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EQUINE ARTERITIS VIRUS: AN OVERVIEW

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SUMMARY

The causative agent of the respiratory disease equine viral arteritis is a small, single-stranded RNA virus with a genome organization and replication strategy related to that of coronaviruses and toroviruses. Clinical signs of infection in horses vary widely and severe infection can lead to pregnant mares aborting. Infected horses generally make good recoveries but stallions may become semen shedders of equine arteritis virus (EAV). These carrier stallions play an important role in the dissemination and perpetuation of EAV. Laboratory tests exist to detect virus and the equine immune response to infection. However, vaccines are not currently licensed in the UK to combat viral arteritis, the incidence of which may increase due to changes in European legislation.

INTRODUCTION

Although equine viral arteritis has been known for almost 40 years, disease outbreaks are identified infrequently and field isolates of the causative agent, equine arteritis virus (EAV), are rare. The only reported host for EAV is the horse in which clinical signs of infection vary widely, but in its most severe form EAV infection causes abortion. In the early veterinary literature viral arteritis was referred to as 'epizootic cellulitis pinkeye' or as 'equine influenza' (Pottie, 1888; Clark, 1892). The EAV type strain Bucyrus owes its name to the town in Ohio where it was first isolated during an abortion storm in 1953 (Doll *et al.*, 1957). Equine viral arteritis is transmitted by a respiratory route (McCollum *et al.*, 1961a); however, a carrier state exists in 30% of seropositive stallions, in which EAV is produced in semen (Timoney, 1985). These 'shedding stallions' may consequently infect broodmares by a venereal route.

Changes in regulations as part of the European Community single market economy could adversely affect the disease free status of the UK with regard to viral arteritis: seropositive animals from continental Europe could introduce this disease into Britain. Excellent reviews on the subject have been published describing the epidemiology and pathology of EAV (Mumford, 1985; Huntingdon *et al.*, 1990a). This article aims to update readers on the results of recent research

investigating the molecular structure of equine arteritis virus and inform on strategies for clinical diagnosis, virus detection, and disease control and prevention.

THE DISEASE

Occurrence

Outbreaks of EAV have occurred in the USA (Doll *et al.*, 1957; McCollum & Swerczek, 1978; Timoney & McCollum, 1985; Timoney *et al.*, 1986; Collins *et al.*, 1987; Hall, 1989), Switzerland (Burki, 1970; Gerber *et al.*, 1978), Poland (Golnik *et al.*, 1981; Golnik, 1991), Austria (Nowotny, 1991) and Canada (Clayton, 1987; Carman, 1988). In addition serosurveys have identified antibody positive horses from England, Japan (McCollum & Bryans, 1973), France, Spain, Ireland, Portugal, Yugoslavia, Egypt, Ethiopia, Morocco, Senegal (Moraillon & Moraillon, 1978), USSR (Akashi *et al.*, 1976), Germany (Kaaden *et al.*, 1989), Sweden (Klingeborn *et al.*, 1991), Australia (Huntingdon *et al.*, 1990b), New Zealand (McKenzie, 1988, 1990), Iran (Maasommeh, 1991), India and Denmark (Chirnside, unpublished), and the Netherlands (de Boer *et al.*, 1978). Semen shedders of EAV are reported present in the USA (Timoney, 1985), Australia (Huntingdon *et al.*, 1990b), Sweden (Klingeborn *et al.*, 1991), and Italy (Autorino *et al.*, 1991). In many serological reports the horses are not categorized into breed types; however in the USA and Australia as many as 80% of standardbreds are seropositive for EAV. In contrast only 3% of Thoroughbreds are seropositive (Timoney & McCollum, 1985; Huntingdon *et al.*, 1990b), indicating different disease exposure or susceptibility within breeds. In many cases import/export serological data are compiled into serosurvey reports: this tends to distort the number of countries harbouring seropositive animals as the horse in question may have originated elsewhere.

To date the United Kingdom remains free of any outbreak of EAV due to (1) its geographical isolation from mainland Europe and (2) strict importation restrictions, introduced in 1984, which require testing of horses for antibodies to EAV in a virus neutralization test. However, the single market economy legislation of the European Community taking effect during 1992 will eliminate the importation requirement for an EAV neutralization test for horses entering the UK from other EC member states. Consequently the EAV-susceptible UK equine population is at an increased risk of disease.

The number of serum samples tested for EAV antibodies at the Animal Health Trust since 1986 is shown in Table I. These data show that the prevalence of UK Thoroughbreds with serum neutralizing antibodies to EAV is very low (<0.6%). The majority of these sera come from horses moving onto stud farms in the Newmarket area and all the EAV seropositive animals detected have histories commensurate with infection whilst on stud duty abroad. In both Australia and New Zealand serosurveys indicate that seropositive animals have been imported (Huntingdon *et al.*, 1990b; McKenzie, 1990) with the associated risk of shedding stallions disseminating virus venereally. The situation regarding UK native horse breeds, other than Thoroughbreds, is unknown but is likely to reflect that of other

European countries with a low prevalence of seropositive animals due to subclinical EAV infection.

Clinical signs

Consequences of EAV infection range from subclinical disease, only recognized by seroconversion (McCollum & Bryans, 1973), to acute illness causing full blown abortigenic disease. Studies of epizootics have shown that subclinical infections are up to sixfold more prevalent than clinical infections (Timoney & McCollum, 1985; Collins *et al.*, 1987).

In acute disease clinical signs are variable including pyrexia, depression, anorexia, oedema of scrotum, ventral trunk and limbs, conjunctivitis, lacrimation, serous nasal discharge and respiratory distress. Other clinical signs associated with arteritis include lameness, coughing, general weakness and ataxia (Doll *et al.*, 1957; Gerber *et al.*, 1978; Clayton, 1987; Collins *et al.*, 1987; Timoney *et al.*, 1987a). Adult horses generally make an uneventful recovery after a viraemic phase which may persist for up to 40 days after infection (Neu *et al.*, 1988); however, EAV infection may cause foal death (Carman, 1988; Timoney & McCollum, 1988; Golnik, 1991).

The major fear in clinical disease is abortion with an incidence in exposed mares of up to 50%. EAV may cause abortions in mares between 3 and 10 months of gestation (Timoney & McCollum, 1985; Cole *et al.*, 1986). Abortion may occur concurrent with or shortly after infection (Doll *et al.*, 1957; Golnik *et al.*, 1981; Timoney *et al.*, 1986; Clayton, 1987) and is a result of myometrial necrosis and oedema leading to placental detachment and fetal death (Coignoul & Cheville, 1984).

Transmission

Until recently the chief mode of virus transmission during EAV outbreaks was reported to be by direct contact via nasal droplet spray (McCollum *et al.*, 1961a) during the acute phase of infection. In the Kentucky outbreak of 1984 a high percentage (36%) of stallions infected by the respiratory route continued to shed virus in their semen after recovery (Timoney, 1985) which resulted in transmission of EAV to susceptible mares to which they were bred. Two carrier states exist in the stallion: a short-term state during convalescence (duration=weeks) and a long-term chronic condition (Timoney, 1985; Timoney *et al.*, 1986, 1987a; Neu *et*

Table I
Incidence of EAV seropositive Thoroughbred horses in the UK

Year	*No. sera tested*	*Seropositive*	*% Seropositive*
1986	494	3	0.61
1987	672	4	0.59
1988	484	1	0.21
1989	402	1	0.25
1990	261	1	0.38
TOTAL	2313	10	0.43

al., 1988) which may persist for years after clinical infection. In experimental infections of stallions Neu *et al.* (1988) achieved long-term carrier rates of 62.5% with persistence of EAV in the vas deferens, ampullae, seminal vesicles, prostate and bulbourethral glands. Mounting evidence suggests that the carrier stallion plays a pivotal epidemiological role in the dissemination and perpetuation of EAV.

There are no reports of mares becoming EAV carriers or chronic shedders, nor of virus passage by the venereal route from a seropositive mare causing clinical disease or seroconversion in a stallion. Foals born to seropositive mares acquire maternal antibodies to EAV via colostrum (McCollum, 1976). These maternal antibodies decline to extinction from 2 to 6 months, resulting in the foal becoming seronegative unless infected with EAV. After clinical recovery from respiratory EAV infection there is no significant decrease in the fertility of shedding stallions (Timoney *et al.*, 1987c; Klingeborn *et al.*, 1991). Mares infected after service by a carrier stallion do not appear to have any related fertility problems.

Diagnosis

Sample selection. In order to confirm diagnosis of viral arteritis the correct samples must be submitted for laboratory testing. In cases of respiratory disease whole and clotted blood, and nasopharyngeal swabs (stored in virus transport medium at +4 °C) should be taken and submitted as quickly as possible to the diagnostic laboratory. The clinical history and recent transport history of the horse may also help in the selection of laboratory tests. Corticosteroids are cytotoxic to some cell types in tissue culture and interfere with the EAV neutralization test, so any drugs already being prescribed should be noted.

In EAV abortion both fetus and placenta are heavily contaminated with virus (Cole *et al.*, 1986) and samples of these should be collected in a sterile manner and submitted for virus isolation. EAV can be isolated from the placenta, fetal spleen, lung, and kidney, and fetal and placental fluids.

Semen samples should be collected if a stallion is suspected of shedding virus. Samples should be from the sperm rich fraction of full ejaculates—not dismount samples—and stored at +4 °C for rapid transport to the laboratory. In addition serum samples from mares to which the stallion has been bred within the last 3 months can provide additional information on the infective status of the stallion.

Virus isolation. Acute viral arteritis may be diagnosed by virus isolation from nasopharyngeal swabs or washings, and from the buffy coat of EDTA-treated or citrated blood samples (Geering & Forman, 1987). Diagnosis of abortion due to EAV is largely dependent on virus isolation from the placenta or fetal tissues.

EAV has been isolated directly on equine kidney, rabbit kidney (RK-13) and Vero cells (Burki, 1965; Golnik *et al.*, 1981) by serial blind passage of material from aborted fetuses and foals. In the latter cases cytopathic effects (cpe) were only recognizable after 2–8 passages.

Isolation of EAV from field cases can prove difficult and in some incidents has failed; nasopharyngeal swabs taken from febrile horses during an outbreak in Switzerland (Gerber *et al.*, 1978) and from swabs and buffy coats during an outbreak in Kentucky (McCollum & Swerczek, 1978) produced no cpe in tissue cul-

ture inoculations. The latter workers were able to show virus transmission by inoculation of blood from febrile horses into susceptible animals followed by virus isolation from nasal swabs and blood of the experimentally infected horses.

The EAV carrier state was first demonstrated in naturally infected stallions by both test matings and virus isolation on RK-13 cells (Timoney *et al.*, 1986, 1987b). Virus culture from semen samples is currently the recognized method of identifying shedding stallions. Recently a new method independent of virus isolation in tissue culture has been used to demonstrate the presence of EAV in semen samples (Chirnside & Spaan, 1990). The methodology can detect 60 plaque forming units/ml of semen and is as sensitive as tissue culture isolation. Purified viral RNA is first transcribed into copy DNA (cDNA) prior to amplification in the polymerase chain reaction (PCR). During cDNA amplification EAV-specific oligonucleotide primers and the enzyme Taq polymerase amplify the quantity of EAV cDNA. The products of the PCR amplification are then separated by agarose gel electrophoresis, the EAV-specific cDNA band visualized under UV light and the gel probed with a radiolabelled oligonucleotide to confirm the cDNA product as EAV-specific. The advantages of this methodology over tissue culture are: (1) in the speed of virus detection (48 h as compared to 1–3 weeks); (2) in specificity (only EAV RNA is detected); (3) in its applicability to a wide range of clinical samples; and (4) it is not dependent on entire virus being present, only viral RNA. Klingeborn *et al.* (1991) have used this methodology to confirm tissue culture isolation results from Swedish semen shedders.

Serology. Antibodies to EAV can be demonstrated by complement fixation (CF) and virus neutralization (VN) tests (Burki, 1965; Matsumoto *et al.*, 1965; Burki & Gerber, 1966; Hyllseth & Petersson, 1970). The CF test is most useful for studying immunity to arteritis during the first 4 months after exposure, as the titre peaks 2–4 weeks after infection and decreases below detectable limits after 8 months (Fukanaga & McCollum, 1977). CF antibodies have a role in diagnosing recent infection because they decrease more rapidly than VN antibody titres. VN antibody titres develop simultaneously with CF titres, are maximal 2–4 months after infection and remain stable for several years (Gerber *et al.*, 1978).

Laboratory diagnosis of seropositive horses is based on the complement dependent VN test using the Bucyrus strain of EAV for tissue culture infection (Senne *et al.*, 1985). Two laboratories in the UK carry out this standardized test: The Central Veterinary Laboratory, Weybridge (for export/import documentation) and The Animal Health Trust, Newmarket. A titre of 1:4 in duplicate sera is deemed EAV seropositive. VN test procedures, although more expensive and laborious than CF tests, are more sensitive for the detection of immunity to EAV as a result of previous exposure to the virus. The VN test is unable to differentiate between serum antibody elicited by vaccination from that due to natural infection.

Enzyme linked immunosorbent assay (ELISA) tests have been developed in Canada (Lang & Mitchell, 1984) and England (Cook *et al.*, 1989) to detect EAV-specific antibody levels. These use purified EAV as the antigen in the ELISA which is recognized by EAV-positive horse sera. The major problem encountered by EAV ELISAs are their failure to provide clear, sensitive results when the horse being tested has been previously vaccinated with any tissue culture derived virus vaccine

such as those used to prevent equine influenza and herpesvirus infections. In such cases the high background colour development due to non-specific binding of antibodies to tissue culture derived antigen can mask the EAV ELISA result.

THE VIRUS

Morphology

EAV is a small, enveloped, positive-stranded RNA virus. The virion has a diameter of 50–70 nm (Hyllseth, 1973) and consists of an isometric core (35 nm) surrounded by an envelope which carries ring-like subunits with a diameter of 12–15 nm (Murphy, 1980; Horzinek, 1981). On the basis of virion morphology, substructure and size, EAV has been classified as a non-arthropod borne member of the Togaviridae (Porterfield *et al.*, 1978; Westaway *et al.*, 1985).

Physiochemical properties

EAV is resistant to trypsin, ether sensitive and is inactivated by 1 M magnesium chloride at 50 °C (Burki, 1970). The buoyant density of the infectious virus has been calculated as 1.17–1.24 g/ml (Hyllseth, 1970). The single-stranded infectious RNA has a molecular weight of 4×10^6 (van der Zeijst *et al.*, 1975) and is 12.7 kb long (den Boon *et al.*, 1991). Currently there is only one recognized serotype of EAV, although there is some evidence of antigenic variation amongst different isolates (Fukunaga & McCollum, 1977; Murphy *et al.*, 1988).

Genome RNA organization

The nucleotide sequence of the genome of EAV contains eight overlapping open reading frames (ORFs) (den Boon *et al.*, 1991). The organization and expression of the EAV genome is remarkably similar to those of coronaviruses (infectious bursal disease virus, IBV; transmissible gastroenteritis virus, TGEV; feline infectious peritonitis virus, FIP; mouse hepatitis virus, MHV) and toroviruses (Berne virus, Breda virus). The 5' terminal part of the genome encodes the EAV polymerase gene responsible for virus replication. This gene contains two large ORFs, 1a and 1b (Fig. 1), with an overlap region of 19 nucleotides. The presence of a 'shifty' heptanucleotide sequence in this region and a downstream RNA pseudoknot structure indicate that ORF 1b is probably expressed by frameshifting. The frameshifting potential of the ORF 1a/1b overlap region has been demonstrated using a reporter gene. Orfs 2 through 7 are expressed from six subgenomic mRNAs (Fig. 1), which are transcribed from the 3' portion of the viral genome. A number of these ORFs are predicted to encode structural EAV proteins (Table II).

EAV replication

Infectious virus is targeted to specific host cells by interactions between virus

proteins located on the virion surface and receptor proteins located on the surface of host cells. After invasion of the host cell the viral genome directs the synthesis of new virus particles by overriding the normal cell functions and directing the cellular machinery to synthesize viral proteins. One of the initial requirements for the synthesis of new EAV protein is the amplification of the viral genome, producing multiple mRNAs encoding virus-specific proteins. The methodology by which the EAV genomic signals are amplified is unique and is facilitated by the organization of the EAV genes on the viral genome; 5' leader–polymerase genes–envelope genes–nucleocapsid gene 3'.

During EAV replication six subgenomic RNAs (sgRNAs) are synthesized (van Berlo *et al.*, 1982). These form a 'nested set' in which all the sgRNAs have a common 3' end and extend towards the 5' end of genomic RNA for different lengths (Fig. 1). A leader sequence of 209 nucleotides is found at the 5' end of each sgRNA (de Vries *et al.*, 1990). The leader sequence is derived from the 5' end of genomic RNA and is joined to the bodies of mRNAs 6 and 7 at positions defined by the nucleotide sequence 5'UCAAC3'. As a result of leader–body joining ORFs 6 and 7 are located at the unique 5' terminal region of each sgRNA. The complete EAV genome contains 18 UCAAC motifs and each genomic ORF (except 1b) is preceeded by one or more of these motifs. The sizes of the bodies of the EAV sgRNAs correspond closely to the positions of UCAAC junction motifs and the positions of ORFs 2–7 on the genome (den Boon *et al.*, 1991). The mechanism of

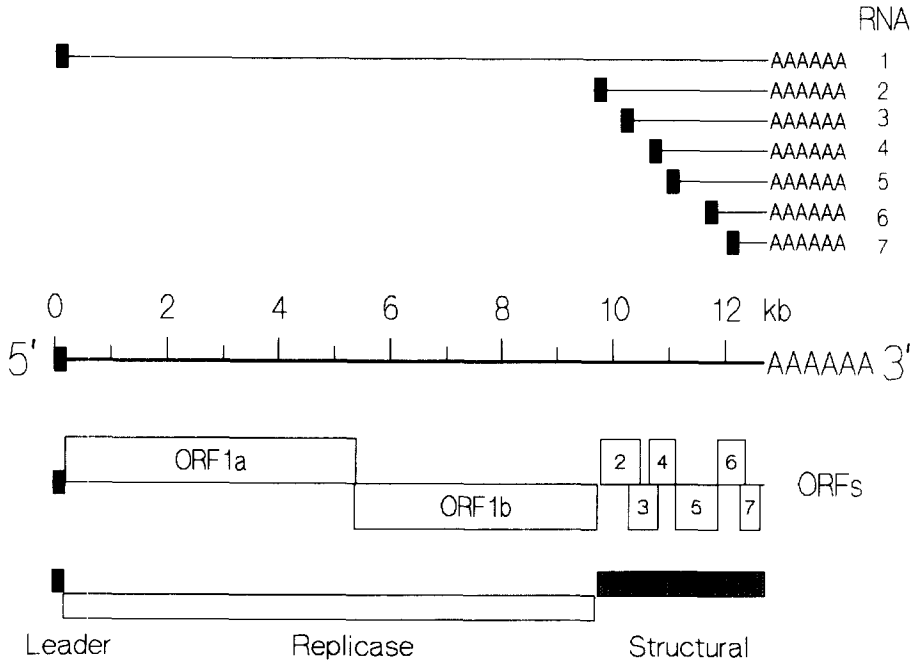

Fig. 1. Organization of the genome of EAV. The upper part of the figure indicates the positions and composition of the EAV sgRNAs. The lower shows positions of ORFs and type of gene product encoded, based on sequence information. The EAV genome is represented by the 12.7 kb bar in the centre of the figure.

Table II
Characteristics of EAV RNAs and ORFs

RNA	*Estimated size (kb)*	*ORF no.*	*Size of product (kDa)*	*Protein function*
1	13	1a	186.9	RNA polymerase
		1b	159.0	
2	3.2	2	25.6	
3	2.7	3	18.0	
4	2.2	4	17.2	Structural
5	1.9	5	28.7	
6	1.2	6	17.7	Small envelope
7	0.8	7	12.3	Nucleocapsid

leader–body assembly has not been fully investigated but is thought to be precise, utilizing only one of the available UCAAC sites upstream of each ORF as there is no detectable heterogeneity in the length of the leader sequence as deduced from primer extension analysis of several subgenomic cDNA clones (de Vries *et al.*, 1990).

Translation

In-vitro translation of EAV genomic RNA results in the production of proteins of 30 kDa and 200 kDa. RNA 1, the intracellular homologue of genomic RNA, synthesizes a 30 kDa protein (van Berlo *et al.*, 1986b) and RNA 7 a 14 kDa protein identified as the nucleocapsid (den Boon *et al.*, 1991; de Vries, unpublished).

The synthesis of multiple sgRNAs each uniquely encoding a different viral ORF at the 5' end resembles corona- and toroviral sgRNA structure and organization (Spaan *et al.*, 1990). With a few exceptions only the most 5' ORF of each coronaviral sgRNA is translated into protein (Spaan *et al.*, 1988). The function of the corona- and toroviral sgRNAs appears to be to position internal genes at the unique 5' end of a mRNA for translation into protein. Although unproven this will presumably be the case with the arteriviral sgRNAs.

Virion proteins

The protein composition of EAV has been analysed by radiolabelling virus proteins during tissue culture infection (Hyllseth, 1973), and comparisons of protein patterns of virus grown in different cell types (Zeegers *et al.*, 1976). These initial studies indicated that 3–9 proteins were virus associated, including a 12 kDa phosphorylated nucleocapsid, a 21 kDa glycosylated protein and a 14 kDa membrane-associated protein (Zeegers *et al.*, 1976) in addition to a protein smear in the 28–40 kDa size range. Monoclonal antibodies have identified three virus specific proteins of mol. wt 14 kDa, 16 kDa and 30 kDa (Chirnside *et al.*, 1988; unpublished) in Western blots and immunoprecipitations of whole virus.

Analysis of the genomic sequence of EAV (den Boon *et al.*, 1991) (Table II) confirms protein sizes detected by earlier methods. EAV ORFs 2, 3, and 4 encode polypeptides with hydrophobic N and C termini and ORF 5 a protein with a

hydrophobic N terminus and an internal hydrophobic domain. The predicted proteins from ORFs 2 through to 5 all contain *n*-glycosylation sites and could therefore encode envelope proteins in the 28–40 kDa size range. ORF 6 encodes an unglycosylated, triple-spanning membrane protein and the product of ORF 7 has been identified as the nucleocapsid protein (van Berlo *et al.*, 1986b; de Vries *et al.*, unpublished). Figure 2 is a schematic diagram of EAV based on the sizes, properties and predicted locations of the virus encoded proteins.

Pulse chase experiments indicate that the viral structural proteins do not arise by processing from larger precursors (van Berlo *et al.*, 1986a), but are primary translation products.

Genetic variation

No data are available at the RNA sequence level to ascertain if differences exist between isolates of EAV in comparison to the published sequence of the Bucyrus prototype. However, EAV isolates from the USA and Europe show differences in oligonucleotide fingerprint patterns (Murphy *et al.*, 1988) with recent North American isolates appearing more homologous to the Bucyrus strain than to a 1968 European isolate. This indicates that sequence differences are likely to exist between isolates.

All EAV isolates cross-react with equine antiserum raised against the Bucyrus strain and no major antigenic variation has been shown between different isolates. Using the CF test Fukunaga & McCollum (1977) have demonstrated minor antigenic differences between virulent Bucyrus, avirulent Bucyrus, Bibuna and Vienna strains of EAV with homologous and heterologous equine antiserum. Burki

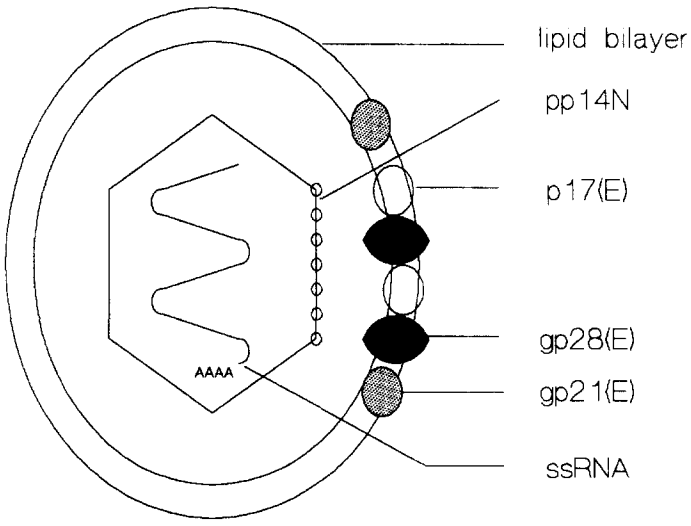

Fig. 2. Schematic diagram of EAV structure: pp14N, nucleocapsid protein; p17E, non-glycosylated envelope protein; gp21E, gp28E, glycosylated envelope proteins.

(1970) and McCollum & Swerczek (1978) commented on the variation of severity of clinical signs of disease caused by Vienna, Bibuna and Kentucky 1978 isolates when compared to those caused by the Bucyrus strain. Reproducibly different tissue culture growth characteristics have been demonstrated with respect to virus shed in the semen of different carrier stallions (Timoney *et al.*, 1987a).

Relationships to other viruses

The Fourth Report of the International Committee for Taxonomy of Viruses has listed EAV as the sole member of the genus arterivirus in the family Togaviridae. EAV fulfils the three prerequisites for being considered a togavirus: (1) a positive ssRNA genome; (2) a lipid envelope; and (3) an icosahedral nucleocapsid. Recently information regarding the genome organization and replication strategy of EAV has been published (de Vries *et al.*, 1990; den Boon *et al.*, 1991) and the current classification now appears unjustifiable. EAV appears ancestrally related to the corona- and toroviruses on the basis of genome organization and replication strategies and has been proposed a member of the 'coronavirus-like' family of viruses (den Boon *et al.*, 1991). However, the genome size and virion architecture of both coronaviruses (Spaan *et al.*, 1988) and toroviruses (Horzinek *et al.*, 1986) are fundamentally different from the arteriviruses.

Attempts have been made to find antigenic relationships between the togavirus lactate dehydrogenase virus (LDV) which affects mice, and EAV (van Berlo *et al.*, 1983). However, these comparisons have had little success. Partial sequencing of the LDV genome indicates that the LDV nucleocapsid protein is located at the extreme 3' end of the genome (Godeny *et al.*, 1990). The genome organization of LDV may therefore resemble that of the arteri-, toro- and coronaviruses rather than the alphaviruses.

DISEASE CONTROL

Treatment

Virtually all naturally infected horses recover from EAV infection although some fatalities have been induced by experimental infections. Pyrexia in stallions can lead to sperm damage and temporary infertility, so treatment with non-steroidal, anti-inflammatory preparations may be indicated to control pyrexia. Little *et al.* (1991) have recently demonstrated by administering testosterone to castrated stallions that the persistence of EAV in chronically infected stallions is mediated either directly or indirectly by testosterone and consequently dependent on the presence of the testes. Additionally several unpublished findings suggest that viral persistence in the accessory sex glands of the stallion is testosterone dependent. These include a failure of virus persistence to develop in prepubertal colts, a seasonal fluctuation in viral output coincident with seasonal fluctuations in serum testosterone levels, the apparent elimination of EAV from the reproductive tract following castration and the absence of any carrier state in the mare. Sexual rest following acute EAV infection may be important in reducing the likelihood of a stallion becoming a chronic EAV carrier (Timoney & McCollum, 1988).

Natural immunity

Previously infected horses are immune to experimental reinfection with virulent virus (McCollum, 1970). Natural immunity persists for up to 7 years (Gerber *et al.*, 1978) and although difficult to demonstrate empirically is presumed to be lifelong. During recovery from EAV infection high levels of serum neutralizing antibody are induced which have long duration. High levels of antibody correlate very well with protection from respiratory and venereal infection (McCollum *et al.*, 1988).

Vaccination

Research work on EAV vaccines has concentrated on the safety and efficacy of a whole inactivated virus vaccine and an attenuated live virus vaccine.

The early studies of Doll *et al.* (1968) demonstrated that virulent EAV could be attenuated by passage in tissue culture whilst retaining immunogenicity. Serial passage through horse kidney, rabbit kidney and equine dermis cells have served to attenuate virulent virus (McCollum, 1969, 1981, 1986; Harry & McCollum, 1981; McCollum *et al.*, 1988) resulting in a modified live vaccine (MLV) (Timoney *et al.*, 1988). This vaccine received widespread use during the Kentucky EAV outbreak of 1984 and is registered for use in some states within the USA (Arvac, Fort Dodge Laboratories). Use of the MLV does not produce any side effects apart from a short-term abnormality of sperm morphology (Timoney *et al.*, 1988), and a mild fever with no overt clinical signs. Virus can be isolated sporadically from the nasopharynx and blood samples generally for up to 7 days but in a few cases for up to 32 days post-vaccination (Fukunaga *et al.*, 1982; Timoney *et al.*, 1988). Vaccine virus has never been isolated from semen or urine after vaccination with MLV (Fukunaga *et al.*, 1982; Timoney *et al.*, 1988). VN antibody titres are induced within 5–8 days of vaccination (Timoney *et al.*, 1988) and persist for at least 2 years (McCollum, 1986).

Vaccination with MLV protects against clinical disease and reduces the amount of virus shed from the respiratory tract in experimental infection. Horses in contact with, and mares served by vaccinated stallions are not infected by EAV (McKinnon *et al.*, 1986; Timoney *et al.*, 1988). Vaccinated mares experimentally challenged by artificial insemination were protected from clinical infection (McCollum *et al.*, 1988). However, the latter workers demonstrated the need for vaccinated animals to be isolated, as one mare in close contact with the vaccinated, venereally challenged group became EAV seropositive.

The modified live EAV vaccine was used to good effect in the containment of the 1984 arteritis outbreak on Kentucky stud farms. However, vaccination of horses induces shedding of virus from the nasopharynx and does not prevent infection. To be universally accepted for use both on stud farms and within training stables these problems should be addressed.

Fukunaga and co-workers (1984, 1990) have pioneered the use of a formalin inactivated EAV vaccine. Secondary immunization 4 weeks after primary vaccination results in VN antibody titres of up to 1:5120. The antibody titre decreases rapidly but a third immunization after 2 months results in a VN titre of between 1:80 and 1:320 persisting for 6 months. The 50% protective level of antibody was calculated at a VN titre of 1:43. However, even though clinical disease was averted

not all horses with high antibody levels were protected from infection as live virus was recovered from blood samples after experimental exposure. The value of this type of vaccine in disease control within stables is unclear because it is unknown if the vaccinated horses showed reduced levels of virus excretion from the nasopharynx. Recently the inactivated vaccine has been shown to prevent stallions from becoming semen shedders after exposure to EAV (Fukunaga *et al.*, 1991).

Disease prevention

The most efficacious EAV disease programme has been conducted in Kentucky during and since the disease outbreak of 1984. During the outbreak the spread of EAV was controlled by movement restrictions, isolation of infected animals on stud farms followed by a quarantine period after recovery, allocation of personnel to deal solely with infected animals, diagnostic surveillance, and the use of a modified live vaccine to immunize animals at risk of infection. The disease outbreak and subsequent vaccination resulted in a population of seropositive animals due to infection or live virus immunization, both groups with the potential to infect susceptible animals brought into close contact with them. As a result a strict EAV control programme has been conducted since 1985. This involves annual vaccination of all non-carrier stallions and teasers prior to the breeding season and vaccination of seronegative mares served by carrier stallions. Following vaccination animals are isolated for up to 21 days from non-vaccinated horses. Additionally mares bred to seropositive stallions are isolated for 14 days after service. Shedding stallions are housed and bred in separate facilities and any seropositive non-shedding stallion is checked for two seasons by monitoring the serological status of mares bred to that stallion. Stallions and mares becoming clinically infected during the breeding season are not used further during that season.

The use of vaccination as an integral part of EAV control programmes is also advocated in the state of New York and in New Zealand (McKenzie, 1990) and remains an option in areas where either clinical disease or seropositive animals exist. However, the widespread vaccination of horses with a modified live EAV vaccine entails the risk that the vaccine virus could revert to virulence and that vaccinates could spread the attenuated strain outwith the vaccination programme either by virus shedding directly after vaccination or by inducing semen shedders. This does not appear to have occurred in Kentucky. However, in an equine population free of diseased and seropositive animals import regulations excluding seropositive and clinically ill animals together with internal diagnostic surveillance is the safest and most effective method of disease prevention. In the event that a new outbreak occurs within a susceptible population management procedures should follow those practised in Kentucky with the substitution of a killed or subunit vaccine for the modified live vaccine.

CONCLUDING REMARKS

Understanding of the virus causing equine arteritis has recently improved due to the determination of the genomic nucleotide sequence of equine arteritis virus (EAV) (den Boon *et al.*, 1991). Additionally, knowledge of the disease process and

its effects have necessarily improved in the wake of an outbreak of viral arteritis amongst Thoroughbred horses on Kentucky stud farms (Timoney, 1985).

Serosurvey and virus isolation data show that equine viral arteritis exists within the EC. Since the disease can be passaged from seropositive stallions to mares it is unreasonable to expect that, with changes in importation requirements, the UK will remain free of this disease. Consequently UK veterinarians will need to be informed on all aspects of EAV disease, diagnosis and management.

EAV-specific proteins identified from the genomic sequence are being studied to investigate their function in virus structure, replication and assembly and to determine antigens stimulating protective immunity in the horse. These studies will provide vital information for the development of novel EAV vaccines and diagnostic tests. Additionally, EAV appears to stimulate protective immunity of very long duration in the horse and immunoprophylaxis suitable for other equine virus infections may be developed directly from research on EAV.

There are no data on the mechanism by which EAV, a respiratory virus, develops into a chronic venereal infection or vice versa. Little is known about strain variation amongst isolates of EAV, or if EAV isolated from the respiratory tract differs genomically or antigenically from virus present in the accessory sex glands. How does EAV avoid immunosurveillance in the carrier stallion, and why do all seropositive stallions not shed virus? What are the antigenic components of the virus responsible for stimulating immunity and how do they interact with the equine immune system to provide host protection? With so many questions still to be answered EAV will remain the subject of continued scientific scrutiny.

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