most current EIA educational materials, including:

- A regularly updated version of this EIA brochure,
- A regularly updated version of the EIA Uniform Methods and Rules (UM&R),
- A current edition of the EIA video, and
- A current EIA laboratory guide.

2. Improve national EIA surveillance by

- Accounting for quarantined EIA reactors,
- Increasing the frequency and accuracy of laboratory reporting,
- Improving tracking system(s) for out-of-State testing, and
- Incorporating portions of the UM&R into the Code of Federal Regulations.

VS is open to suggestions from the horse industry, State regulatory officials, and other interested parties concerning what more USDA can do to control EIA in the United States.

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Since 1996, USDA–APHIS–VS has published several documents on the subject of EIA and is maintaining the most important of these on the World Wide Web. Our original black-and-white brochure, "Equine Infectious Anemia: Status Report on Its Control, 1996" (APHIS 91–55–032), is available in .pdf format at <http://www.aphis.usda.gov/nahps/equine/eia/eia-1996.pdf>. We encourage readers of the 2001 brochure on EIA to download this older publication, which contains a great deal of basic information on the disease that has not been repeated in the 2001 pamphlet or the 2004 revision of it. Finally, users who need a short, easily photocopied document about EIA may find APHIS' factsheet on this subject helpful. It can be viewed at http://www.aphis.usda.gov/lpa/pubs/fsheet_faq/_notice/fs_aheia.html and printed from a .pdf version at the blue hotlink at the bottom of that Web page.

Readers looking for more technical information on the detection and control of EIA and the transportation of equines that have tested positive for the disease may wish to consult "Equine Infectious Anemia: Uniform Methods and Rules, Effective March 1, 2002" (APHIS 91–55–064). This publication can be downloaded at <http://www.aphis.usda.gov/oa/pubs/eiaumr.pdf>.

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