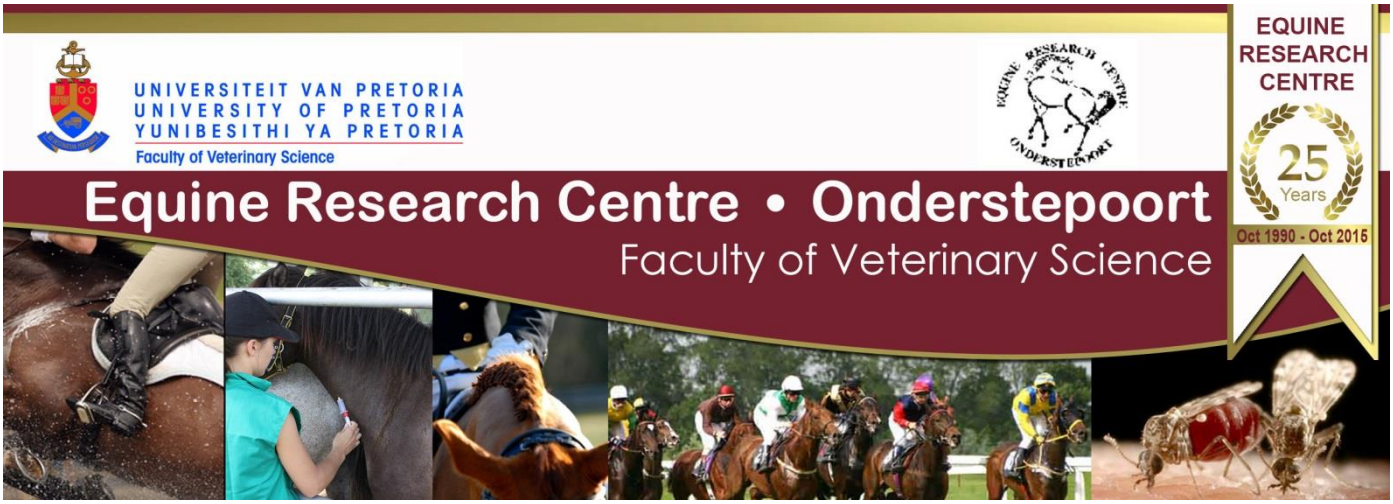

EQUINE RESEARCH … what you need to know

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The ERC Team is pleased to bring you two more recent research papers for your interest :

SUMMARISED PUBLICATIONS

DETECTION OF EHV-4 AND PHYSIOLOGICAL STRESS PATTERNS IN YOUNG THOROUGHBREDS AT SOUTH AFRICAN AUCTION SALES

Equine respiratory infection is an important cause of disease and economic loss worldwide. EHV-1 and -4 are important agents associated with infectious upper respiratory tract disease (IURD). Risks associated with IURD include host, environmental, management and pathogen-specific factors. In addition, higher detection rates of EHV-1 and -4 occur in juvenile horses and during the colder winter months. Physiological stress is arguably one of the more important risk factors, with associations reported between EHV-1 and -4 shedding and externally derived stressors. Stress may further increase the susceptibility of naïve animals to new infections. Transport and the subsequent confinement, handling and management at sales events may contribute to physiological stress.

Little is known about the prevalence of equine herpesvirus-1 and -4 (EHV-1 and -4) in South African Thoroughbreds at auction sales. The bringing together of young Thoroughbreds from various populations, together with the stress associated with transporting them to sales could very well cause shedding and transmission of EHV-1 and -4. This study sampled 90 young Thoroughbreds consigned from 8 farms from 3 different South African provinces representative of the South African breeding demographic.

Horses were tested by qPCR via :

- nasal swabs for EHV-1 and -4 DNA;
- blood samples for EHV-1 and -4 antibodies on arrival and departure;
- nasal swabs obtained serially from those horses with high temperatures and/or nasal discharge.

In addition daily faecal samples were used to determine faecal glucocorticoid metabolite (FGM) concentrations as a measurement of physiological stress.

Results

EHV -1 and -4 detection: No EHV-1 was detected, but EHV-4 DNA was detected in 13/90 (14.4%) of the horses from 7 out of 8 of the farms. Repeated incidents of EHV-4 DNA detection occurred in 30.8% of the positive horses. The longest period of continuous detection was four days. Similarly, the longest interval between consecutive detections was four days. Nasal swabs from 1.1% of the horses were positive for EHV-4 DNA on the day of arrival, while 7.8% were positive on the day of departure.

EHV-4 detection and clinical signs: All 13 EHV-4 DNA positive horses showed either nasal discharge alone, or both high temperatures and nasal discharge. However 84.4% of the remainder of the horses that were EHV-4 DNA negative, also showed one or both of these clinical signs. The high temperature lasted for less than 24 hours in 7/8 horses with concurrent detection of EHV-4 DNA.

EHV-1 and -4 serology: Upon arrival at the sales complex 1.1% of the study population showed serological evidence of prior exposure to EHV-1, and 93.3% had previously been exposed to EHV-4. No instances of seroconversion were recorded between arrival and departure. Only one horse of the 13 EHV-4 DNA positive horses was EHV-4 seronegative on arrival and remained so on departure seven days later.

FGM concentration : The average FGM concentrations for the eight farms increased (with increased variability) after arrival, before decreasing in concentration and variability for most of the remainder of the study period. During the adaptation phase, FGM concentrations were 64% higher on the day of arrival, and 93% higher on one day after arrival, when compared with three days after arrival. No discernible increase in FGM concentrations was associated with the auction phase.

Conclusions

EHV-4 DNA was detected in some young Thoroughbreds consigned to a South African auction sale. Most of these horses had been previously exposed to EHV-4 and very few to EHV-1 prior to their arrival at the sale. The combination of stressors associated with their transport and arrival was associated with most horses showing a physiological stress response. These, other stressors and commingling inherent to the current worldwide consignment process increase the risk association with IURD in young horses. The transport and arrival phases are key areas for future investigation into management practices to reduce the impact of physiological stress on the health and welfare of young Thoroughbreds during sales consignment.

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Research Team :
Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria : Marcha Badenhorst, Patrick Page;
Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria : Andre Ganswindt, Peter Laver;
Equine Research Centre, Faculty of Veterinary Science, University of Pretoria : Alan Guthrie;
Section of Reproduction, Faculty of Veterinary Science, University of Pretoria : Martin Schulman

Distribution & prevalence of *Taylorella equigenitalis*, the causative agent of Contagious Equine Metritis (CEM) in South African horses, determined by qPCR-based National Surveillance Programme

Following the 2011 outbreak of CEM , further investigation was required to define the pre-existing status of *T. equigenitalis* in the South African equine population and the progression of the epidemiological investigation via the implementation of a molecular diagnostic-based surveillance programme.

T. equigenitalis is a bacteria that causes contagious equine metritis (CEM), a venereally transmitted disease of horses. The bacteria is spread directly during natural mating or artificial insemination (AI) with semen from a carrier stallion. Stallions show no clinical signs and carrier status may last for months or even years. Mares typically show a varginal discharge and irregular interoestrus periods or may show no clinical signs. Most mares rid themselves of infection, however a small population of infected mares may attain carrier status.

Bacterial culture is the test currently recognised by the World Organisation of Animal Health (OIE for international trade purposes. However, *T. equigenitalis* is slow growing and is readily overgrown by other microorganisms present in the reproductive tract of horses, therefore affecting the sensitivity and utility of bacterial culture. Due to these difficulties, various qPCR tests have been developed to detect the presence of the bacteria.

Since the first reported cases of CEM in Thoroughbred horses in the UK, the disease has been found to have a worldwide distribution. The most recent outbreaks in non-endemic countries include the 2008-2010 outbreak in the USA and the 2012 outbreak in Gloucestershire, UK. South Africa was considered free of CEM until 3 May 2011 when a suspected outbreak was confirmed. This paper describes the subsequent outbreak investigation and shows how molecular tools were utilised during a national surveillance programme and outbreak management.

Background to SA Outbreak

The South African Veterinary Authority notified the OIE in May 2011 of an outbreak of CEM in South Africa. The index case, a stallion released from post-arrival quarantine in February 2011, was moved to an equine breeding centre in Gauteng Province where semen was collected for AI, which resulted initially in successful embryo transfer (ET) from several mares. Subsequently, following unsuccessful ET attempts, tests were performed and the stallion and a mare inseminated with his semen tested positive on culture for *T. equigenitalis*.

Subsequently all in-contact horses at the breeding centre (index property) were traced, quarantined and sampled for *T. equigenitalis* testing. Two stallions that had indirect contact with the index case on the index property tested positive on a second property. A national screening programme was subsequently introduced in August 2011 to facilitate investigation of the extent of *T. equigenitalis* within the South Africa equine population.

National Screening Programme : The SA Directorate of Animal Health instituted a national screening programme and issued an order that no stallion was allowed to be used for breeding without the issue of an official CEM clearance certificate. To support this ruling, only offspring from stallions with a valid CEM clearance certificate for that breeding season were eligible for registration by their relevant breed society. The screening programme used qPCR for testing, with any positive horses being retested using a combination of qPCR and confirmatory bacterial culture.

Traceback of horses in contact with positive stallions identified during the national screening programm: Following the identification in the Western Cape Province of a *T. equigenitalis*-positive stallion originating from the SA Lipizzaner Centre (SALC) in Gauteng Province, a traceback screening of all 33 stallions resident at the SALC was instituted and all stallions were swabbed in accordance with the method prescribed in the legislation.

Archived semen samples of 7 stallions from the SALC that had formed part of a 1996 investigation of equine arteritis virus were accessed in August 2014 and submitted for qPCR and culture.

A trace-back of 70 mares that were classified as having been 'in-contact' with *T. equigenitalis*-positive stallions commenced approximately 18 months after the index case was reported, with an additional trace-back of 48 offspring of *T. equigenitalis*-positive stallions.

Results

In addition to the animals identified on the two properties in the initial outbreak investigation, *T. equigenitalis*-positive horses were identified on a further 11 properties. During the interval from July 2011-July 2013 the national screening programme tested 3703 animals and identified 9 additional *T. equigenitalis*-positive stallions. The traceback of stallions resident at the SALC identified 24 *T. equigenitalis*-positive stallions and the traceback of in-contact mares identified 2 positive for *T. equigenitalis.*

. All 35 *T. equigenitalis*-positive horses identified during the national screening programme and the traceback of in-contact horses tested positive on qPCR, while 24 were positive and 11 negative on bacterial culture. The countrywide prevalence of *T. equigenitalis* by testing was 0.97% in stallions and 2.56% in mares. Overall, thirty-nine horses were identified positive for *T. equigenitalis* by qPCR screening with 28 being confirmed positive using bacterial culture.

The findings of the national screening programme and traceback of in-contact horses demonstrated that the index stallion was not the source of this outbreak. Records showed negative bacterial cultures for *T. equigenitalis* during both pre- and post importation quarantine testing, and it was only after initially successful AI and ET's at the index property that the observed genital infection was confirmed as *T. equigenitalis.* More recently the archived semen samples collected from Lipizzaners in 1996 were shown to be positive for *T. equigenitalis* on both bacterial culture and qPCR, indicating the organism's presence in South Africa prior to the 2011 outbreak.

Several stallions from the SALC had visited the index property for semen collection during 2001-2006; however prior to this, the SALC had maintained a closed herd since the first introduction of Lipizzaners into South Africa in 1948. It would appear that following the introduction of the organism into South Africa, the infection persisted at the SALC and thereafter, the index property was repeatedly contaminated during visits by colonised stallions for semen collection, with further stallions being infected by fomite transmission, i.e. via grooming equipment, personnel during semen collection and handling, tack etc. No evidence of *T. equigenitalis* was detected among Lipizzaner mares and offspring at a satellite property, thus supporting the theory that fomite transmission was the probable primary cause of transmission among stallions resident at the SALC.

Prior to the introduction of the national screening programme, a review of the laboratory resources available for bacterial culture and the anticipated time for transport of samples to the laboratory from remote areas in South Africa was performed. Due to the impracticality of transporting a large proportion of the samples from the collection points to the laboratory within 48 hours, and due to the limited laboratory capacity for bacterial culture it was decided to implement a qPCR test for screening to overcome both the logistical issues and to provide laboratory capacity. Following the identification of 9 positive stallions in the national screening programme, arrangements were made for samples for bacterial culture to be transported to the bacterial laboratory timeously. The qPCR and bacterial culture results concurred.

In the case of the traceback of in-contact horses, all 26 tested positive on qPCR while 15 were positive on bacterial culture. Based on the history, and in an effort to expedite resolution of the outbreak, treatment was initiated shortly after the animals were shown to be positive on qPCR, and prior to completion of the bacterial cultures. Therefore,

while these practical limitations could be partially responsible for the difference in detection rate between qPCR and bacterial culture it has been reported that the sensitivity of qPCR exceeds that of bacterial culture.

A web-based platform coordinating key aspects of stallion screening was set up and proved indispensable in disseminating information, ensuring compliance of stakeholders and providing a central database for data collation facilitating epidemiological investigation. Successful treatment of all positive horses supported redrafting of pertinent CEM legislation to include a derogation permitting the treatment of any infected equines subject to compliance with the legislation where prior legislation has stated that any *T. equigenitalis*-positive stallion needed to be castrated or euthanased and any positive mares were to be euthanased.

Conclusions

This study showed that *T. equigenitalis* was present in the South African Lipizzaner population prior to outbreak identification in 2011. During investigation of outbreaks, targeted surveillance of stallions and their in-contact mares is recommended and qPCR was proven to be the more sensitive testing method. Ongoing bacteriological surveillance of all horses entering assisted reproduction facilities is recommended despite the *T. equigenitalis* status of a country.

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Research Team:
Correspondence : C E May, M L Schulman
Equine Research Centre, University of Pretoria : A J Guthrie, C Joone, M Monyai
Companion Animal Clinical Studies Department, University of Pretoria : B Keys

Nora-Jean (N-J) Freeman on behalf of the Equine Research Centre – nfreeman@witshealth.co.za