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Equine encephalosis virus (EEV): A Review

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ABSTRACT

Equine Encephalosis (EE) is an arthropod borne febrile non contagious disease of equines. The causative virus, *Equine encephalosis virus* (EEV), has several serotypes (EEV1-EEV7) and the virus has been reported from southern Africa including Kenya, Botswana and South Africa. EEV was first isolated in 1967 from horses in the Republic of South Africa. Like the *African horse sickness virus* (AHSV) EEV is also endemic in southern Africa. In most of the country, EE virus in comparison to AHSV has a higher transmission rate. Two species in the *Culicoides imicola* species complex, *C. imicola* (*senso stricto*) and *C. bolitinos* are known to transmit EEV. Zebra and elephants can act as maintenance host of the virus, making the elimination of the virus difficult. Outbreaks of EEV infection is reported to be associated with equine foetus abortion during the first 5-6 months of gestation. ^{32}P-labelled genomic probes of EEV are used for detection of viral Ribonucleic Acid (RNA). Sero-epidemiological tools for the detection of antibodies against EEV include Serum Neutralization Test (SNT) and Enzyme Linked Immunosorbent Assay (ELISA). A novel real time Polymerase Chain Reaction (PCR) assay has also been developed for the detection of EEV by targeting the gene Viral Protein (VP)-7. There is no specific treatment or vaccine available for this virus. Supportive treatment can only be provided. Management of horses in the stable is the key to control the spread of EEV in equines along with follow up of good biosecurity measures. The present review deals with all these aspects of the infection caused by this virus to enrich knowledge of researchers and equine/stud farm owners and the industry.

Key words: *Equine encephalosis virus*, epidemiology, abortion, ecology, icterus, repellants, vector, diagnosis, prevention, control

INTRODUCTION

Equine Encephalosis (EE) is a disease which is vector-borne in nature and Culicoides midges are responsible for transmission of the virus. The disease is similar to African Horse Sickness (AHS) as well as Blue Tongue (BT). All equidae are infected by the virus but horses only exhibit clinical signs. The *Equine encephalosis virus* (EEV) was identified as well as isolated solely in South Africa

prior to 2008 where characterization of seven serotypes that are distinct antigenically (EEV 1-7) have been identified. The disease African Horse Sickness (AHS) has got great similarity with EE. Vector activity is mainly responsible for transmission and especially in southern parts of Africa. It is seasonal occurring mainly during the months of December-July. Seropositivity ranges from 50-60 per cent in donkeys and zebras along with horses. EEV antibodies may be rarely found in elephants as well. As the disease is not primarily a neurological disease the name equine encephalosis is misleading. Less than 5% mortality is usually observed in animals that are affected. Infection due to EEV is mostly subchinical in nature for which confirmation in most of the instances is done by sero-conversion in serum samples (paired). Due to absence of effective vaccine, control of vector remains as one of the primary methods of prevention (Geering *et al*., 1995; Venter *et al*., 2006; Oura *et al*., 2012).

Etiology: Equine encepalosis is an arthropod borne febrile non contagious disease of equines which was documented 100 years before when A. Theiler named it as Equine Ephimeral fever (Theiler, 1910). The *Equine encephalosis virus* (EEV), a member of the Orbivirus genus of the Reoviridae family, is an insect-borne orbivirus that is transmitted by a variety of biting midges of *Culicoides* spp. (Erasmus *et al*., 1970; Venter *et al*., 1999b; Paweska and Venter, 2004; Hinchcliff, 2007). African Horse Sickness (AHS) and EEV are the two orbiviruses that infect equids. EEV is an agriculturally important orbivirus associated with per acute illness of horses in southern Africa. Virus isolation from a horse with clinical symptoms of neurologic disease led to the acquisition of the name "*Equine encephaolosis virus*". EEV is closely related to bluetongue and epizootic hemorrhagic disease viruses than to African Horse Sickness Virus (AHSV) (Viljoen and Huismans, 1989). The virus has characteristics in cell culture similar to AHSV virus (Laegrid, 1996). Theiler could make a differentiation between AHS and EE based on the transmission pattern and clinical signs noticed.

The genus *Orbivirus* is the largest genus within the family Reoviridae. *Orbiviruses* encode at least seven structural and four non-structural proteins from 10 dsRNA genome segments that are encapsidated by a double-layered icosahedral shell. The seven structural proteins of the protein capsid vary in MW from 36,000-120,000 (Viljoen and Huismans, 1989). The inner capsid proteins VP3 and VP7 are serogroup-specific antigens, while the outer capsid protein, VP2, harbors serotype-specific antigenic epitopes that segregate a particular serogroup into distinct serotypes. S10 is the smallest segment of the genome encoding NS3 and NS3A (the non-structural proteins) from two alternate in-phase open reading frames (Van Staden and Huismans, 1991). The NS3 protein in orbiviruses mediates the release of virus particles from infected cells. The protein may also be involved in virus virulence and can significantly influence vector competence thus influencing the natural dispersion of these viruses. Phylogenetic analysis of EEV NS3 indicated the presence of two distinct clusters that correlate with the distribution of two different species of the *Culicoides* vector (Van Niekerk *et al*., 2003).

The level of variation found in the NS3 gene and protein of *Equine encephalosis virus* (EEV), was higher than that found for Bluetongue Virus (BTV) and reached 25 and 17 % on the nucleotide and amino acid levels, respectively (Van Niekerk *et al*., 2003). The EEV isolates formed a lineage independent from that of the other orbiviruses which segregated further into two clusters that corresponded to the South African northern and southern regions. The distribution of these isolates geographically may be related to the distribution of the Culicoides subspecies that transmit them. EEV is not a zoonotic pathogen and so does not possess any public health significance.

Potgieter *et al.* (2002) reported the first EEV sequence data which established that EEV genes have the same conserved termini (5' GUU and UAC 3') and coding assignment as AHSV and BTV. Co-expressed EEV VP3 and VP7 assembled into core-like particles that have typical orbivirus capsomeres. This kind of progress with cloning of complete genome of dsRNA genes is important for recombinant vaccine development and determination of the role of terminal sequences for replication and gene expression. Recently, Potgieter *et al.* (2009) reported significant improvements in the efficacy of sequence-independent amplification and quality of sequencing of viruses with segmented double-stranded RNA (dsRNA) genomes. They reported the complete consensus sequence of seven viruses from four different dsRNA virus groups. They include the first complete sequence of the genome of *Equine encephalosis virus* (EEV), the first complete sequence of an African Horse Sickness Virus (AHSV) genome determined directly from a blood sample and a complete human rotavirus genome determined from feces. Maan *et al.* (2007) described the techniques which are applicable to any viruses with segmented dsRNA genomes and conserved RNA termini. This has made it possible to generate sequence data rapidly from multiple isolates for molecular epidemiology studies.

Seven serotypes of virus (EEV1-EEV7) have been reported and the virus has been reported from southern Africa including Kenya, Botswana and South Africa (Viljoen and Huismans, 1989; Barnard and Paweska, 1993; Barnard, 1997; Venter *et al.*, 1999b; Laegrid, 1996; Howell *et al.*, 2002; Crafford *et al.*, 2003). The seven serotypes are EEV-1 (Bryanston), EEV-2 (Cascara), EEV-3 (Gamil), EEV-4 (Kaalplaas), EEV-5 (Kyalami), EEV-6 (Potchefstroom) and EEV-7 (E21/20) (Howell *et al.*, 2002). Kyalami, Bryanston and Cascara are the best known. Localized foci with an increased seasonal seroconversion in groups of horses to a specific serotype and the detection of an ongoing low level of infection from other serotypes within the population, confirm the independent persistence of the viruses in a maintenance cycle. Gerdes and Pieterse (1993) reported the isolation and identified a new member of the equine encephalosis group of orbiviruses the Potchefstroom virus. Virus was isolated from the blood of horses showing fever and jaundice. In cross neutralisation tests, a new serotype related to Gamil, one among the 6 *Equine encephalosis virus* serotypes has been identified wherein the isolates can be placed. The name Potchefstroom was proposed for this new serotype.

Depending on strain of the virus and the species of midges, EEV replicates to varying degrees in midges (Venter *et al.*, 2002). EEV has the potential for the emergence of new strains or recognition of currently undetected strains. Variations in pathogenicity might exist.

Epidemiology: EEV was first isolated in 1967 from horses in the Republic of South Africa that died from an unknown per acute illness. For many years before that AHSV was the only orbivirus of significance known to infect horses (Erasmus *et al.*, 1970). Equine encephalosis infection is most common in southern part of Africa and it is endemic to these areas (Barnard, 1997; Venter *et al.*, 1999a). Recently outbreaks of EEV have been reported in regions of Israel (Wescott *et al.*, 2013). The outbreak in 2008 in Israel involved 150 cases of equines but this does not cost for any mortality as per the data reported (Aharonson-Raz *et al.*, 2011). Since 1967, equine encephalosis took on epidemic proportions in some years, with more than 75% of horses tested having antibodies against EEV (Erasmus *et al.*, 1970). Subsequent serological studies have shown this orbivirus to be both widespread and prevalent in southern Africa (Venter *et al.*, 1999b). Seroprevalence rate has been recorded to be high in Equidae of South Africa. Horses, donkeys and zebra in Southern Africa frequently have antibodies to a group of EEV, indicating widespread infection of these equids

(Barnard and Paweska, 1993; Barnard, 1997; Paweska *et al*., 1999; Venter *et al*., 1999b; Howell *et al*., 2002; Lord *et al*., 2002; Paweska and Venter, 2004) with EEV appearing to be more prevalent than AHSV in South Africa (Venter *et al*., 1999b). One of the contributing reasons for the higher prevalence of EEV in southern parts of Africa in particular is due to immunization of horses with AHSV or related viruses (Mellor, 1993; Venter *et al*., 1999a). Cascara was the place which entered the book of history reporting the outbreak of EEV where animals were suspected for AHSV based on the clinical signs. Serological survey undertaken immediately after the isolation of cascara isolate suggested a higher geographical coverage of the virus within a short span of 3 months from the first report. Reports suggest that free ranging equines are more susceptible to the EEV infection when compared to stabled equines pointing the involvement of arthropods in the transmission (Venter *et al*., 1999a). Recent studies in Israel concluded that Israel strain was different and it does not come under the group of South African isolates (Kedmi *et al*., 2010; Mildenberg *et al*., 2009). This leads to the hypothesis that the virus has been transmitted to Israel by vector or airborne. It has also evolved itself to adapt to the conditions and the transmission was not acute as it took time for its evolution (Hendrickx *et al*., 2008; Aharonson-Raz *et al*., 2011).

Like the African Horse Sickness (AHS), Equine Encephalosis (EE) virus is also endemic to southern Africa. In most of the country, EE virus in comparison to AHS had a higher transmission rate. For EE virus the force of infection increased between 1985 and 1993 (Lord *et al*., 2002). Two species in the *Culicoides imicola* species complex, *C. imicola* (*senso stricto*) and *C. bolitinos* are known to transmit EEV (Venter *et al*., 1998, Venter *et al*., 1999a). In one study, of the 1456 horses tested, 1144 (77%) had antibody to EEV (Paweska and Venter, 2004). Homologous virus-neutralizing antibodies to six serotypes (EEV1 to EEV6) were detected in individual horses from different geographical regions. The distribution, prevalence and the rate of exposure to individual serotypes varied significantly between regions. The potential for several *Culicoides* species as vector for EEV with unique ecologies and lack of cross-protection to re-infection with multiple serotypes are likely to play a role in the virus natural maintenance cycle and the transmission amongst South African horses. *C. imicola* and *C. bolitinos* are spread throughout the length and breadth of sub Saharan Africa, regions of Northern America, Southern Europe and also in South Asian continent as well (Meiswinkel *et al*., 1994; MacLachlan and Guthrie, 2010). These two are also the well studied vectors for the transmission of other two orbivirus infections like BTV and AHSV (Venter *et al*., 1999a). A study conducted for a 10 year period from 1990-2000 showed a prevalence of 56.9% for one or more serotypes of EEV (Howell *et al*., 2002). Recently, Howell *et al*. (2008) studied the prevalence of serotype specific antibody to EEV in thorough bred yearlings in South Africa (1999-2004). Seasonal sero-prevalence varied between 3.6 and 34.7%, revealing both single and multiple serotype infections in an individual yearling. Serotypes 1 and 6 were most frequently and extensively identified while the remaining serotypes 2, 3, 5 and 7 were all identified as sporadic and localized infections affecting only individual horses. Antibodies to EEV have also been reported in elephants, though not often (Barnard, 1997). Oura *et al*. (2012) conducted a study wherein they showed the evidence for EEV circulation beyond South Africa; Ethiopia; Ghana and the Gambia for the first time. This indicates that it is most likely that circulation of EEV has occurred freely in East as well as West Africa. It has been revealed by the analysis of sequence that the isolate of EEV circulating in the Gambia is closely related to an isolate of EEV in Israel isolated during the year 2009 giving an indication of the common ancestry of the two viruses. Negative results have been obtained in case of horses in Morocco when studies have been conducted for the presence of EEV antibodies. This indicates that the Sahara desert may act

as a barrier geographically to the virus spread to countries in North America thereby shedding light on the spread of the virus in Israel causing the outbreak recently in the year 2009. Outbreaks of EEV in horses are recorded mostly in the late summer and autumn seasons because these seasons favor the replication of the vectors which transmit the virus (Coetzer and Guthrie, 2004; Rogers and Randolph, 2006). Every outbreak can have isolation of different serotype from the affected horses and it varies year round in the African continent. The most common isolate to be isolated from Southern Africa is the serotype 4. Other serotypes like 1, 6 and 7 are the major isolates in the recent outbreaks in Africa while the serotype 2, 3 and 5 are comparatively rare (Howell *et al*., 2002, 2008). There is a constant flip flop of the virus between the host and the vector especially in case of zebra which is considered as the potential maintenance host of the virus. Thus zebra helps in sustenance of the virus in the equine population in the African continent (Barnard and Paweska, 1993). The major problem in elimination of the EEV from Africa will remain on the extend with which it can identified in wild animals like zebra and elephants as they can act as maintenance host of the virus (Williams *et al*., 1993; Barnard, 1997). Another problem lies in the vector which transmits both AHSV and EEV (Williams *et al*., 1993; Zimmerli *et al*., 2010).

As far as the molecular epidemiology of the virus is concerned phylogenetic analysis has been conducted and a considerable degree of variation has been observed in non-structural (NS)-3 and structural protein (S)-10 gene segment. A 16.7% variation has been observed in the NS3 protein of EEV. Based on the sequence of S10/NS3 a lineage has been formed by NS3 of EEV which is independent to that of other orbiviruses. This lineage has segregated into two clusters corresponding to the northern as well as southern regions of Africa.

Disease: Necropsy examinations revealed general venous congestion, fatty liver degeneration, brain edema and highly conspicuous and sharply demarcated areas of catarrhal enteritis, especially in the small intestine's distal half. The microscopic findings are confirmed by histopathological examination (Lecatsas *et al*., 1973; Anderson, 2000). Outbreaks of EEV infection is reported to be associated with equine foetus abortion during the first 5-6 months of gestation. An early undetected abortion could therefore be misdiagnosed as infertility in a mare. Electron microscopic studies on thin sections of EEV-infected baby hamster kidney cells revealed that mature virus particles are released from the granular inclusion bodies seen in the cytoplasm. The cytopathology is further characterized by a swelling of the rough endoplasmic reticulum and by the presence of inclusion bodies in the mitochondrial matrix (Lecatsas *et al*., 1973). Clinical signs are noticed just 24 h before the death of the animal. The signs include stiff facial expression, stiffness of mouth and commissures. There is a bit of increase in temperature and heart rate but it was not marked improvement. Incubation period is short but variable between 3-6 days. Clinical symptoms attributed to EEV infection comprise fever, lassitude, edema of the lips, acute neurologic disease and enteritis. Few horses will develop redness of the mucus membrane of the eye which may be due to icterus (Erasmus *et al*., 1970; Chaimovitz, 2009). Facial swelling, swelling of supra orbital fossa and swelling of eyelids are also other signs noticed in some cases. Most of the cases died of EEV are more than 7 years of age and the reason behind it is not clear. Morbidity is higher and the mortality rate is usually minimal which is under 5% (Howell *et al*., 2004). Post mortem examinations of the affected horses show cerebral edema, localized enteritis, degeneration of cardiac myofibres and myocardial fibrosis. It is however not fully clear that whether these abnormalities are specifically due to EEV infection only (Laegrid, 1996). Thus clinical significance of the EEV appears to be limited. Asymptomatic or subclinical infections of EEV are mostly seen, as evidenced

by lack of clinical disease in majority of horses and higher sero-conversion rates. Disease associated with EEV infection is not well understood. Disease outbreaks have not been reported. However, due to the high prevalence of infection, EEV might be falsely diagnosed in some situations. In zebra or donkeys, EEV associated disease has not been reported (Paweska and Venter, 2004; Hinchcliff, 2007).

Diagnosis: The development of ^{32}P-labelled genomic probes of EEV for the detection of virus-specified RNA in infected cells have been reported with the probes derived from the genome segment encoding non-structural protein NS1 found to be the most sensitive (Venter *et al*., 1991). The cell-hybridization method is a rapid detection system for small quantities of infectious virus particles. Sero-epidemiological tools for the detection of antibodies against EEV include Serum Neutrahization Tests (SNT) and Enzyme Linked Immuno Sorbent Assay (ELISA). SNT could detect different serotypes, while ELISA is a group specific test. Williams *et al*. (1993) reported a group-reactive ELISAs for selectively detecting antibodies to African horse sickness and *Equine encephalosis virus*es in horse, donkey and zebra sera. Crafford *et al*. (2003) reported the development of a group-specific indirect sandwich ELISA for the detection of EEV antigen. A group specific competitive ELISA for detection of EEV is developed already (Crafford *et al*., 2011). In ELISA, cross-reactions were not observed with antigens of African horse sickness, blue tongue or epizootic hemorrhagic disease viruses. Clinical and pathological characteristics of the disease not known in depth and poorly defined and because of the high seroprevalence rates, the confirmatory diagnosis of the disease is difficult to make. Serotyping can be done using plaque inhibition neutralization assay (Quan *et al*., 2008). Though there are some reports regarding the development of serological assays for detection of EEV but there are limited reports regarding the nucleic acid amplification tests. A novel real time PCR assay has been developed for the detection of EEV by targeting the gene VP7. A bunch of 38 isolates were used for the detection using the developed real time PCR assay and it was found to be specific and sensitive for the diagnosis of EEV. For amplification of the structural (S)-7 gene segment of EEV, Fast PCR software has been used. From tissue culture isolates of EEV extraction of RNA has been done. For this purpose Mag Max Express particle processor as well as MagMax-96 total RNA isolation kits have been used. It has been found that EEV real-time RT-PCR is specific as it does not detect AHS or BT virus. Because of the convenience along with sensitivity and ability of rapid production of results the real-time format is selected for diagnosis. The assay however must be validated properly for establishment of diagnostic assay routinely (Rathogwa, 2011; Rathogwa *et al*., 2014).

EEV Bryanston NS3 gene has also been studied by means of expression in a translation system *in vitro* using the denatured double stranded ribonucleic acid (dsRNA) genome along with use of expression system (baculovirus). It has been indicated by these expression studies that EEV NS3 as well as NS3 A have been expressed approximately at equal levels during translation studies *in vitro*. Such study has made the confirmation of the presence of EEV in clinical specimen more accurate.

Treatment: The veterinarian must be contacted once the body temperature goes above 39 degrees. Supportive treatment by the use of anti-inflammatories as well as vitamins and appetite stimulants is required. Recovery in case of 90 per cent horses occurs without any complication. Horses can also remain as billiary carriers (i.e., without showing any clinical signs) for which it is important to treat such horses. Hospitalization of those horses that do not respond to supportive treatment may also be required (http://www.horsejunction.co.za/questions And Answers/view Thread?topic ID = 21527).

Prevention and control: Currently, no vaccine is available for preventing EEV in equines. As with most of the other viral infections, no treatment is recognized for EEV associated disease. Control measures are also not well defined (Hinchcliff, 2007). Management of horse in the stable is the key to control the spread of EEV in equines. The stables should be kept clean so as to prevent the breeding of the vectors. Horse should be brought to the stable before sunset and should not be allowed outside before sunrise. The period of dawn and dusk is the time when the vector is more active and this helps in control of EEV (Coetzer and Guthrie, 2004). Windows should be screened to keep in check the entry of vectors and also lights should be put off to minimize the attraction of vectors towards the light. Vector repellants can be used in the stable as well as over the horse to control its breeding. Diagnosis, treatment of affected animal and quarantine, isolation measures also help to control the spread of infection within the stable.

Present issue of global warming along with amplifying vector populations warrants the necessity for implementing rapid and recent advances in diagnosis and surveillance/monitoring tools (Rogers and Randolph, 2006; Crafford *et al*., 2003, 2011; Schmitt and Henderson, 2005; Belak, 2007; Rathogwa, 2011; Deb and Chakraborty, 2012; Deb *et al*., 2013; Dhama *et al*., 2008, 2012, 2013a, b, c, 2014; Rathogwa *et al*., 2014), safer, effective and advanced vaccine regimens and novel/alternative treatment modalities (Dhama *et al*., 2013d, e, f; Mahima *et al*., 2012; Tiwari *et al*., 2014) for safeguarding equines against this disease. Appropriate disease prevention and control measures with strict biosecurity practices also need due attention to tackle this virus.

Conclusion and future perspectives: Equine encephalosis is an endemic disease just like African horse sickness. Transmission rate of EEV is however more than AHSV in most of the countries. As infection usually occurs sub clinically confirmation of most of the cases is done by serological assays. Complement Fixation Test (CFT) has been recommended along with use of SNT and ELISA. Collection of heparinized blood and tissues is important for conducting diagnosis in clinical cases. The use of baculovirus expression system recently has allowed elaborate molecular study on EEV. The control of the disease solely depends on how the horses are managed in the stables. Implementation of good biosecurity measures in stable is of great importance to prevent disease in time. Along with this effective control of midges is important. By examining prevalence of infection in various age classes of vertebrate host much can be inferred about transmission of the virus. For this reason measurement of antibody titre is of utmost importance that gives an estimate of the exposure to the virus and thereby helps to undertake effective prevention and control measure. There is no vaccine available currently but with the advent of molecular technology and vaccinology the development of effective vaccines is expected in near future.

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