Epidemiology and reproductive outcomes of EHV-1 abortion epizootics in unvaccinated Thoroughbred mares in South Africa

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Ethical animal research

This was a retrospective study performed on data collected from privately owned animals that received the highest standards of care. All activities were conducted with informed owners.

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Authorship

M.L.S. developed the hypotheses, designed the data collection protocol, interpreted the data and was the principal author contributing to writing the manuscript; B.VDM. and A.B. collected all data; AJG performed the data analysis and TAES assisted with the data interpretation and contributed significantly to the writing of the manuscript

Summary

Reasons for performing study*:* EHV-1 is one of the most common causes of infectious abortion in mares. Analyzing the demography of outbreaks and detailing subsequent reproductive performance of affected mares will assist in the management of future (threatened) epizootics.

Objectives: To examine the epidemiology and reproductive outcomes of two EHV-1 abortion epizootics with very different patterns of morbidity.

Study design: Epidemiological and reproductive data were analyzed retrospectively following abortion epizootics associated with EHV-1, but initiated via different routes, among unvaccinated mares on two Thoroughbred farms in South Africa.

Methods: Aborting mares were assigned to either the EHV-1 abortion cohort via positive immunostaining (farm 1 and 2) or qPCR (farm 2) on tissue samples, or to the non-EHV abortion cohort.

Results: During their respective epizootics, EHV-1 abortions affected 9/30 (30.0%) and 18/316 (5.7%) of the pregnant mares on farms 1 and 2 respectively; there were also 25 (7.9%) non-EHV abortions on farm 2. Epizootic differences included: durations (farm 1 = 135 d; farm 2 = 34 d), intervals between first and subsequent abortions (farm 1 = 39 d; farm 2 = 2 d), and intervals to confirmation of EHV-1 (farm 1 = 40 d; farm 2 = 2 d). The median age of EHV-1 abortion mares (8.0; 5-18 years) in both epizootics was similar, but significantly younger (P= 0.004) than the 25 non-EHV-1 abortion mares (11.0; 4-24 years) on farm 2. Gestation stage (median; range) of EHV-1 (291.5; 277-313) and non-EHV-1 (211.9; 61-339 d) abortions were significantly different (P=0.001). The post-abortion complications and subsequent reproductive outcomes had no significant association with EHV-1 abortion.

Conclusions: The marked difference in morbidity between the two epizootics may be associated with routes of introduction or intervention strategy dictated by availability of molecular diagnostic techniques. Unexpectedly, EHV-1 was not more commonly associated with post-abortion complications.

Introduction

Equine herpes virus type 1 (EHV-1) is the most important viral cause of equine abortion, both because it is relatively common and because of its potential for epizootic spread, particularly within naïve populations [1, 2, 3, 4]. Under natural conditions, EHV-1 abortion rarely occurs before four months of gestation: 95 % of diagnosed EHV-1 abortions occur in the last third of pregnancy and 75 to 80 % between eight and 10 months of gestation [2]. The reported incubation time varies between nine and 121 days and abortion typically occurs without any premonitory signs [2, 4, 5]. Mare factors associated with susceptibility to EHV-1 abortion are largely undefined, but previous reports have suggested that primiparous mares and Lipizzaners are at higher risk [6, 7]. In Thoroughbred mares, age and reproductive status (maiden, barren or foaling) influence the likelihood of pregnancy loss [8] and, although associations between breeds and EHV-1 or infectious abortion in general are difficult to prove, abortion rates of up to 87% during an EHV-1 abortion epizootic have been reported [9, 10, 11].

Following abortion, EHV-1 is cleared from the genital tract, and future breeding capacity is not impaired unless reproductive tract damage has occurred as a result of dystocia [4, 12]. Premature placental separation (PPS) is reported in 1.6 % of all foaling's [13], but the likelihood is thought to be higher in the case of EHV-1 abortion or still-birth [4, 14,15]. The post-abortion incidence of retained fetal membranes (RFM), one of the most common post-partum complications in mares, is reported to be greater than after normal foaling [16]; indeed, a 66.6% rate of RFM in mares that aborted due to EHV-1 was recently reported [15]. On the other hand, no increase in

the incidence of post-abortion complications was evident during an extensive abortion epizootic involving unvaccinated Thoroughbred mares [10].

In this study we retrospectively analyzed the relationships between epidemiological factors and reproductive outcomes following EHV-1 abortion epizootics on two separate Thoroughbred farms in South Africa. The two epizootics featured divergent environmental and management factors and outbreak interventions. The aim was to improve the understanding of infectious abortion epizootics in horses in order to enhance future prevention and management strategies.

Materials and methods

Background and study design

Aspects of the epidemiology and post-abortion reproductive outcomes in Thoroughbred broodmares were analyzed retrospectively for two temporally-unrelated EHV-1 abortion epizootics on farms in geographically separated areas of South Africa. The paddock sizes and stocking density were similar on both farms. Prior to the epizootics, neither of the farms vaccinated broodmares against EHV-1.

Epidemiology

Farm 1: This farm in the Western Cape Province recorded abortions in the period 10th May to 22nd September 2007 in nine of a resident population of 30 broodmares confirmed pregnant at the end of the 2006 Southern Hemisphere breeding season. All abortions occurred within the same group of 21 mares located originally in one of two extensive paddocks of approximately eight hectares in area, and in which the

farm's resident pregnant broodmares were maintained. No abortions occurred among the nine mares in the second paddock located a considerable distance away. Following the first abortion on 10th May (D0), the affected mare was moved to an isolated quarantine paddock for three weeks while the in-contact mares were sub-divided into smaller groups that were separated geographically to limit the risks of further spread while awaiting the outcome of diagnostic tests. The fetus and associated membranes were immediately sealed in plastic drums to prevent environmental contamination and removed for diagnostic sampling and disposal. A second abortion occurred on D39, one day prior to receiving confirmation that the first abortion was caused by EHV-1, and 40 days after sample submission. The interval between the first (on D0), and last abortion was 135 days. The protracted interval from first abortion to confirmation of EHV-1 biased the intervention strategy and, in all likelihood therefore, the subsequent course of the epizootic. The cause of abortion in all cases was confirmed as EHV-1 by immunostaining of formalin-preserved fetal tissue and membrane samples. No other abortions were recorded during 2007, and the remaining 21 mares foaled uneventfully.

Farm 2: This farm, situated in KwaZulu-Natal Province experienced an abortion epizootic between 20th June and 24th July 2009. Eighteen of the 316 pregnant resident broodmares aborted as a result of EHV-1 infection. The suspected instigator of the epizootic was the introduction of a group of eight mares, including six in late-gestation, acquired at a broodmare sale when the resident mares were at least seven months pregnant. Immediately upon arrival, the eight newly acquired mares were quarantined as a group for three weeks, in accordance with the farm's disease

prevention protocol. Thereafter, the two non-pregnant mares were introduced into an isolated group of barren mares, while the six pregnant mares were dispersed over three groups, each of approximately 30 pregnant mares, which were kept in grass paddocks ranging from 2.5 to 11 hectares in area. The grouping of pregnant mares was based on anticipated foaling dates, as is customary in Thoroughbred management systems. The first abortion occurred 12 days after the introduction of two newly acquired mares into one of these three groups on 20th June (D0). The fetus and associated membranes were not recovered, presumably because of scavenging e.g. by the black-backed jackals (Canis mesomelas), present in the area. Two days later (D2) three abortions occurred in the same paddock as the first. Ultimately, the 18 mares affected by EHV-1 abortions included two of the six introduced-mares. The fetuses and membranes in these cases were retrieved intact, and samples were submitted to the University of Pretoria's Equine Research Centre (ERC) for diagnostic tests. The results of a real-time-quantitative PCR (qPCR) assay on the fetal tissues were available by D3 of the outbreak, i.e. within 24 h of the second group of abortions. Confirmation that EHV-1 was the cause of abortion in all cases was provided by a duplex qPCR assay for EHV-1 and EHV-4 that employed previously described primers and probes [17], and immunostaining of both fetal tissue and membrane samples.

  The rapid etiological diagnosis enabled the farm's management to justify the immediate institution of specific EHV-1 outbreak-control measures aimed at isolating the focus of infection, interrupting virus transmission and reducing the susceptibility of high-risk late-gestation mares [3, 18]. This response was based around a number of key decisions. The first was to vaccinate all pregnant broodmares with an

inactivated EHV-vaccine (Pneumabort-K+1B)[a] and to re-vaccinate at two-month intervals until foaling. In addition, the mares were sub-divided, within each paddock, into groups of four to six using electrical tape barriers and maintaining a distance of $\geq$ 20 m between each enclosed area while ensuring that the groups still had visual and auditory contact until the mares either foaled or aborted. This manner of separation aimed to balance the risks of virus transmission between mares, particularly at the time of foaling or abortion, against additional separation stress that could otherwise conceivably induce viral recrudescence or lead to abortion for other reasons. Night-watchmen maintained constant observation and removed aborting or foaling mares immediately. After delivery, the mares were placed in a quarantine area for three weeks prior to relocation to the farms' barren mare groups at a considerable distance from pregnant mares. After an abortion, in-contact group-mates were immediately relocated to another electrically-fenced enclosure $\geq$ 20 m away, precluding direct contact with fetal fluids and reducing the risk of respiratory spread via aerosol [5]. The aborted fetus and fetal membranes were immediately sealed in plastic drums and removed for diagnostic sampling and disposal. An EHV-1-appropriate biosecurity protocol was applied concurrently [3]. The fetus and associated membranes were examined by the farms' veterinarian, and tissue samples were obtained during a standardized autopsy protocol and submitted for laboratory diagnosis as outlined above.

Molecular characterization of viruses

DNA for sequencing was extracted from the supernatant of virus cultures performed on all samples that tested positive for EHV-1 by qPCR. To amplify the ORF 30

region for sequencing, 0.5 µL of extracted DNA was added to 0.5 µL 20 mM dNTP mix, 1.2 µL 25mM MgCl, 2 µL 10xPCR Buffer[b], 0.05 µL Taq polymerase (Super-therm gold DNA Polymerase) and 0.2 µL EHV-1 ORF30 forward (GCG CTA CTT CTG AAA ACG) and reverse (CCA CAA ACT TGA TAA ACA CG) primers, in a total volume of 20 µL. The PCR reaction involved denaturing at 95°C for 10 min, followed by 35 cycles of 95°C for 45 sec, 51°C for 1 min and 72°C for 2 min, before a final cycle of 72°C for 8 min in an ABI 9700 PCR thermocycler[c]. The amplification products were purified with an Invitek PCRapace kit[d] according to the manufacturer's instructions. The sequencing reaction consisted of 2 µL ABI Prism® BigDye® Terminator v3.1 Ready Reaction mix[c], 1 µL sequencing buffer, 1 µL 3.2 pmol forward and reverse primer and 6 µL of purified DNA. The sequencing reactions were run in an ABI 9700 PCR[c] machine according to the manufacturer's instructions. The product was ethanol precipitated and air-dried for 10 min before denaturing using formamide and bi-directional sequencing with an ABI 3130xl Genetic Analyser[c]. EHV-1 and EHV-4 virus isolates grown on equine lung cells were used as positive controls, and pyrogen-free water as negative control.

Reproductive outcomes

The reproductive outcomes recorded in all aborting mares, whether abortion was due to EHV-1 (both farms) or other causes (farm 2 only) were compared to those of the other resident broodmares, both during and following the epizootics. Subsequent to the epizootic-affected seasons, both farms vaccinated all pregnant mares with Pneumabort-K+1B[a] at two-monthly intervals, commencing at five months of gestation and continuing until foaling.

Assignment of mares to the EHV-1 abortion cohort was based on a positive diagnosis *via* immunostaining (farm 1), or both immunostaining and qPCR assay (farm 2) on fetal and fetal membrane samples. Mares aborting due to other causes in the seasons when EHV-1 abortions were reported were classified as non-EHV-1 abortion mares. The sum of these two constituted the "total aborting mares" cohort. The mares were categorized by age (years) and their reproductive status in the preceding breeding season. Reproductive status allocation was as: i) maiden (never bred previously); ii) barren (bred at least once previously but did not foal during the current season); and iii) foaling (foaled during the current season). The reproductive variables chosen to describe the pattern and outcomes of abortion were: stage of gestation (median and range at abortion), incidence of RFM and incidence of PPS.

Data analysis

The data sources were the broodmare records maintained by the respective farms' manager and veterinarian. Data was analysed using SigmaPlot V11[e] software. Basic descriptive statistics and between-groups Mann-Whitney Rank Sum Tests were conducted. For all analyses, $P \leq 0.05$ was considered to indicate a statistically significant difference.

Results

Epidemiological data

Farm 1: The initiating events for the first abortion were undefined, but most likely resulted from EHV-1 reactivation in the first aborting mare [19]. Alternatively,

because recrudescence and secondary reinfection within a group are difficult to differentiate, the first abortion may have occurred subsequent to horizontal respiratory exposure from an asymptomatic-paddock mate that underwent sub-clinical viral recrudescence in response to an unknown stressor [20, 21]. In either case, after the first abortion, group exposure must have occurred as a result of high virus loads within the aborted fetus, fluids and membranes [21]. At least two additional mares must have been infected at, or before, this first abortion because, even though this initial group was subdivided almost immediately, abortions were subsequently recorded in two of three resultant sub-groups.

Farm 2: This epizootic was closely associated with recent introduction and dispersal of pregnant mares into established groups of resident pregnant broodmares. The first abortions occurred soon after the introductions without premonitory clinical signs. Ultimately, EHV-1 abortions occurred in four groups of mares; these included two of the three groups into which acquired broodmares were introduced; the aborting mares included two of the newly-introduced mares.

  The initiating pathogenesis may have been associated with social disruption causing stress-induced EHV-1 recrudescence among one or more of the newly introduced or the previously-resident mares [18, 21]. Alternatively, or additionally, virus may have circulated among the introduced group during quarantine prior to dispersal. There was complete agreement between diagnostic methods for all abortions on farm 2 as to whether EHV-1 was implicated or not.

Comparison between farms: Selected reproductive performance parameters were compared for the breeding season in which the epizootics took place and for the following breeding season, for mares that aborted due to EHV-1 or due to other causes. There was no significant difference in the median age of the mares that aborted due to EHV-1 infection between farm 1 and farm 2 (8.0 years), nor was there any difference in the distribution of mare reproductive status or the previous use of EHV-1 vaccination. The most notable mare-level difference between the affected farms was that the median gestational stage at the time of EHV-1 associated abortion on farm 1 was lower than that of farm 2. This most likely reflected the stage of gestation of the respective mare groups when they were infected with EHV-1. However, other factors including EHV-1 strain differences in virulence or incubation intervals may have contributed. The other significant differences between the two farms were: the duration of the outbreaks (farm 1 = 135 d; farm 2 = 34 d), the intervals between the index case and subsequent abortions (farm 1 = 39 d; farm 2 = 2 d), and the time taken to confirm a diagnosis (farm 1 = 40 d; farm 2 = 2 d).

Molecular characterization of viruses
Retrospectively, both epizootics were confirmed to be associated with abortogenic EHV-1 virus, with the ORF 30 sequence characterized by the G^{2254} genotype for all cases [15].

Reproductive outcomes
The reproductive outcomes in the pregnant mare cohorts are summarized in Tables 1 and 2. On farm 1, there were no recorded cases of non-EHV-1 abortion, PPS or RFM. During the course of 2009, 43 (13.61 %) of the pregnant mares on farm 2

aborted. These abortions included 18 due to EHV-1 and 25 that resulted from other causes. There were significant differences in both the median age (P = 0.001) and gestational stage (P = 0.044) between mares that aborted due to EHV-1 and those that aborted for other reasons. Specifically, EHV-1 abortions were, on average, recorded in younger mares at a later stage of gestation than non-EHV-1 abortions. The cause of non-EHV-1 abortions was diagnosed in 10 of 25 (40.0 %) cases, and included six (24.0 %) ascribed to ascending placentitis, two (8.0 %) to twinning, and

Table 1: Reproductive performance of the pregnant broodmare populations during the abortion epizootic in 2007 and the subsequent post-epizootic breeding season on farm 1				
	Mare populations			
Reproductive performance	All mares	Total aborting	EHV-1 abortion	Non-EHV-1 abortion
Pregnant prior to epizootic (n)	30	9	9	-
Age - median (range) in years	10.2 (4-19)	8.0 (5-18)	8.0 (5-18)	-
Status at breeding in 2006				
- foaling	20	6	6	-
- maiden	5	2	2	-
- barren	5	1	1	-
Gestation stage at abortion median (range) in days	-	227 (200-291)	227 (200-291)	-
RFM post-abortion or foaling	0	0	0	-
Cycles per pregnancy (mean, range)	1.4 (1-5)	1.8 (1-5)	1.8 (1-5)	-
Cumulative pregnancies (%)	41 (91.1)	8 (100.0)	8 (100.0)	-
Live foals in 2008 (%)	27 (60.0)	5 (62.5)	5 (62.5)	-
EHV-1 equid herpesvirus type-1 RFM retained fetal membranes				

Table 2: Reproductive performance of the pregnant broodmare populations during the abortion epizootic in 2009 and the subsequent post-epizootic breeding season on farm 2				
	Mare populations			
Reproductive performance	All mares	Total aborting	EHV-1 abortion	Non-EHV-1 abortion
Pregnant prior to epizootic (n)	316	43	18	25
Age - median (range) in years	10.5 (3-24)	10.0 (4-24)	8.0[†] (5-16)	11.0[†] (4-24)
Status at breeding in 2008				
- foaling	214	25	7	18
- maiden	44	8	4	4
- barren	58	10	7	3
Gestation stage at abortion median (range) in days	-	278.0 (61-339)	291.5* (277-313)	211.9* (61-339)
RFM post-abortion or foaling (%)	16 (4.4)	5 (1.2)	1 (0.6)	4 (16.0)
Cycles per pregnancy (mean, range)	1.7 (1-6)	1.9 (1-4)	1.9 (1-4)	1.8 (1-4)
Cumulative pregnancies (%)	319 (85.1)[§]	37 (94.9)	17 (94.4)	20 (95.2)
Live foals in 2010 (%)	279 (74.4)	34 (87.2)	16 (88.9)	18 (85.7)

†sig, P= 0.004; *sig, P= 0.001
§ five pregnant mares subsequently died
EHV-1 equid herpesvirus type-1
RFM retained fetal membranes

one each as a result of umbilical cord pathology and placental oedema, respectively. The overall diagnostic rate for all abortions was high, at 65.1%. On farm 1, one mare died acutely as a consequence of a uterine prolapse that presented a few hours after abortion and, on farm 2, one non-EHV-1 abortion mare was euthanized as a result of a rectal prolapse following abortion due to placentitis. Retained fetal membranes were more commonly reported in mares that aborted for reasons other than EHV-1 (Table 2). The frequency of PPS in the current study was 7.3% (21 incidents) which was markedly higher than the historical average of 5.3 events per year during the

previous three seasons from a similar number of foaling mares at the same farms (data not shown).

Discussion

There are conflicting reports in the literature with regard to the contribution of both age and parity of the mare to the subsequent risk of EHV-1-associated abortion [6, 7]. In the current study, the EHV-1-affected mares in both epizootics were relatively young and evenly distributed over reproductive status categories, thereby partially supporting a previous report of an association of EHV-1 abortion with primiparity [7], but opposing the suggesting of an association with older mares [6].

The variations in epidemiology and outcome between the two South African epizootics described in this report, even though they affected demographically-similar mare populations, may have been a result of a critical host-factor difference, namely gestational stage at exposure. The outcome may have been further biased, independently or as a co-dependent, by differences in management interventions on the respective farms, including allocation of mares into groups on the basis of gestation stage and the history of introducing high-risk animals on farm 2. On farm 1, the gestation stage at EHV-1 abortion was markedly earlier and the incubation, inter-abortion intervals and epizootic duration were greater than on farm 2 or in previous reports of epizootics affecting similar-sized groups of unvaccinated late-gestation Thoroughbreds or mares of other breeds [6, 10]. The long interval between presumed exposure and abortion on farm 1 is difficult to explain but may in part have been associated with initial exposure at an early gestational stage less susceptible to immediate abortion, or to viral factors such as abortogenic potential [22]. The

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previously reported difficulty in recognizing reactivation followed by horizontal spread, because of the absence of obvious clinical signs [20] was also supported. On farm 2, the occurrence of the abortions in a temporal cluster was similar to an Australian Thoroughbred epizootic affecting unvaccinated mares managed on extensive pastures [10]. The abortion rate in the Australian epizootic was, however, much higher. The availability of PCR-based diagnosis for farm 2 allowed a markedly shorter interval to definitive diagnosis, and thereby enabled rapid implementation of an intervention response specific to EHV-1. This justified management decisions that carried significant economic, resource and manpower implications, including the vaccination, constant observation and subdivision of approximately 300 pregnant broodmares. It is tempting to speculate that availability of a validated rapid PCR test was critical in limiting the duration and relative impact of the EHV-1 abortion epizootic on farm 2. An additional difference was the decision to immediately vaccinate all pregnant broodmares. Vaccination in the face of an outbreak had no apparent adverse response, in contrast to anecdotal reports during neurotropic EHV-1 epizootics, and may have helped to limit the spread and extent of the epizootic. Continuous observation of the mares with immediate separation of any aborting or foaling mare and subdivision of affected groups *in situ* by electric tapes almost certainly limited subsequent exposure via fetus, membranes and fluids [5, 22]. The traditional scheduling of broodmare sales in the winter months (coincident with advanced gestation) was highlighted as a risk-factor in Thoroughbreds. A key risk-mitigation strategy would be strict separation of newly-acquired mares from the resident population until after foaling.

The overall abortion rates for the epizootic-associated years were, respectively, within (farm 2) or markedly higher than (farm 1), the reported ranges for Thoroughbreds in South Africa and other countries [2, 23]. The marked differences between the two outbreaks in duration and abortion patterns may have been biased by differences in the outbreak responses which in turn may have been affected by the interval to confirmation of EHV-1. The speed with which the diagnosis was confirmed was a product of the availability (farm 2), or absence (farm 1), of a specific diagnostic qPCR test.

Mares that aborted due to EHV-1 were both significantly younger and aborted significantly later and over a narrower range of gestation than mares that aborted due to other causes. This was probably a product of a number of factors including the general increase in the frequency of abortion with increasing age [24], but also to the range of both infectious and non-infectious aetiologies that could have caused the non-EHV-1 abortions [2, 25].

Both the overall success of diagnosing the apparent cause of abortion and the relatively-high representation of placentitis among the positive diagnoses were similar to other reports [2, 25]. The diagnostic rate was however; appreciably lower than reports that considered non-EHV-1 abortions alone. Moreover, the predilection of placentitis for older mares [26] may have contributed to the higher mean age among the non-EHV-1 abortion cohort.

EHV-1 did not noticeably affect the incidence of RFM, which was similar to that in normal post-foaling mares; this contrasts to a recent report [15]. There was however an increased incidence of RFM in mares that aborted due to causes other than EHV-1, which supported an earlier report of an elevated risk of RFM in aborting mares

[16]. PPS was reported in association with both "normal foaling" and non-EHV-1 abortions, whereas in contrast to previous reports [14, 19, 27] EHV-1 was not associated with PPS. Both farms reported a single mare fatality associated with abortion, one uterine prolapse (EHV-1) and one rectal prolapse (subsequent to placentitis), respectively.

A previously-reported outcomes-based analysis of the post-epizootic reproductive performance on these two farms demonstrated that abortion due to EHV-1 (or due to any other cause) did not predict the pregnancy or foaling rates among the affected mare populations during the subsequent breeding season [12].

Conclusions

This study recorded several novel features, and confirmed some previous associations, of EHV-1 abortion in unvaccinated mare populations, and indicated intervention strategies that may affect the spread of infection. The following preventative and response measures appeared to help: i) subdivide into the smallest practicable groups early in gestation, ii) avoid additions to late-pregnant mare groups, and maintain newly-acquired late-pregnant mares as separate groups until after foaling, iii) investigate all abortions and prioritize EHV-1 diagnosis, iv) rapidly implement EHV-1- appropriate biosecurity measures until an aetiological diagnosis is obtained (availability of a rapid diagnostic test facilitates compliance with these measures), v) submit appropriate samples for molecular diagnostics (including fetal tissues and membrane samples), vi) if the index abortion occurs in a large group of mares, subdivide as soon as possible to reduce the number of mares exposed to

horizontal transmission by any subsequent abortions; vii) minimize separation-associated stress by maintaining group-mates within "sight and sound' of aborting mares, and vii) institute preventative vaccination in all at-risk pregnant mares.

Manufacturers' addresses

[a] Pfizer Animal Health, Sandton, South Africa

[b] Roche Products, Randburg, South Africa

[c] Lifetech, Johannesburg, South Africa

[d] Celtic Molecular Diagnostics, Cape Town, South Africa

[e] Systat Software Inc, San Jose, California, USA

References

1. Foote, A. K., Ricketts S.W. and Whitwell, K. E. (2012) A racing start in life? The hurdles of equine feto-placental pathology. *Equine Vet. J.* **44**, 120-129.

2. Laugier, C., Fouche, N., Sevin, C., Leon, A. and Tapprest, J. (2011) A 24-year retrospective study of equine abortion in Normandy (France). *J. Equine Vet. Sci.* **31**, 116-123.

3. Lunn, D. P., Davis-Poynter, N., Flaminio, M. J. B. F., Horohov, D. W., Osterrieder, K., Pusterla, N. and Townsend, H. G. G. (2009) Equine herpesvirus-1 consensus statement. *J. Vet. Intern. Med.* **23**, 450-461.

4. Smith, K.C. (1997) Herpesviral abortion in domestic animals. *Vet. J.* **153,** 253- 268.

5. Mumford, J.A. (1991) The epidemiology of Equid herpesvirus abortion: a tantalising mystery *Equine Vet. J.* **23,** 77-78.

6. Barbić, L., Lojkić, I., Stevanović, V., Bedeković, T., Starešina, V., Lemo, N., Lojkić, M. and Madić, J. (2012) Two outbreaks of neuropathogenic equine herpesvirus type 1 with breed-dependent clinical signs. *Vet. Rec.* **170,** 227.

7. Hartley, W. J. and Dixon, R. J. (1979) An outbreak of equine herpesvirus type I: pathological observations. *Equine Vet. J.* **11,** 215-218.

8. Bosh, K. A., Powell, D., Neibergs, J. S., Shelton, B. and Zent, W. (2009) Reproductive performance measures among Thoroughbred mares in central Kentucky, during the 2004 mating season. *Equine Vet. J.* **41**(9) 883-888.

9. Brück, I., Anderson, G. A. and Hyland, J. H. (1993) Reproductive performance of thoroughbred mares on six commercial stud farms. *Australian Vet. J.* **70**, 299-303.

10. Carrigan, M., Cosgrove, P., Kirkland, P. and Sabine, M. (1991) An outbreak of equid herpesvirus abortion in New South Wales. *Equine Vet. J.* **23,** 108-110.

11. Hemberg, E., Lundheim, N. and Einarsson, S. (2004) Reproductive performance of Thoroughbred mares in Sweden. *Reprod. Dom. Anim.* **39**, 81–85.

12. Schulman, M.L., Kass, P. H., Becker, A. and Van der Merwe, B. (2013) A predictive model for reproductive performance following abortion in Thoroughbred mares. *Vet. Rec.* **172**, 44.

13. McCue, P.M. and Ferris, R. A. (2012) Parturition, dystocia and foal survival: A retrospective study of 1047 births. *Equine Vet. J.* **44,** 22-25.

14. Irwin, V. L., Traub-Dargatz, J. L., Newton, J. R., Scase, T. J., Davis-Poynter, N. J., Nugent, J., Creis, L., Leaman, T. R. and Smith, K. C. (2007) Investigation & management of an outbreak of abortion related to equine herpesvirus type 1 in unvaccinated ponies. *Vet. Rec.* **160,** 378-380.

15. Walter, J., Seeh, C., Fey, K., Bleul, U. and Osterrieder, N. (2013) Clinical observations and management of a severe equine herpesvirus type 1 outbreak with abortion and encephalomyelitis. *Acta Vet. Scandinavica* **55,** 19.

16. Provencher, R., Threlfall, W.R., Murdick, P.W. and Wearly, W.K. (1988) Retained fetal membranes in the mare: A retrospective study. *Canadian Vet. J.* **29,** 903-10.

17. Diallo, I.S., Hewitson, G., Wright, L.L. Kelly, M.A., Rodwell, B.J. and Corney, B.G. (2007) Multiplex real-time PCR for the detection and differentiation of equid herpesvirus 1 (EHV-1) and equid herpesvirus 4 (EHV-4). *Vet. Micro.* **123**, 93-103.

18. Allen, G.P. (2002) Epidemic disease caused by Equine herpesvirus-1: recommendations for prevention and control. *Equine Vet. Educ.* **4,** 177-184

19. Smith, K.C., Whitwell, K.E., Binns, M.M., Dolby, C.A., Hannant, D. and Mumford, J.A. (1992) Abortion of virologically negative foetuses following experimental challenge of pregnant pony mares with equid herpesvirus 1. *Equine Vet. J.* **24**, 256-259.

20. Edington, N., Bridges, C.G. and Huckle, A. (1985) Experimental reactivation of equid herpesvirus 1 (EHV I) following the administration of corticosteroids. *Equine Vet. J.* **17,** 369-372.

21. Gardiner, D.W., Lunn, D.P., Goehring, L.S., Chiang, Y-W, Cook, C., Osterrieder, N., McCue, P., Del Piero, F., Hussey, S.B. and Hussey, G.S. (2012) Strain impact on equine herpesvirus type 1 (EHV-1) abortion models: Viral loads in fetal and placental tissues and foals. *Vaccine* **30,** 6564-6572.

22. Patel, J.R. and Heldens, J. (2005) Equine herpesviruses 1 (EHV-1) and 4 (EHV-4) epidemiology, disease and immunoprophylaxis: A brief review. *Vet. J.* **170**, 14-23.

23. Schulman, M. L., Marlow, C. H. and Nurton, J. P. (2003) A survey of reproductive success in South African Thoroughbred horse breeding from 1975 to 1999. *J. South African Vet. Assoc.* **74**, 17-19.

24. Chevalier-Clement, F. (1989) Pregnancy loss in the mare. *Anim. Reprod. Sci.* **20**, 231-244.

25. Hong, C. B., Donahue, J. M., Giles, R. C. JR., Petrites-Murphy, M. B., Poonacha, K.B., Roberts, A. W., Smith, B. J., Tramontin, R. R., Tuttle, P. and Swerczek, T. W. (1993) Equine Abortion and Stillbirth in Central Kentucky during 1988 and 1989 Foaling Seasons. *J. Vet. Diagn. Invest.* **5,** 560-566.

26. LeBlanc, M.M. (2010) A study of placentitis in the mare: An update. *Reprod. in Dom. Anim.* **45**, 28-34.

27. Smith, K. C., Whitwell, K. E., Blunden, A. S., Bestbier, M. E., Scase, T. J., Geraghty, R. J., Nugent, J., Davis-Poynter, N. J. and Cardwell, J. M. (2004) Equine herpesvirus-1 abortion: atypical cases with lesions largely or wholly restricted to the placenta. *Equine Vet. J.* **36,** 79-82.