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Clinical signs, clinical pathology and outcomes in horses infected naturally with equine encephalosis virus

Graeme Piketh | Adrienne Viljoen | Christina Eberhardt

Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

Correspondence
Graeme Piketh, Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa.
Email: graeme.piketh@up.ac.za

Abstract

Background: Equine encephalosis (EE) is caused by an *Orbivirus* from the family *Sedoreoviridae* and is thus similar to African horse sickness (AHS) and Bluetongue viruses (BTV). These viruses are transmitted by *Culicoides* midges. Equine encephalosis can infect horses, donkeys and zebras sub-clinically while only horses develop clinical disease. The vector's distribution is climate-dependent with evidence for circulation in Southern Africa, the Middle East and India. Global warming could facilitate the expansion of this distribution and consequently the potential spread into Europe should not be overlooked.

Objectives: To describe clinical signs, clinicopathological abnormalities, and outcomes in horses naturally infected with EE.

Study Design: A retrospective, descriptive, observational study.

Methods: Data were obtained from the Onderstepoort Veterinary Academic Hospital's clinical database to identify cases with EE from 2013 to 2023. Data including the history, clinical signs and clinicopathology were analysed.

Results: Equine encephalosis cases predominantly occurred from February to April. Twenty-five horses were included. Throughout the disease, 25 (100.0%) horses had pyrexia (mean maximum temperature 39.3°C; SD 0.86°C), 16 (64.0%) horses had tachycardia (median maximum heart rate 52/min; range 36–100/min), 19 (76.0%) horses had tachypnoea (median maximum respiratory rate 24/min; range 12–60/min). Within 24 h of presentation, horses predominantly displayed lymphopenia (median 1.17×10^9 cells/L; range $0.15–9.21 \times 10^9$ cells/L), thrombocytopenia (median 67.5×10^9 cells/L; range $3–303 \times 10^9$ cells/L), and leukopenia (median 5.44×10^9 cells/L; range $2.08–18.07 \times 10^9$ cells/L).

Main Limitations: Retrospective study design with a small number of cases and many of these evaluated at differing times after infection.

Conclusion: Pyrexia, tachycardia and tachypnoea are the most common clinical signs associated with EE. Haematological evaluation appears valuable in EE cases, with leukopenia, lymphopenia, and thrombocytopenia commonly observed. Equine encephalosis is a relevant differential diagnosis for other infectious diseases in horses in geographical regions where EEV and *Culicoides* vectors are potentially present.

KEYWORDS
horse, leucopaenia, neutrophilia, pyrexia, tachycardia, vector

1 | INTRODUCTION

Equine encephalosis (EE) is a vector-borne viral disease that is endemic to the southern part of Africa.[1–5] The disease was first described in 1910 by Sir Arnold Theiler as a disease with similarities to African horse sickness (AHS). It was then referred to as 'Ephemeral Fever'.[6] Equine encephalosis virus (EEV) is an *Orbivirus* of the family *Sedoreoviridae*.[7] Like AHS and Bluetongue virus (BTV), which are also *orbiviruses*, EEV is transmitted by *Culicoides* midges.[8] Midges become infected when feeding on a viraemic host. The virus is suspected to replicate in the midgut, spread to the salivary glands, and be transmitted to new hosts during feeding.[9] Transmission is highly dependent on climate and environmental conditions that support vector survival and breeding. Warm, humid conditions favour midge population growth, thereby increasing the risk of virus spread. Initially considered to be confined to the southern part of Africa, recent serological evidence has confirmed the presence of the virus in the Middle East and India in association with disease outbreaks.[1–5,10] The epidemiology of the virus is closely linked to the ecology of its vector, whose climate-dependent distribution may expand with global warming.[11–13] For example, since 2007, multiple epidemics of BTV have occurred throughout Europe.[14] Given the shared vectors and similar epidemiology between BTV and EE, the risk of EE spreading into Europe warrants attention.[14–19]

Equine encephalosis virus has been categorised into 7 different serotypes based on variations in the viral protein-2 (VP-2) structural protein.[7,20–23] Equine encephalosis can sub-clinically infect horses, donkeys and zebras while in some cases, horses can progress to develop clinical disease.[2,24,25] Several small studies outline clinical signs and pathological findings in EE cases. Common clinical signs reported include fever, inappetence, dullness, peripheral oedema and neurological signs.[22–24,26–29] One study suggests that certain EE serotypes may be associated with specific clinical signs and pathology.[26] The largely non-specific clinical signs make EE an important differential diagnosis for other infectious diseases primarily presenting with pyrexia of unknown origin.[30] Treatment is focused on symptomatic and supportive management including managing pyrexia and ensuring normal hydration status. Vaccination is currently unavailable, and preventative measures focus on vector control to reduce horse contact.[30] No specific studies have investigated clinicopathological abnormalities or disease progression in cases of EE.[22–24,26–29] This study aimed to perform a retrospective analysis of records from horses diagnosed with EE by the Onderstepoort Veterinary Academic Hospital (OVAH) of the University of Pretoria. The aims were to assess horses naturally infected with EE in order to describe associated clinical signs, clinicopathological abnormalities, disease progression and outcomes.

2 | MATERIALS AND METHODS

2.1 | Animals

Hospital records from horses presented to OVAH in South Africa were reviewed to identify cases that tested positive for EE by real-time polymerase chain reaction (RT-PCR) from January 2013 to March 2023. Horses were included in the dataset if they met the following inclusion criteria:

1. The horse was examined by OVAH's referral or ambulatory service due to clinical signs for which EE was considered a differential diagnosis and clinical examination records were available.
2. A diagnosis of EE was confirmed based on a positive RT-PCR performed on whole blood with a cycle threshold (Ct count) less than 35. The cut-off point is considered by the laboratory to be the point above which the RT-PCR is unlikely to identify viral genetic material even if it is present.[31]
3. Other obvious concurrent disease was excluded based on a standard diagnostic approach and evaluation including clinical examination, routine haematology, and biochemistry, thoracic and abdominal ultrasound, abdominocentesis, blood smear as well as RT-PCR testing for AHS, Equine Herpes Viruses 1 and 4, piroplasmosis and where possible for West Nile, Sindbis, Shuni, Middelburg and Wesslesbron viruses. All haematology was conducted on blood with ethylenediamine tetra-acetic acid (EDTA) as an anti-coagulant. All cases with thrombocytopenia were also identified to have a low platelet count on manual assessment of a blood smear made from blood obtained from the jugular vein.

The sample size was determined by including the maximum number of cases that met the appropriate inclusion criteria.

2.2 | Examination protocol

Information obtained from the medical records included age, breed, sex, time of the year at presentation, historical abnormalities, and duration of illness and medications administered prior to presentation to the hospital. Clinical examination findings, results of diagnostic tests performed within 24 h of admission including RT-PCR for EE and Ct count, complete blood count, venous blood gas analysis and biochemistry, and results of subsequent examinations were recorded. Abnormalities on the follow-up clinical examinations were recorded the first time they occurred, if not present on initial examination. Total days of pyrexia (rectal temperature >38.5°C) from when it was first

identified, total length of hospitalisation and patient outcome were also recorded.

2.3 | Data analysis

Qualitative (nominal) data was summarised in the form of a frequency table and then expressed in the form of a percentage.

Quantitative data was assessed for normality using the Shapiro–Wilk test. Normally distributed data was then summarised as mean ± standard deviation. Non-parametric data was summarised in the form of median ± range.

When assessing the clinical parameters and haematology, abnormalities were defined based on the normal reference range for the specific age categories, the reference ranges supplied by the appropriate laboratory or as can be found in the literature when not available for the laboratory in question. Pyrexia was defined as a temperature >38.5°C, tachycardia as heart rate (HR) >44 beats per minute (BPM) and tachypnoea as respiratory rate (RR) ≥20 breaths per minute (BPM).[32]

3 | RESULTS

3.1 | Study population

Analysis of the hospital records revealed a total number of 37 patients with positive test results on RT-PCR for EE. Of these 37 positive cases, 28 cases (75.7%) met the inclusion criteria. Five cases were excluded based on the presence of concurrent disease, and 4 cases were excluded based on the lack of clinical data in the medical records. Of the 28 cases, 3 cases were <12 months of age. These cases were excluded from graphical and descriptive data analysis based on differing age-related reference intervals, to allow appropriate categorisation. Data for these cases (<12 months of age) is presented as Table S1. A flow chart outlining the study population's inclusion is available as Figure 1.

The sex distribution of the remaining study population (25 horses) consisted of 4 (16%) stallions, 11 (44%) geldings and 10 (40%) mares. The breed distributions are reflected in Table 1. The ages ranged from 1 to 20 years old with a median age of 9 years.

3.2 | History

Case occurrences between the years of 2013 and 2023 are presented in Figure 2.

Each year had a median of 2 cases (range 0–6). Multiple EE cases were reported to occur between the months of February and April inclusive, with isolated cases in January, June and December. None of the cases included in the study occurred on the same premises in close temporal proximity. Pyrexia (rectal temperature >38.5°C) was the sole historical abnormality in 8/25 (32%) horses. Based on history, 16/25 horses (64%) had fever and concurrent clinical signs including colic, inappetence, neurological abnormalities, lethargy, supraorbital swelling and coughing prior to presentation. One horse that did not have fever as a historical abnormality, reportedly had colic prior to presentation. This horse later developed fever after presentation. In 15/25 (60%) cases, horses were examined on the same day that clinical signs were first noted by the owner. In 9/25 (36%) cases, there was an average delay of 2.2 ± 1.48 days before presentation. One case (4%) lacked information on the onset of clinical signs. Twelve of

TABLE 1 Distribution of breeds of horses admitted to Onderstepoort Veterinary Academic Hospital of the University of Pretoria during 2013–2023 that met the inclusion criteria of positive RT-PCR for equine encephalosis virus.

Breed	Number	Percentage (%)
Thoroughbred	6	24
Warmblood	9	36
Friesian	4	16
Boerperd	2	8
Nooitgedacht	2	8
Nordic	1	4
Trakehner	1	4
Total	25	

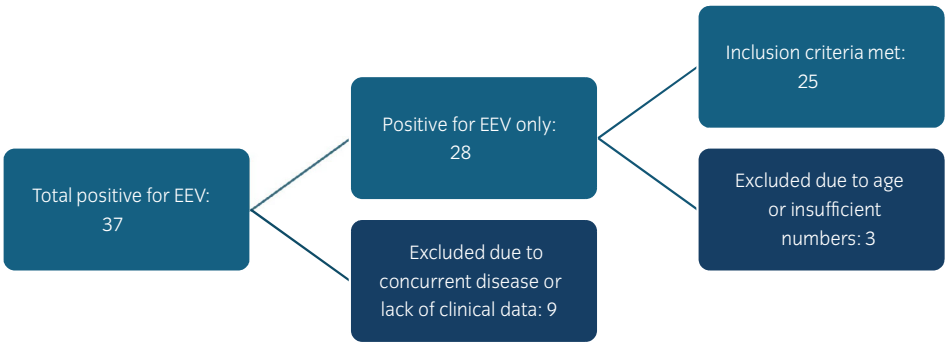

FIGURE 1 Number of cases included or excluded in the analysis of horses naturally infected solely with equine encephalosis virus (EEV).

25 horses had received treatment by a veterinarian prior to presentation to OVAH. Treatment consisted primarily of non-steroidal anti-inflammatory drugs (NSAIDs), with some horses also receiving dexamethasone or dipyrone. Specific details of historical abnormalities and treatments can be found in Table S2.

3.3 | Clinical examination findings and diagnostics

Twenty-one of 25 horses (84%) were examined at the hospital facility and 4 cases (16%) were examined and treated by the hospital's ambulatory service at the horse's yard. Seven cases (28%) were examined on a first-opinion basis, with four of these seen by the ambulatory service and three presenting directly to the hospital. The remaining 18 cases (72%) were referred by an outside veterinarian due to limited diagnostic capabilities in the field. None of the horses had a positive diagnosis for EE prior to presentation.

All 25 cases had data of the clinical examination available from the day of presentation to the hospital services. Follow-up examination performed during hospitalisation was available from 21 (84%) cases. Table 2 shows the number of horses with specific clinical abnormalities identified during the clinical examination at presentation and over all examinations performed from the time of presentation (each horse only had the abnormality recorded once).

At the time of presentation, the average rectal temperature was 38.5°C (SD 0.93°C), the median heart rate was 48 beats per minute (range 36–100) and the median respiratory rate was 24 breaths per minute (range 12–60). All horses that were normothermic on presentation had received antipyretic medications prior to clinical examination.

The number of horses with pyrexia, tachycardia and tachypnoea over the first 7 days after presentation can be seen in Figure 3.

Throughout the illness, horses with EE had an average maximum temperature of 39.3°C (SD 0.86°C), a median maximum heart rate of 52 beats per minute (range: 36–100), and a median maximum

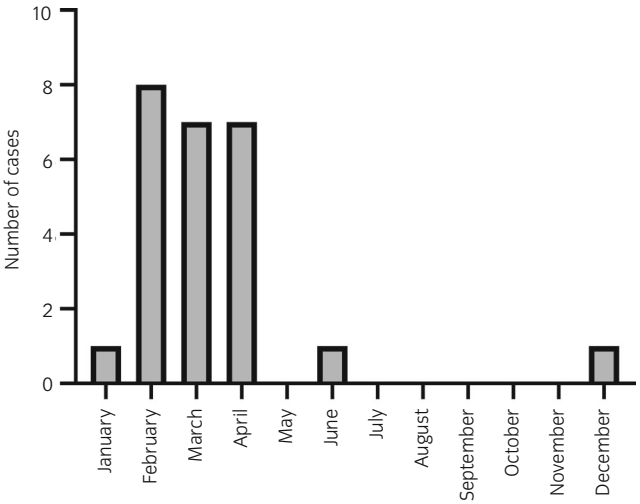

FIGURE 2 Monthly distribution of equine encephalosis cases of the 25 horses that presented to the Onderstepoort Veterinary Academic Hospital of the University of Pretoria between the years 2013 and 2023.

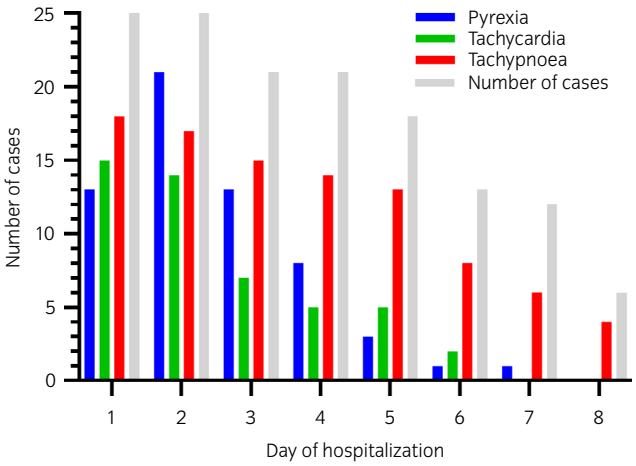

FIGURE 3 Number of horses with equine encephalosis that displayed pyrexia, tachycardia, and tachypnoea and the total number of horses still receiving veterinary care over the first 7 days.

TABLE 2 Frequency of clinical abnormalities identified at the time of presentation and over the entire course of the disease process in 25 adult horses with equine encephalosis.

	Presentation		Entire disease course	
Clinical abnormality	**Number (*n*)**	**Percentage (%)**	**Number (*n*)**	**Percentage (%)**
Pyrexia (>38.5°C)	13	52	25	100
Tachypnoea (≥20/min)	15	60	19	76
Tachycardia (>44/min)	18	72	16	64
Colic	11	44	11	44
Signs of dehydration	9	36	9	36
Neurological signs	6	24	6	24
Lethargy/dullness	5	20	5	20
Icterus	3	12	3	12
Peripheral oedema	3	12	3	12
Petechiae	1	4	1	4

respiratory rate of 24 breaths per minute (range: 12–60). EE cases had a median of 3 (range: 1–9) days of fever.

Neurological abnormalities recorded included ataxia, stupor, anxiety and hyperaesthesia, abnormal head position with the head lowered, head tilt, tongue paresis, seizure-like activity, and weakness. Peripheral oedema involved the supraorbital fossae, lips, ventral abdomen, pectoral muscles, and distal limbs. All 11 (44%) horses that displayed colic signs underwent examination per rectal palpation and abdominal ultrasound. Eight (32%) horses had no significant abnormalities, and 3 (12%) horses had a large colon impaction, ileal impaction, and large colon displacement, respectively. One horse also had grade 4 squamous gastric ulcer syndrome and glandular gastric disease. Thoracic ultrasound was performed in 19 (76%) cases of which 6 (24%) had bilateral ring-down artefacts and 13 (52%) had no abnormality recorded. Electrocardiographic evaluation was performed in 3 (12%) horses. An accelerated idioventricular rhythm and isolated ventricular premature complexes were present in one horse. The other two horses displayed sinus tachycardia with no recorded arrhythmias.

RT-PCR testing for neuroarboviruses (West Nile, Sindbis, Shuni, Middelburg and Wesslesbron viruses) was only performed in the 6 horses with neurological signs, of which 4 cases had a negative result and in 2 horses, no results were recorded.

3.4 | Clinical pathology findings

Twenty-three cases (92%) had haematology results available from the day of presentation. Twenty-two of these cases had results from a complete blood count and 1 had results from a partial blood count. Biochemistry results were available for a subset of cases to a varying extent; in some horses, only selected parameters were recorded. The descriptive statistics and frequencies of laboratory findings for cases with EE are summarised in Table 3.

3.5 | Hospitalisation, treatment and outcome

Twenty-one of 25 cases (84%) were hospitalised. The median number of days that horses were hospitalised was 7 days (range 3–19). Complications developed in 3/25 (12.0%) cases. The median number of days of hospitalisation, excluding the cases with complications, was 6.5 days (range 3–15).

Common medications administered included NSAIDs (17/25 cases; 68%), antimicrobials (8/25 cases; 32%), and corticosteroids (6/25 cases; 24%). Fluid therapy was administered in 12 cases (48%). Specific details about medications, additional treatments and complications in individual cases can be found in Table S3.

All horses survived and were discharged from veterinary care. At discharge, 24/25 (96%) horses were clinically healthy. One horse had grade 3 ataxia at discharge, which improved to grade 1 after 9 weeks.

4 | DISCUSSION

4.1 | Clinical and economic relevance

Equine encephalosis is widely purported to be a viral disease of limited clinical importance and minor economic relevance.[13,30] Based on the clinical findings in this research however, the disease should not simply be deemed as inconsequential. Considering its close clinical resemblance to some cases of AHS as well as the similarities in epidemiology, EE should be considered as an important differential diagnosis for AHS.[6,22,30] As reported here, EE can lead to a variety of clinical signs and as such may be confused with a multitude of other diseases.[6,22,30] This, coupled with the possibilities of co-infection means these potential cases of EE often require multiple diagnostic modalities to ensure thorough diagnosis.[6] This contributes not only to the economic aspects of case management but also to the need for a diverse array of diagnostic capabilities.

The clinical relevance of EE for the individual patient is more difficult to define with considerable variation in clinical signs. However, in the current study, pyrexia, tachypnoea and tachycardia were reported in over half of cases. Although not frequently reported, more severe clinical signs associated with EE have been identified in the literature including neurological disease and sudden death.[22,24,26] This combined with the clinical signs such as ventricular arrhythmias identified here suggests that further research into the pathogenesis is required before a definitive statement about the true clinical impact of infection with EEV can be made.

4.2 | Epidemiology and clinical signs

Infections in this data set were noted to be most common from February to April each year, corresponding to the late summer and early autumn period in Southern Africa. Similar findings have been reported for AHS,[43] as well as BTV regarding the times of peak infections.[34] This is likely a function of the epidemiology of the virus and its vector transmission. Considering the similarities in vector transmission and seasonal occurrence, EE could be considered a sentinel for arboviral disease such as AHS and West Nile. Transmission of EE suggests that the appropriate epidemiological elements are present for many vector-borne diseases to occur, with clinical EE cases portending other disease occurrences in the near future for this reason. Because of this, horses in the vicinity of positive EE cases should be considered at risk of exposure to other arboviral diseases. This is particularly relevant for AHS considering their congruous epidemiology.[30] The specific reasons for the years with the highest number of cases were not investigated here as it was beyond the scope of this project.

All of the cases displayed at least one episode of pyrexia over the course of the disease. This supports previous findings indicating that there was a significant association between pyrexia and infection with EE, although the actual proportion of cases that were positive for EE and had pyrexia was greater in the present study.[22]

TABLE 3 Descriptive statistics and frequency for the clinicopathological findings identified within the first 24 h after presentation in 25 horses with equine encephalosis.

Variable	No. of horses with data	Reference range	Median (range)	Mean (standard deviation)	Number of cases above reference range	Number of cases below reference range
Haematology						
Haematocrit (L/L)	23	0.24–0.44[a]	0.34 (0.24–0.54)		2	0
RBC (10^{12} cells/L)	23	5.5–9.5[a]	7.75 (5.53–12.34)		2	0
WBC (10^9 cells/L)	23	6–12[a]	5.44 (2.08–18.07)		2	14
Mature Neu (10^9cells/L)	22	3.54–7.08[a]	3.69 (1.09–12.79)		2	9
Immature Neu (10^9cells/L)	22	0–0.24[a]	0.08 (0–1.32)		6	0
Lym (10^9 cells/L)	22	1.8–3.6[a]	**1.17 (0.15–9.21)**[b]		2	17
Mon (10^9 cells/L)	22	0–0.72[a]	0.18 (0–1.26)		3	0
PLT (10^9 cells/L)	23	>100[c]	**67.5 (3.0–303.0)**[b]		0	15
Biochemistry						
GGT (IU/L)	9	2–25[a]	15 (0–73)		1	0
GLDH (IU/L)	9	1–8[a]	3 (0–11)		1	0
Albumin (g/L)	10	28–39[a]		30.99 (2.64)	0	2
Globulin (g/L)	10	28–44[a]		28.91 (7.07)	1	5
TSP (g/L)	13	66–78[a]		**61.85 (6.88)**[b]	0	10
Creatinine (μmol/L)	8	105–170[a]		105.00 (20.81)	0	4
Urea (mmol/L)	5	3.8–7.7[a]		3.92 (0.63)	0	1
Total bilirubin (mmol/L)	5	8.3–30.2[a]		**63.96 (15.61)**[b]	5	0
SAA (mg/L)	6	0–24[a]	**386 (5.2–1817)**[b]		5	0
Blood lactate (mmol/L)	6	<2[c]	1.2 (0.47–4.10)		1	0
Sodium (mmol/L)	12	136–144		**133.67 (3.65)**[b]	0	8
Potassium (mmol/L)	12	2.1–5.1[a]		3.42 (0.45)	0	0
Chloride (mmol/L)	7	96–110[a]	103 (92–104)		0	1
*i*Calcium (mmol/L)	11	1.48–1.65[d]		1.5 (0.068)	0	0
pH	12	7.32–7.44[a]		7.43 (0.04)	4	0
Bicarbonate (mEq/L)	12	27.06–32.94[d]		**24.56 (1.81)**[b]	0	10

Abbreviations: GGT, γ-glutamyltransferase; GLDH, glutamate dehydrogenase; *i*Calcium, ionised calcium; Neu, neutrophils; pCO_2, partial pressure of carbon dioxide; PLT, platelets; RBC, red blood cells; SAA, Serum Amyloid A; TSP, total serum protein; WBC, white blood cells.
Note: Bold values indicate values outside of the reference range.
[a]Reference range obtained from Clinical Pathology Laboratory.
[b]Measure of central tendency outside the reference range.
[c]Reference range obtained from Bayly et al.[32]
[d]Reference range obtained from Lascola et al.[33]

Additional common clinical signs identified on clinical examination included tachycardia as well as tachypnoea. This coincides with the findings reported in the literature.[6,26–28] Although pyrexia is a known cause for sinus tachycardia, a large portion of the horses had tachycardia without the concurrent presence of fever.[43] Further investigations of the tachycardias using electrocardiography were only performed in a small number of cases but identified some evidence of other abnormal rhythms. This further raises the question of whether more of the cases had pathological arrhythmias that were not identified because no ECG was performed. To the authors' knowledge, no other studies have examined ECG findings in cases of EE. Combining this information and considering the literature which reports that cases of infection with the Bryanston serotype (serotype 1) of EE had histopathological evidence of myocardial degeneration, fibrosis and haemorrhage,[26] a more thorough evaluation of the cardiovascular system may have the potential to contribute valuable information to understanding the pathogenesis and cardiovascular consequences of this disease.

Tachypnoea was observed as a common clinical finding in EE similar to AHS. This finding can be correlated to the presence of pyrexia; however not all cases had tachypnoea and pyrexia concurrently at all times. This and findings in the literature of an association between EE and dyspnoea seems to support the idea of EE leading to pulmonary pathology.[22] Additional pulmonary evaluations (thoracic ultrasound) in the present data set only revealed subjectively minor clinical indicators of pathology. In particular, the severe pulmonary oedema and

pleural effusions often present in cases of AHS were conspicuously absent in the present cases of EE.[35]

Colic was identified as a clinical sign in 44% of cases with EE. Specific gastrointestinal tract (GIT) lesions were only confirmed in a small number of cases. The remaining cases had no specific GIT pathology identified on routine evaluation. Colic signs have been reported previously in cases of EE, but the literature does not expand on reasons for these signs in association with the disease.[28] These findings may suggest that colic is not solely due to the development of secondary GIT lesions such as large colon impactions. Signs of colic can result from extra-gastrointestinal causes but might also reflect a state of general discomfort of no specific cause. Scant reports in the literature of segmental areas of catarrhal enteritis lend credence to the idea of a primary lesion caused by EE in the GIT.[26,30]

Neurological signs were recorded as an infrequent clinical sign in this study (24%). This is in contrast to other reports that 47% of EE cases had neurological signs.[22] Inadequate knowledge of the true pathogenesis of these clinical signs in EE leaves the reason for this discrepancy unresolved.

Several other non-specific clinical signs including lethargy, congested mucous membranes and dehydration were identified at very low frequencies (<36%). These signs can arise due to a multitude of reasons and as such may or may not be directly ascribed to EE. Although the specific serotypes responsible for the cases of EE and therefore the specific clinical signs identified in this data set were not evaluated, review of the literature does suggest specific clinical signs ascribed to certain serotypes of EE.[23,26] Considering this, it is plausible to hypothesise that the presence of specific clinical signs in a population can be influenced by the serotype of EE which is causing the infection, with the prevalence of these clinical signs correlating to the prevalence of specific serotypes in the region.

4.3 | Clinical pathology and diagnostic testing

The veterinary literature is sparse regarding the description of clinical cases of EE with no specific publications examining clinicopathological data. This is the first study, to the authors' knowledge, that directly describes clinicopathological findings in cases naturally infected with EE. In this study, more than half of the cases tested showed leukopenia. Similar leukopenia characterised by lymphopenia, neutropenia, and left shift towards a predominance of immature neutrophils is seen in experimental cases of AHS and may be due to increased leukocyte margination but may also result from stress-induced endogenous corticosteroid release.[35] However, horses experimentally infected with AHS over time do not show clinically significant leukopenia (WCC < 4700 cell/μL).[43] Bluetongue Virus, another *Orbivirus*, is also associated with inconsistent findings of leukopenia.[39] This may reflect viral immune evasion through host immune downregulation in these diseases.[35,38] It is noteworthy that EE, typically regarded as milder than AHS or BTV in susceptible mammalian hosts, exhibited marked leukogram changes with 10/25 (40%) cases showing clinically significant leukopenia.[34] The reasons for this are likely to be multifactorial including EEV serotype and pathogenesis, host genetics, stage of disease on admission, potential immunomodulation and others.

The fact that leukopenia was evident within 24 h of disease onset in several cases (52%) suggests a rapid leukocyte response from the onset of clinical disease. In general, horses with clinically significant leukopenia especially associated with neutropenia are also at increased risk of complications relating to secondary infections,[38] and may be part of the reason for the complications that developed in a small number of cases identified in this study (12%). These complications can and did compound the severity of the disease, resulting in prolongation of recovery as well as increased costs associated with additional treatments and care. The low frequency of complications relating to secondary infection may in part be due to the use of antimicrobials in 32% of cases.

Thrombocytopenia was also a common clinicopathological finding. A definitive cause for the thrombocytopenia is unknown. Considering the endothelial tropisms of related viruses such as AHSV and BTV, endothelial injury is a possible initiator of platelet consumption and thereby thrombocytopenia in EE.[39,40] More importantly, 6/25 (24%) cases showed thrombocytopenia below the threshold suggested to be associated with clinical signs of petechiae that is, $<30 \times 10^9$ cells/L.[41,42] However, only one case showed clinical petechiation in this study.

The findings in this study demonstrate that horses with EE display clinically significant haematological abnormalities. Therefore, suspected EEV cases should have haematology performed as part of their diagnostic assessment.

Mild hypoproteinemia was identified in 10/25 (40%) horses tested with the predominant cause being hypoglobulinemia (20%). Only 3 cases showed oedema clinically of which only one had concurrent hypoproteinemia, making it an unlikely mechanism of oedema formation. Mild hyperbilirubinemia was present in all 5 cases tested. Icterus was only identified in 3 EE cases which is supported by the literature.[22] Only one of these cases displaying clinical icterus had corresponding biochemistry results available for assessment (results were indicative of hyperbilirubinemia). Liver enzymes were normal in most cases tested and no signs of haemolysis were present. This corresponds with the likelihood of the development of hyperbilirubinemia and icterus being due to anorexia.[32] Although Serum Amyloid A was only assessed in 6 cases, 5/6 (83%) horses had moderate increases in SAA. This suggests that EE may be associated with systemic inflammatory responses and increases in acute phase proteins. Mild metabolic disturbances were infrequently detected. However, mild hyponatremia was a commonly identified electrolyte abnormality in EE cases.

4.4 | Outcomes

A large number of horses in this data set were hospitalised although this should be interpreted with caution as no information about the total number of EE cases or its seroprevalence in the respective areas was available for comparison during this time period. The results from this study do however reflect that some cases of EE require hospitalisation and intensive care and monitoring. Prolonged periods of

hospitalisation were identified in some cases of EE, especially when associated with the development of secondary complications.

No cases of mortality in adult horses infected with EE occurred. Low levels of mortality for EE are described in the literature although a definitive diagnosis of EE was not irrevocably proven in the majority of these publications.[21,25] The majority of horses were reported to make a complete recovery.

5 | STUDY LIMITATIONS

Several limitations can be identified with regard to the study design. The retrospective nature of the study results in an innate set of limitations. Selection bias is a limitation of retrospective studies including the study in question, as cases were selected based on their known positive test results for EEV. Selection bias may also be applicable as there is a propensity to only test horses for EE if they develop fever even though it may be possible for horses to show other clinical signs and no fever. The total number of cases included was relatively small and likely only represents a small proportion of the total cases present in South Africa over this time period. Although all horses included in the data set were RT-PCR positive for EEV and had the majority of other potential infectious causes for their clinical signs excluded, an exhaustive exclusion of potential causes and/or co-infections was impractical especially considering the retrospective nature of the research. This can lead to substantial confounding in associations. This is particularly true when considering the fact that RT-PCR testing for the neuro-arboviruses can result in substantial false negative results. Due to the fact that many of the cases were examined on a referral basis, some cases were evaluated at differing stages of the disease process. This means that many of the cases could have had abnormalities that had resolved prior to referral. Medications were administered prior to referral in several cases and could have resulted in discrepancies in the true frequency of clinical signs and potentially some influence on haematology performed at presentation. Thrombocyte counts were determined on EDTA blood and thrombocytopenia was confirmed on manual assessments of blood smears. A more robust approach would have been to perform thrombocyte counts on blood collected into a sodium citrate anticoagulant to avoid inaccuracies due to platelet clumping. Although a Ct value was determined for the majority of cases, the horses in this study were all tested at different stages of the disease process and as such a direct correlation between Ct value and disease severity or clinical signs could not be evaluated. The duration of hospitalisation on the development of clinical signs was not assessed. Hospitalisation and its associated management changes and stress can lead to the development of additional disease and associated clinical signs that are not linked with EE.

6 | CONCLUSION

Equine encephalosis is a relevant differential diagnosis for infectious diseases in horses within the geographical distribution of the *Culicoides* vector. Similarities were identified in the epidemiology and clinical signs specifically relating to AHS. Pyrexia, tachycardia and tachypnoea were the most common clinical signs associated with EE. Haematological evaluation appears valuable in EE cases, with leukopenia, lymphopenia, and thrombocytopenia commonly observed. These findings on haematology are potentially valuable to help differentiate EE from cases of AHS. Further research into the pathogenesis and pathophysiology of EE is vital, to better describe these haematological changes and the reasons for their development and quantify the clinical significance of this disease.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Graeme Piketh: Conceptualization; investigation; writing – original draft; writing – review and editing; methodology; formal analysis; project administration; data curation; resources; visualization; validation. **Adrienne Viljoen:** Conceptualization; methodology; supervision; writing – review and editing; project administration. **Christina Eberhardt:** Conceptualization; supervision; writing – original draft; writing – review and editing; methodology; investigation; visualization; project administration; formal analysis.

DATA INTEGRITY STATEMENT

Graeme Piketh had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

ETHICAL ANIMAL RESEARCH

This study was assessed and approved by the University of Pretoria, Research Ethics Committee, reference number REC123-22.

INFORMED CONSENT

Consent to use clinical information for research purposes is provided on admission of patients for veterinary evaluation at the University of Pretoria.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/evj.70117.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the University of Pretoria research repository at http://doi.org/10.25403/UPresearchdata.25942681, reference number 25942681.

ORCID

Graeme Piketh https://orcid.org/0000-0002-1556-3418

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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