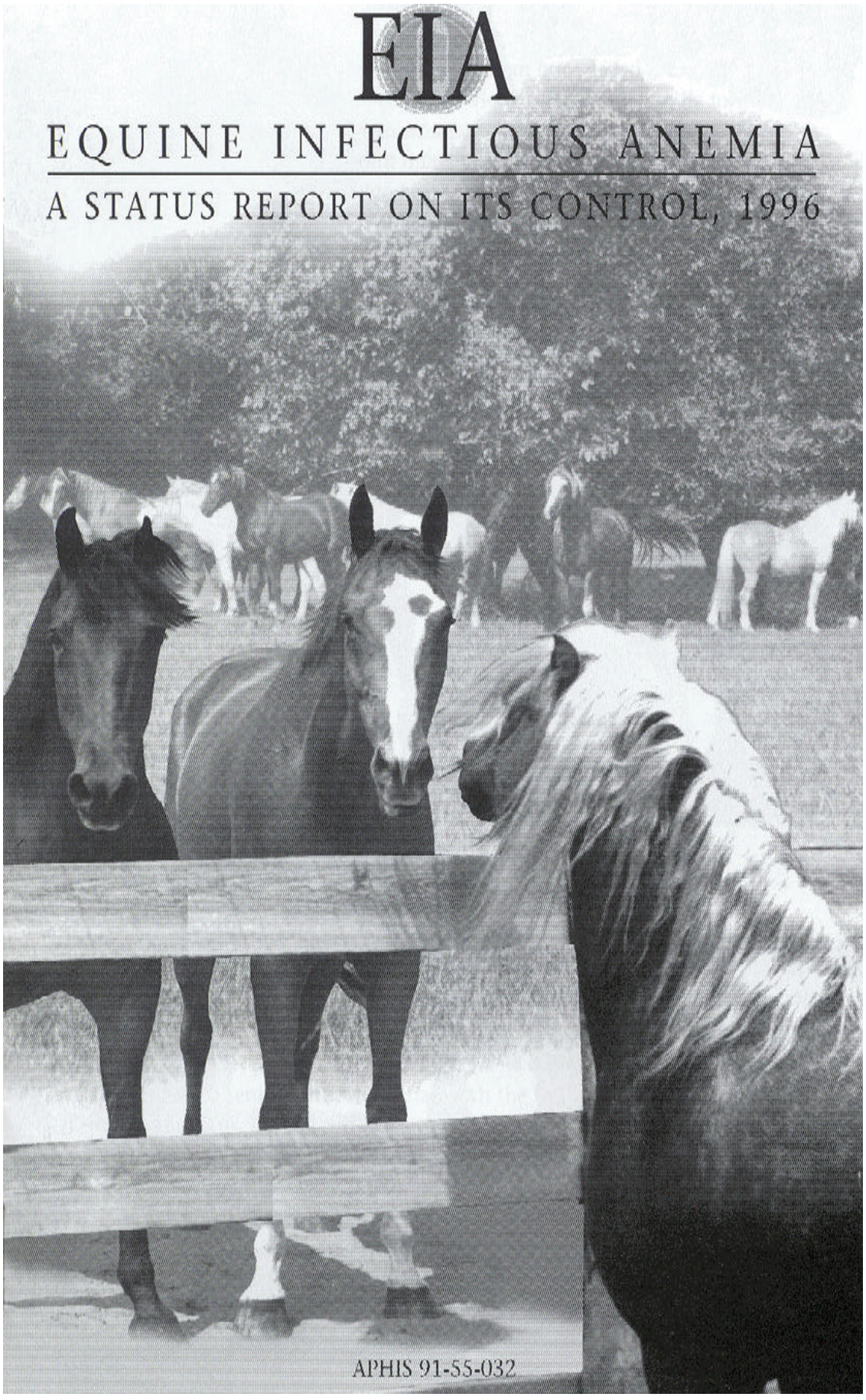
EIA

EQUINE INFECTIOUS ANEMIA

A STATUS REPORT ON ITS CONTROL, 1996



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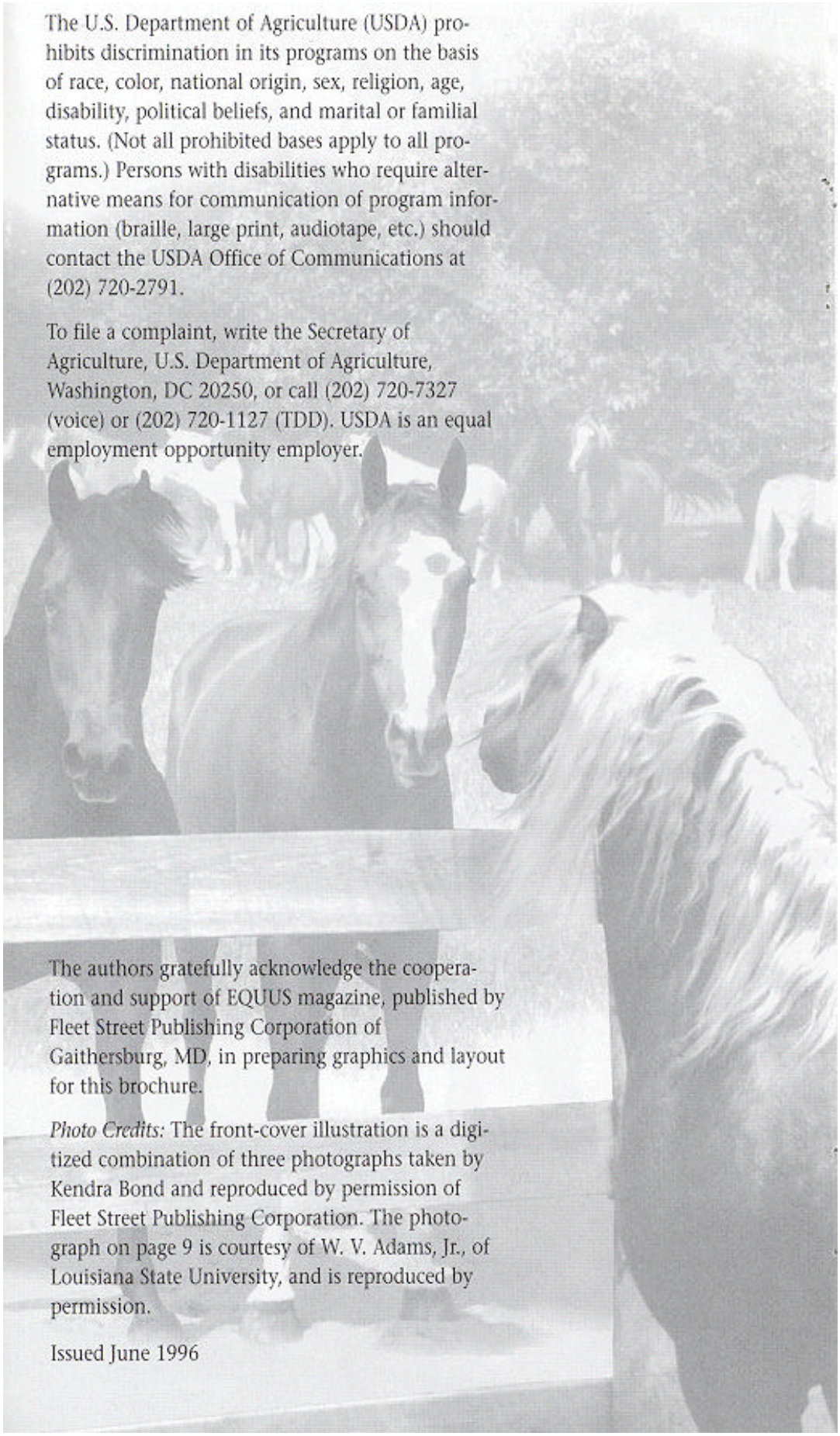

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EIA

Equine Infectious Anemia

A Status Report on Its Control, 1996

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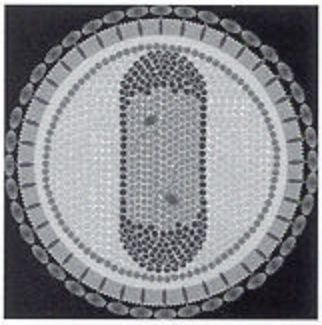


Introduction

Assume the photograph on the cover of this brochure shows *your* horse welcoming a new stallion to the neighborhood at a safe distance across the fence. What risks does the new horse present? Could he transmit disease to your horse if no additional precautions are taken?

Although vaccines can help protect horses against many common infectious diseases, no vaccine is marketed for equine infectious anemia (EIA), a potentially fatal viral disease. Is the new horse a carrier? If he is infected, how likely is it that your horse will become a carrier? How can owners best protect all horses against EIA? These questions have been asked repeatedly over the years, and we felt it was time to address these issues through a series of educational tools and discussions to help set national priorities for additional safeguards.

One step in that direction is this brochure, designed to inform veterinarians, students, and horse owners about issues related to the control of EIA in the United States in 1996. Our intention is to

- describe the structure and function of the equine infectious anemia virus (EIAV),
- outline the details of its transmission, and
- describe methods used to diagnose the infection.

It has been possible to control EIA in horses through the use of effective serologic procedures with cooperation from veterinarians, horse owners, and State and Federal disease-control authorities. We hope that this brochure and a companion video, both produced and distributed by the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS), will promote continued cooperation and lead to a better understanding of the relationship between the horse and EIAV. We believe that this educational outreach effort will lead to an environment where U.S. Horses are at a lower risk to acquire this infection.

We intend to build on this document on a regular basis by providing data on the distribution of newly diagnosed cases, by reporting progress made in defining additional reservoirs of the infection, by outlining methods of detecting infected equids, and by announcing exciting discoveries through research on this viral disease.

EIA: The Virus and Its Traits

General Information

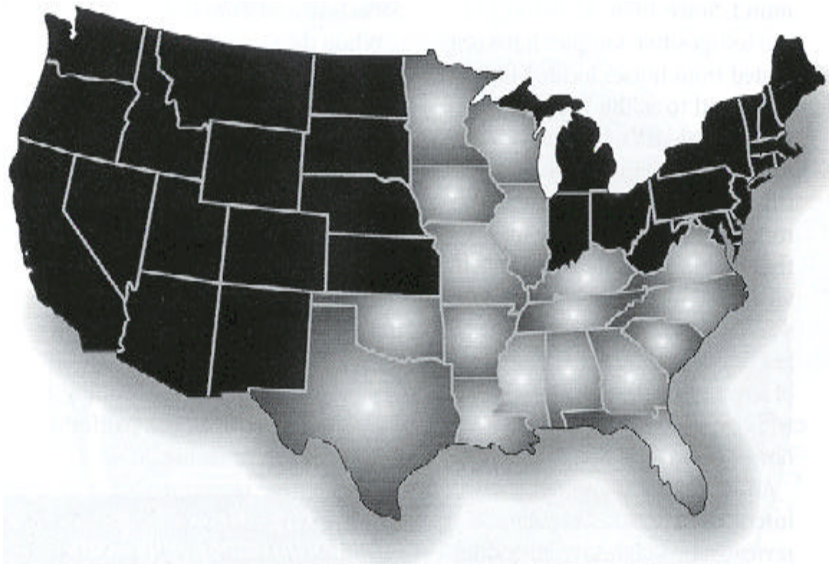
EIA is a viral disease of members of the horse family. Identified in France in 1843 and first tentatively diagnosed in the United States in 1888, it has commanded a great deal of attention over the years.

The EIAV is categorized as a retrovirus: it contains the genetic material RNA, which it uses to produce DNA. This DNA is then incorporated into the genetic makeup of infected cells.

EIA is significant historically because it si the first disease of horses proven to be caused by a "Filterable virus" - one that can survive a special laboratory filtering procedure and remain infectious (Vallee and Carre 1904). EIA is the first retrovirus-induced disease proven to be transmitted by insects (Stein et al. 1942). And EIAV is the first persistent virus for which "antigenic drift" was defined (Kono 1972). (Antigenic drift is the virus' ability to change its form sufficiently so that it is no longer vulnerable to existing antibodies.) Finally, EIA is the first retrovirus-induced disease for which a diagnostic test was approved (Coggins and norcross 1972).

More recently, the EIAV has been recognized as a lentivirus, the type that causes slowly progressive, often fatal diseases. It is a close relative to the human immunodeficiency virus (HIV), which causes acquired immunodeficiency syndrome (AIDS). In fact, EIAV was the first virus shown to be related to the HIV through cross-reaction in tests of blood serum
(Montagnier et al. 1984). These two lentiviruses share many structural and biochemical features, and EIAV is thought to serve as a useful model for many aspects of HIV research, especially for discovery of common mechanisms of immunologic control (Montelaro and Issel 1990).

Hot Zone for EIA (figure1)



An effective test for antibodies specific to EIAV was described in 1970 by Leroy Coggins, D.V.M., for collaborators (Cogins and Patten 1970) and was rapidly adopted by authorities around the world. The agar-gel immunoddiffusion (AGID) or Coggins test was shown to correlate with horse inoculation test

results for EIAV and therefore, could be used to identify EIAV carriers (Coggins et al. 1972). Because only members of the horse family were shown to be infected, programs based on serologic testing were designed and adopted to help control the spread of EIAV (Campbell 1971). Since 1972, more than 15 million blood samples have been collected from horses in the United States and tested for antibodies against EIAV (Cordes, pers, commun.), partially in response to State, Federal, and/or international regulations concerning EIA.

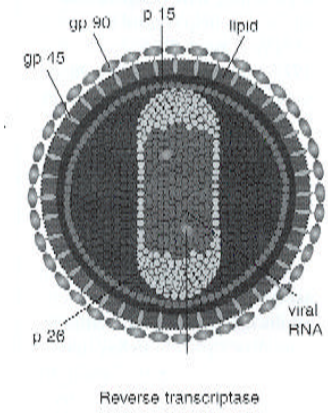
In the United States, the percentage of samples with positive results has decreased dramatically over 3 percent to less than 0.2 percent since testing was initiated in 1972 (Cordes, pers. commun.) Since 1978, 92 percent of the test-positive samples have originated from horses located in what is referred to as the "hot zone" (fig 1). The risk of becoming infected with EIAV is greatest in this region, in part because environmental conditions are ideal for the insect vectors that transmit the virus, and, presumably, because a significant number of untested reservoirs of EIAV exist. The threat of exposure to EIAV-infected horses will continue to exist wherever horses congregate.

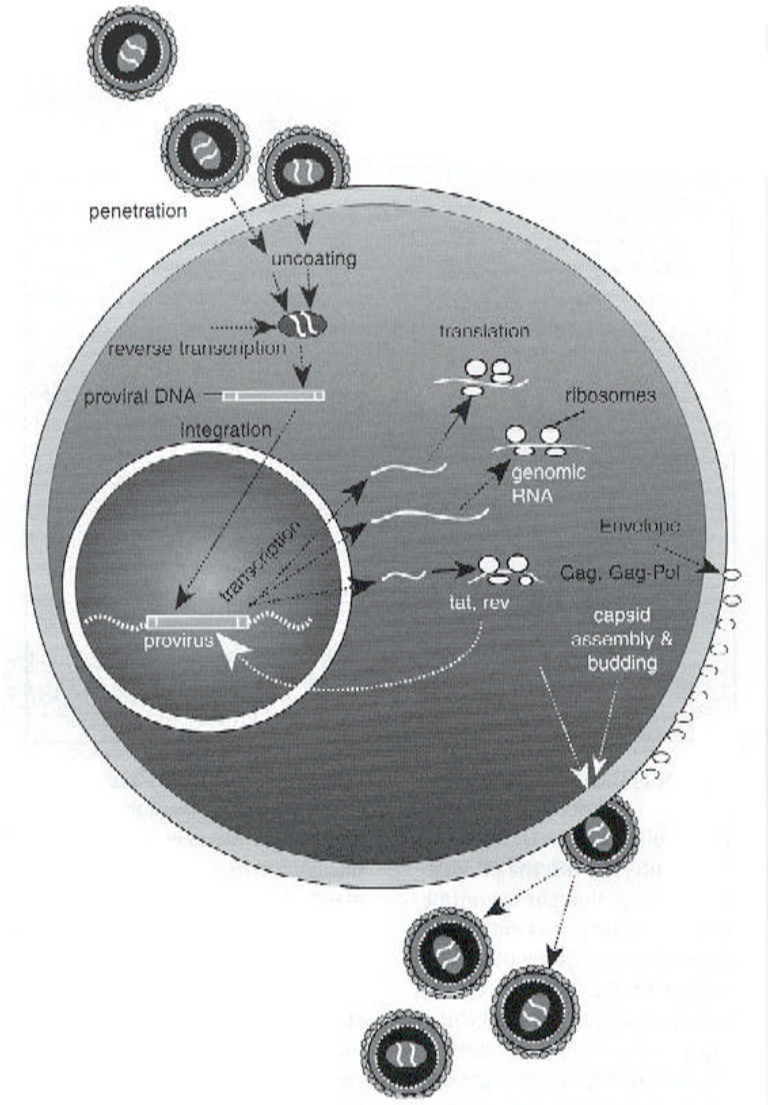
Although this pamphlet is not intended to be an exhaustive review, it is designed to introduce the database on EIAV, the infection and the disease it causes, and programs designed to control its spread. We hope that this document will augment the information published previously by USDA staff (Hourrigan and Knowles 1974), EQUUS magazine (Equine Health Publications 1977), the American Quarter Horse Association (Tashjian 1985), and the American Association of Equine Practitioners and Bayer Corporation (1995), and serve as a catalyst for additional progress in the international control of this infectious disease.

Structure and Function

While the structure and function of the EIAV particle (fig.2) are similar to those of other lentiviruses, the organization and replication of its genetic material are less complex (fig.3). The viral RNA serves as a template for the viral reverse transcriptase enzyme to catalyze the formation of a DNA copy (proviral DNA) that can integrate with the host cell genetic material. This stable integration may allow the virus to persist in the cell for the remainder of its life.

EIA Virus - (Figure2)
Major Structural Components





Viron RNA is released into the cell and, through the process of reverse transcription, a ds priviral DNA is formed that has been modified in its terminal regions. The ds proviral DNA can integrate with the host cell genome and remain latent or be transcribed to form a variety of messenger RNA's that code for the regulatory and structural proteins of EIAV. Additional full-length genomic RNA's can be packaged into new virions.

Table 1. Stuctural and regulatory proteins coded by the EIAV genome

Gene	Protein	Molecular weight	Function
gag	p26	26,000	Major capsid
	p15	15,000	Matrix
	p11	11,000	Nucleoprotein
	p9	9,000	Capsid
pol	PR		Protease
	RT		Reverse transcriptase
			RNAse H
	DU		dUTPase
	IN		Integrase
env	SU	90,000	Surface unit
	TM	45,000	Transmembrane
S1	tat		Transactivator
S2	?		?
S3	rev-like		Inhibits RNA splicing

Under optimal conditions, the proviral DNA codes for a variety of viral proteins (table 1), some of which interact with the proviral DMA and are thought to control and/or facilitate virus multiplication. Complete viral synthesis requires the transcription of several classes of viral RNA, some of which code for viral regulatory or structural proteins and some of which can be packaged with the new structural proteins into virions, which bud from the infected cell membranes.

The viral RNA is surrounded and protected by several proteins, organized into structures referred to as nucleoprotein, nucleocapsid, matrix, and envelope, with low copy numbers of the reverse transcriptase and integrase enzymes found near the RNA in the core of each particle.

Although some laboratory strains of EIAV are cultivated in fibroblastic equine cells, EIAV usually multiplies only in equine macrophages, the main target cell for all EIAV strains is vivo.

The Infection and the Disease

When horses are exposed to EIAV, they may develop severe, acute signs of disease and die within 2 to 3 weeks. This acute response is rarely seen in natural situations, where blood-feeding insects transmit low doses of virus.

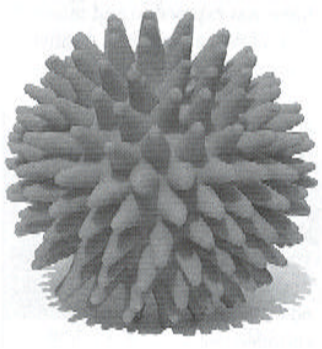
Nonetheless, this form of the disease is the most damaging and the most difficult to diagnose because the signs appear rapidly, and often only an elevated body temperature is noted.
At this early stage of the infection, the horse usually tests negative for antibodies to EIAV, and blood samples must be collected at a subsequent date (generally 10 to 14 days later) to confirm or exclude EIA as a diagnosis. During this period, it is prudent to quarantine the horse (or the farm) if EIA is strongly suspected on the basis of history or signs.

The clinical signs of the acute form of EIA are rather nonspecific; and in mild cases, the initial fever may be short lived (often less than 24 hours). As a result, horse owners and veterinarians may not observe this initial response when a horse is infected with EIAV. These infected horses often recover and continue to move freely in the population. The first indication that a horse was exposed to and infected with EIAV may well be a positive result on a routine annual test.

If the horse survives this first acute bout, it may develop a recurring clinical disease with

- **fever -** An infected horse's temperature may rise suddenly to about 105 degrees F. or**,** rarely, as high as 108 degrees F. Then it may drop back to normal for an indeterminate period until the onset of another episode.
- **petechial hemorrhages-** Minute blood-colored spots appear on the nucos membranes.
- **depression** - The horse appears more or less dejected (head hangs low) and generally listless.
- **weight loss-** The horse may refuse feed or may eat an inordinate amount but still continue an obvious decline from normal weight.
- **dependent edema-** The horse may develop swelling, evidence of fluid collecting under the skin in the legs, under the chest and other underbody surfaces.
- **anemia**- The horse's blood may experience a marked drop in its red corpuscle count and appear thin and watery. The animal may also have an irregular heartbeat, and a jugular pulse may become evident.

(Figure4)



The EIAV particle with surface projections is analogous to this squeaky dog toy. The proteins on the surface mutate at a high rate and complicate strategies for immunization.

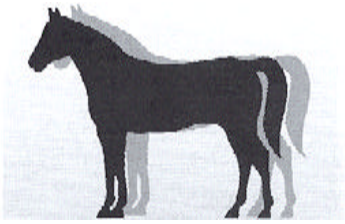
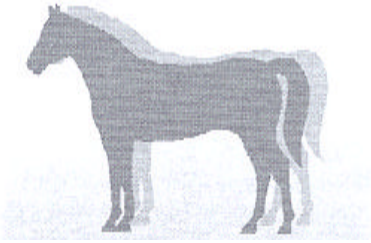
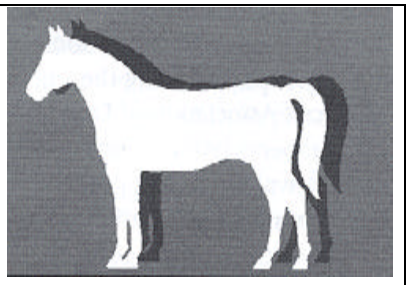
At this time, the horse will be positive for antibodies to EIAV. This chronic form of the disease is the most interesting because the successive clinical bouts are thought to be caused by new, mutant EIAV strains generated in the infected horse (Kono 1972, Payne et al. 1987). These mutants are thought to arise because of changes in the genes that code for critical surface determinants (fig. 4). The resultant structural modifications allow the mutant virus to multiply despite high levels of antibody and other immune effectors produced against the preceding strains (Rwambo et al. 1990).

The horse with chronic EIA is the classic "swamper" who has lost condition, is lethargic and anorexic, has a low hematocrit, and demonstrates a persistent decrease in the number of blood platelets (thrombocytopenia), especially coincident with fever induced by EIAV (Clabough et al. 1991).

By far the majority of horses found to be positive on serologic tests to EIAV are inapparent carriers: they show no overt clinical abnormalities as a result of infection. Their serum contains antibody against EIAV, their blood consistently contains EIAV, albeit in concentrations dramatically lower than in horses with active clinical signs of disease, and they survive as reservoirs of the infection for extended periods.

As all horses infected with EIAV are thought to remain virus carriers for life tend to become positive for antibodies to EIAV, all EIA test-positive horses are treated as if they pose the same risk for transmission of the virus, even though at certain moments there may be million-fold differences in the virus content of the blood of individual test-positive, infected horses (Issel and Foil 1991) (fig. 5). The decision to treat all test-positive horses by the same rules was reached because it is known that each infected horse may develop clinical signs of EIA upon treatment with immunosuppressive drugs and/or in response to natural stressors (Kono 1972). These clinical bouts occur in response to a lifting of immunosurveillance mechanisms within the body and the release of higher levels of virus into the blood, which increases the risk of transmission and causes fever. The mechanisms that control EIAV multiplication in the horse, therefore, are not totally effective and are subject to modification in response to the biological and chemical environment of the horse. In other words, because no one can predict accurately the risk posed by each infected horse over time, veterinarians take the conservative position and assume that each infected horse poses the same threat at all times.

Different Degrees of Infectiousness: (Figure5)

		
Acute One-fifth of a teaspoon (one milliliter) of this horse's blood contains enough virus to infect 1 million horses.	**Chronic Case** One-fifth of a teaspoon of blood from a chronic case during a feverish episode contains enough virus to infect 10,000 horses.	**Inapparent Carrier** Only one horse fly out of 6 million is likely to pick up and transmit EIAV from this horse.

An acutely ill horse harbors a substantial concentration of EIAV in its bloodstream, as does a chronic case experiencing a fever. But an inapparent carrier may have only an infinitesimal amount of virus in its blood.

In general terms, there is a low risk of acquiring EIA from an inapparent carrier of EIAV. In fact, the perceived *threat* of transmission from this source often exceeds the actual *risk* by several orders of magnitude. Many owners of healthy test-positive horses (with no history of clinical EIA) who opt to keep them a safe distance from other horses find the isolation and social stigma associated with maintaining the "swamper" unacceptable, and within a relatively short time make alternative decisions about the horse's fate. Because of these considerations, many groups, including the American Association of Equine Practitioners (1995), recommend the removal of EIA test-positive horses from the population.

How EIAV is Transmitted

EIA is considered a classic blood-borne infection. People have played an important role in EIAV transmission over the years by using blood-contaminated materials on different horses. Although this mode of transmission was more prevalent before serologic tests to identify EIAV carriers were available, it is wise for owners and veterinarians to apply the same universal precautions that are used to reduce the risk of spreading blood-borne disease agents in humans (U.S. Department of Labor 1992).

The EIAV most frequently is transmitted between horses in close proximity by large biting insects, such as horse flies and deer flies (tabanids), which inflict painful bites (Issel and Foil 1984). The bites from these tabanids stimulate defensive movement by the horse, which often results in an interruption of the blood-feeding. When interrupted, the fly is motivated to complete the feeding as soon as possible. It then attacks the same or a second host and feeds to repletion. In this manner, any infective material from the blood of the first host which is present on the mouthparts of the insect can be mechanically transmitted to the second host.

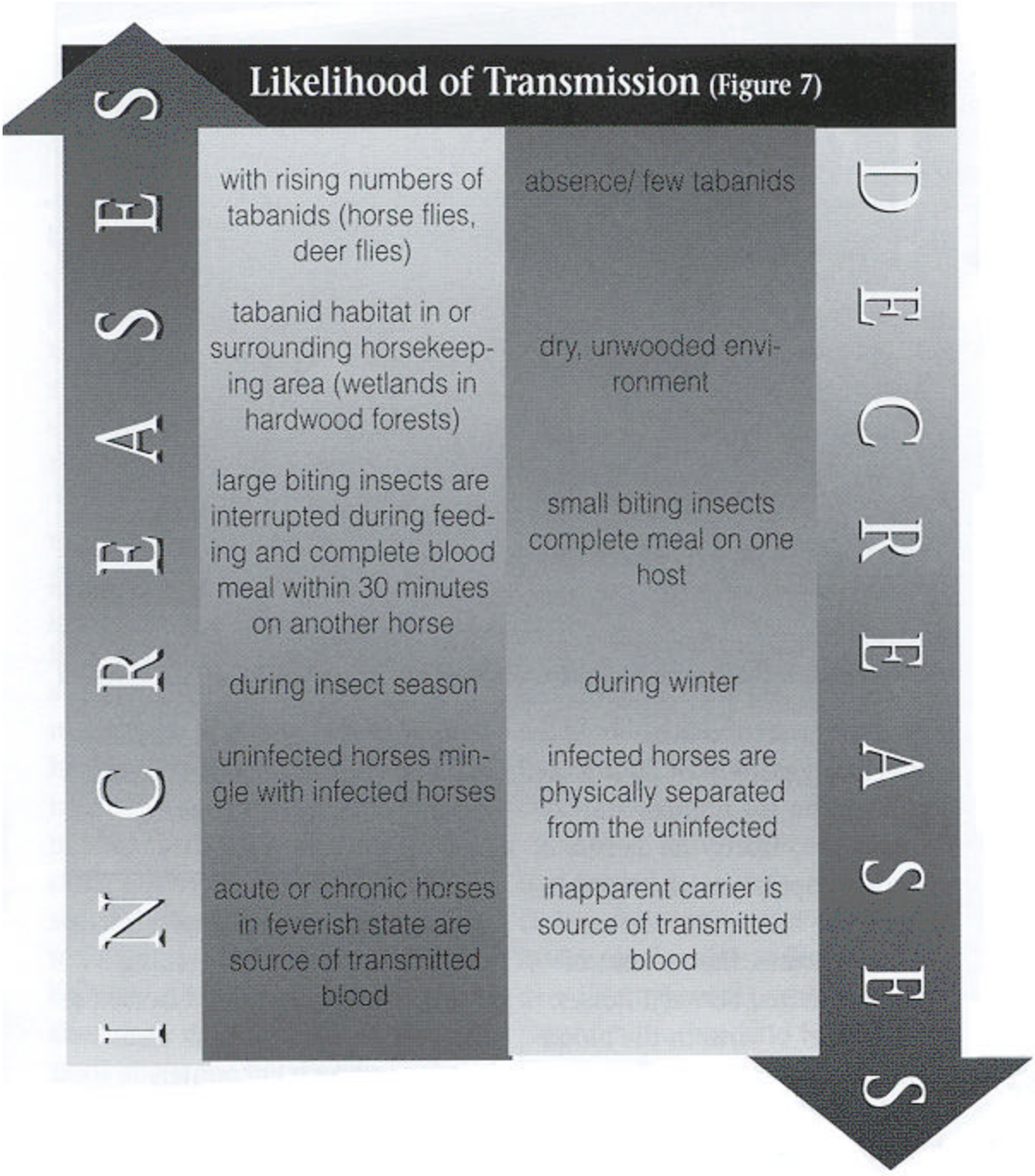
A group of 25 horse flies was able to transmit EIAV from this healthy-looking, inapparent carrier.

Insect transmission of EIAV is dependent on the number and habits of the insects, the density of the horse population, the number of times the insect bites the same and other horses, the amount of blood transferred between horses, and the level of virus in the blood of the infected horse from which the initial blood meal was obtained (Issel and Foil 1991, Foil et al. 1987, Knaus et al. 1993).

Under ideal conditions a single horse fly has been shown to transmit the virus from a horse with acute signs of EIA, and a group of 25 medium-sized horse flies (*Tabanus fuscicostatus*) transmitted EIAV from a horse without clinical signs of disease (Hawkins et al. 1976, Issel et al. 1982) (fig. 6)

Because each encounter of a test-positive horse, a blood-feeding insect, and an uninfected horse has the potential for EIAV transmission, all such encounters should be avoided. Under natural conditions where test-positive horses are expected to be present in the population and where vector pressures may result in more than 1,000 horse fly bites per hour, it is reasonable to expect EIAV transmission between horses. The rate of transmission, however, cannot by predicted accurately because of the variables previously mentioned (fig. 7). If, for example, all infected horses have EIAV replication under control and the virus is present in only 1 infective dose per mL of blood, then the predicted chance that any 1 fly

that feeds on the horse would have virus on its mouthparts in about 1 in 100,000 (see fig. 5). It's all a game of chance. But why take chances?



To optimize the natural transmission of EIAV, crowd uninfected horses and horses with acute signs of EIA in an environment where large blood-fedign insects are numerous.

Conversely, to prevent transmission, commingle only test-nagative horses after suitable quarantine periods and maintain a separation of 200 yards from horses whose EIA test status is unknown.

Immune Responses to EIAV and Serologic Diagnosis of Infection

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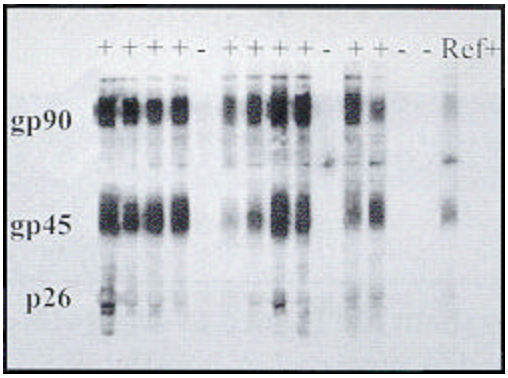
Horses exposed to EIAV generally develop detectable immune responses to EIAV antigens within 45 days following infection (Issel and Coggins 1979). Antibodies to the spectrum of EIAV antigens can be detected by using all of the virus proteins in a n immunoblot test (Issel and Cook 1993). In this protocol, gradient-purified EIAV is subjected to sodium dodecyl sulfate polyacrynlamide gel electophoresis. The proteins separate by their apparent molecular weight and are transferred to a membrane. Serum samples from suspects are tested for their ability to react with the individual EIAV proteins in the membrane. This process, known as immunoblotting, has the power to discriminate antibodies to the variety of EIAV proteins because of their physical separation on the membrane (fig. 8).

The major antigens of EIAV recognized by the horse are the two envelope proteins, gp90 and gp45, and the major core protein, p26. Even though the envelope gene mutates at high rates, it appears to contain highly conserved genetic sequences that are found in all strains of EIAV. As a result , the envelope proteins of the laboratory strain can be used to detect antibodies generated by horses in response to infection with all field strains. Similar group-specific determinants were found in the major core protein, p26, which permitted its use in the first specific tests for EIA in 1970.

Diagnosis of the disease EIA and of infections with EIAV was not possible until the AGID test was shown to have an excellent correlation with horse inoculation tests for EIAV. Since 1970, the AGID test, which detects the presence of antibodies against the p26 protein, has been used and is recognized internationally as the gold-standard serologic test for the diagnosis of EIA. Is specificity is high because nonspecific reaction cause the formation of lines of "non-identity" in the agar matrix.

Other serologic tests have been defined and approved for the diagnosis of EIA. At the time of this writing, the competition enzyme-based CELISA, which detects antibodies against p26, and the SA-ELISA, a synthetic antigen ELISA which detects antibody against gp45, have been licensed both of these tests have the advantage of being based on ELISA formats, which are inherently more sensitive than AGID tests.



Samples from horses with known positive (+) and negative (-) reactions in the agar gel immunodiffusion (AGID) test for antibodies to EIAV are compared with a reference positive sample from a horse infected with EIAV but with an equivocal AGID test reaction. all samples were diluted 1:20 and tested against the prototype cell-adapted strain of EIAV. In the positive samples, high levels of staining are seen against the surface unit and transmembrane proteins (gp90 and gp45, respectively) even though they are found in the virus in relatively low quantities compared to the major core protein (p26). the immunoblot test is the test of choice to confirm the antibody status of people to the human immunodeficiency virus.

The more sensitive tests also are less specific; that is, a higher percentage of the positive reactions are expected to be false-positive. Because of these considerations, all positive reactions in the ELISA-based formats must be confirmed in the AGID (Pearson an Gipson 1988).

The use of additional tests for the diagnosis of EIAV infections has assisted in the control of EIA as ELISA test results can be obtained on the same day the samples are collected; in contrast, AGID test results cannot be obtained for at least 24 hours. Unfortunately, but not unexpectedly, these additional tests have added a certain degree of confusion about the diagnosis of EIA because results in the different test formats do not always agree.

As a result of the availability of additional tests and study of horses whose test results are discordant, it is clear that false-negative and false-positive reactions are reported at a very low rate. These could occur for several reasons: human error in the collection and handling of samples or in the interpretation and reporting of test results, or inherent sensitivity and/or specificity considerations in the tests themselves. By addressing the issues associated with human errors in testing and vigorously searching for clarification of the status of horses with discordant test results for EIA, we hope to determine if additional safeguards for our equine population are needed. Additionally, we hope to determine if additional safeguards for our equine population are needed. Additionally, we wish to remain responsive to the needs of the horse industry. We are committed to these goals, and this document is one of the first steps in this process.

Preventing the Spread of EIAV

Controlling the spread of EIAV involves minimizing or eliminating contact of horses with the secretions, excretions, and blood of EIAV-infected horses. This has been effected in most areas of the world by testing and segregating test-positive horses from those that are test-negative. When this separation is done, it is imperative to retest the test-negative band at 30- to 60-day intervals until new cases fail to appear. Once the reservoirs of EIAV are identified, separated, and maintained a safe distance from other horses, the transmission of EIAV is broken. This sounds easy, but until all horses are tested, one must assume that each horse is a potential reservoir of EIAV and take precautions to commingle only with horses whose background is impeccable, i.e., they come from farms where only test-negative horses are found and have never been exposed to test-positive horses or other equids.

Federal and State Regulations Concerning EIA

USDA regulations regarding EIA are listed in Title 9 of the Code of Federal Regulations (CFR). Tittle 9, Part 75 contains provisions for the interstate movement of EIA reactors and the approval of laboratories, diagnostic facilities, research facilities, and stockyards. Section 75.4(a) of these regulations defines an EIA reactor as any horse, ass, mule, pony, or zebra that is subjected to an official test and found positive.

Until recently, under 75.4(b) of these regulations, no EIA reactor could be moved interstate unless the reactor was official identified. Section 75.4(a) of these regulations defines “officially identified” as the permanent identification of a reactor with markings permanently applied by an APHIS representative, a

State representative, or an accredited veterinarian using a hot iron or chemical brand, freeze-marking, or a lip tattoo. In 1995, an amendment added the provision stating that "Official identification is not necessary if the horse is moved directly to slaughter, traveling under a permit and in sealed conveyance."

Essentially, an EIA reactor may be moved interstate to only one of three places: (1) a federally inspected slaughtering facility, (2) a federally approved diagnostic or research facility, or (3) the home farm of the reactor.

The major regulatory actions to control EIA are carried out by the States. States' rules, while encompassing a much broader scope of EIA concerns, vary considerably and lack uniformity among individual State control programs. Recently, the United States Animal Health Association (USAHA) passed a resolution urging USDA to facilitate the development of a uniform control program for EIA and the interstate movement of horses. In 1996, a committee consisting of State Veterinarians and the authors of this pamphlet convened to initiate and coordinate these actions.

Conclusion

It is our hope that this document and the companion video, which is available from APHIS, will stimulate further discussion on EIA at the national and international levels and lead to more effective control programs. Please contact us and assist in refining and implementing measures for the control of EIA. A list of priorities for the control of EIA includes the following:

- Encourage the development and use of the best possible tests to diagnose EIA.
- Encourage the continued education of diagnosticians involved with testing for EIA.
- Cooperate with States to identify additional reservoirs of EIAV.
- Support research efforts to develop vaccines for EIA to protect the population at risk better.
- Help establish scientifically sound control programs for EIA with optimal benefit-cost ratios.
- Develop and encourage the adoption of uniform guidelines for the control of EIA nationally.
- Establish means to communicate with horse owners on issues concerning EIA control.

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