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Equine Research Centre • Onderstepoort

Faculty of Veterinary Science

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EQUINE RESEARCH … what you need to know

Brought to you by the Equine Research Centre, University of Pretoria

Welcome to all our new Subscribers – thank you for being responsible and choosing to be kept informed. Also a big Thank You to Western Shoppe for distributing our Newsletter, from which we received hundreds of requests to be added to the mailing list. Thank you also to various associations who have recognised the importance of keeping your members informed. To have your details added to the mailing list please e-mail Nora-Jean (N-J) Freeman on nfreeman@witshealth.co.za.

WHAT ALL HORSE OWNERS WANT TO KNOW … PROGRESS ON VACCINE DEVELOPMENT

The good news is that the Equine Research Centre, under Prof Alan Guthrie, in collaboration with researchers from USA and France, has successfully developed a preliminary recombinant canarypox virus vectored vaccine. Horses immunised with this vaccine showed complete resistance to infection with a virulent strain of AHSV-4, There are ongoing studies underway, with researchers currently evaluating two further serotypes (in addition to AHSV-4).

The not-so-good news is that there is a long and costly road still to be travelled before this vaccine can become commercially available. The next steps will include a proposal for the Department of Agriculture, Forestry and Fisheries (DAFF) to approve the field evaluation of the safety and efficacy of the vaccines Only once the vaccine has passed all the relevant tests, will a decision be taken with regard to progressing to registration and licensing of the vaccine. This process is necessary for ensuring that our horses are treated with a safe and tested product, and once available, will be a huge leap forward in the control of this insidious disease. *(See summarised publication later in this Newsletter).*

SA HORSE EXPORT STRATEGY WORKSHOP - 15/16 APRIL 2014

SA HORSE EXPORT STRATEGY WORKSHOP - 15-16 APRIL 2014

Racing South Africa, in collaboration with the Equine Research Centre (University of Pretoria) and Wits Health Consortium, will be hosting a strategic horse export workshop on 15 and 16 April 2014.

OBJECTIVE OF WORKSHOP : ***To develop a strategic plan that unites all stakeholders in the horse export industry and clearly identifies the way forward***

Why is the Workshop necessary?

The unique challenges facing horse exports from South Africa are well-known and the restrictions to trade crippling to the equine industry and to the country as a whole. On the positive side, there have been favourable adjustments to the regulatory environment and significant scientific advancements which has led to a far greater understanding of the risks associated with exporting horses from AHS endemic countries.

In 1995, a workshop was held at the Faculty of Veterinary Science in Onderstepoort to explore the possibility of exporting horses from South Africa. This ultimately led to the establishment of the SA Horse Export Protocol which was ratified by the EU in 1997 and subsequently other trading partners.

In light of the changing scientific and regulatory landscape, Racing South Africa and strategic partners are reviewing the current status with a view to providing a future framework for South African horse exports**. The primary aim is to develop a strategic plan to unite all stakeholders in the horse export industry and clearly identify the way forward to get export directly to the EU back up and running in a sustainable way.**

The first day (15 April) is restricted to key technical role players in Government and from industry whilst the second day will be open to the industry at large where the strategic plan will be presented for wider discussion and endorsement.

The end goal of this process is to ensure an inclusive and aligning process, to develop long-term strategic direction and a detailed set of enabling Action Plans.

This is a matter of national importance and for a united vision to be achieved, participation is vital.

For further information on the workshop, please contact Nora-Jean (N-J) Freeman on nfreeman@witshealth.co.za.

HOW SEROTYPE SPECIFIC PCR TESTING CAN HELP CONTROL THE SPREAD OF AHS

The beauty of recently developed serotype specific PCR testing, , is that this method can identify exactly which serotype has infected a horse, without the lengthy process involved in viral isolation and serotyping. This means that horse owners/vets will know which of the AHS I or II vaccines to administer to the rest of the horses in the area. AHS "Bottle I" includes Serotypes 1, 3 and 4, and AHS "Bottle II" includes serotypes 2, 6, 7 and 8. Serotypes 5 and 9 are not included in the vaccine but there is cross protection with serotypes that are included. Identifying serotypes involved in an outbreak can also give us an indication of which serotypes are not being sufficiently protected against with the current vaccine.

Although the test has not been DAFF approved yet, it has been used for some time with reliable results.

If AHS is suspected, it is vital that horse owners ensure that blood samples are submitted as soon as possible to make a diagnosis and help bring the disease under control in their area. The test can be completed within twenty four hours of sample submission, and can be performed on whole blood samples. Don't forget that the ERC Team is available to advise you on the submission of samples – contact them on erc@up.ac.za or camillaweyer@gmail.com.

WHAT IS THE AHS CONTROLLED AREA, AND WHAT IS ITS PURPOSE?

An area of the Western Cape Province is declared an 'African horse sickness control area' in accordance with the provisions of the Animal Disease Act. The AHS controlled area is divided into the African horse sickness free zone, the surveillance zone and the protection zone.

Free Area :

The metropolitan area of Cape Town is an African horse sickness free area which is delineated as follows:

- *Northern boundary*: Blaauwberg Road (M14);
- *Eastern boundary*: Koeberg Road (M14), Plattekloof Road (M14), N7 Highway, N1 Highway and M5 Highway;
- *Southern boundary*: Ottery Road, Prince George's Drive, Wetton Road, Riverstone Road, Tennant Road, Newlands Drive, Paradise Road, Union Drive, Rhodes Drive up to the Newlands Forestry station and across Echo Gorge of Table Mountain to Camps Bay;
- *Western boundary*: Coastline from Camps Bay to Blaauwberg Road.

This area is completely AHS free and must be kept this way, which is why movement control is so important from all the other zones. The export quarantine station is situated in the Free Area. Horses living in the free area may only be vaccinated for AHS if they receive written permission from the state

veterinarian. Permission will be granted if the horses will be moving from the free area into the infected area in order that they are protected when they are in the infected area.

African horse sickness surveillance zone:
The African horse sickness free area is surrounded by a surveillance zone which includes the magisterial districts of Cape Town, Vredenburg, Hopefield, Mooreesburg, Malmesbury, Wellington, Paarl, Stellenbosch, Kuilsrivier, Goodwood, Wynberg, Simonstown, Somerset West, Mitchells Plain and Strand and is defined by the Berg Rivier to the north, the Hottentots Holland and Hawekwa Mountain Ranges to the east and the coast to the south and west.

By virtue of its title, surveillance of equids in this zone is done monthly to monitor the occurrence of disease. Horses living in the surveillance zone may only be vaccinated for AHS if they receive written permission from the state veterinarian. Permission will be granted if the horses will be moving from the surveillance zone into the protection zone or infected area in order that they are protected when they leave and then return.

African horse sickness protection zone:
The African horse sickness surveillance zone is surrounded by a protection zone which includes the magisterial districts of Clanwilliam, Piketberg, Ceres, Tulbagh, Worcester, Caledon, Hermanus, Bredasdorp, Robertson, Montagu, Swellendam.

All horses living in the protection zone must be vaccinated with the OBP polyvalent modified live AHS vaccine against AHS between the ages of 6 and 12 months, then between the ages of 12 and 18 months and then again once every year according to the Animal Diseases Act.

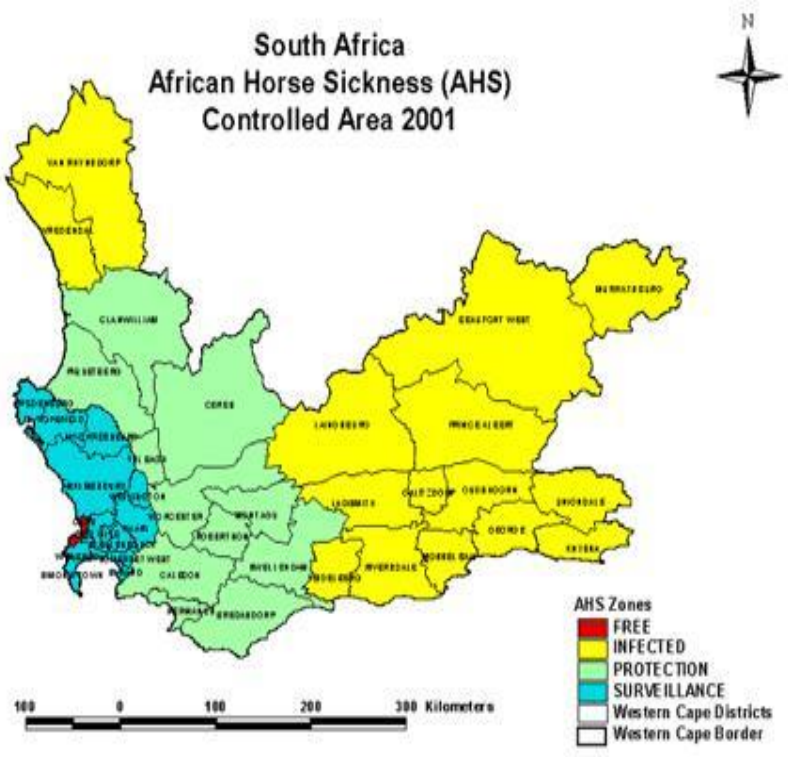
Horses may not move into the AHS controlled area or between the zones in the AHS controlled area unless they comply with the movement conditions as detailed in the movement control protocol. These movement controls are in place to protect the area where horses can potentially be exported directly to the EU and other Trade Partners. Another benefit is that the resident horse population of the AHS Controlled area are protected from AHS outbreaks as long as movement protocols are in place. Without these controls horses will be able to move freely from the infected zone into the AHS Controlled area, and outbreaks will happen much more frequently.

Infected area:
There is a fourth listed area, which is excluded from the AHS Controlled Area- the Infected Area. This area includes the part of the Western Cape outside the AHS Controlled area (the magisterial districts of Vanrynsdorp, Vredendal, Laingsburg, Ladismith, Heidelberg, Riversdale, Mossel Bay, Calitzdorp, Oudtshoorn, George, Knysna, Uniondale, Prince Albert, Beaufort West and Murraysburg) and the rest of South Africa.

Free movement of equids within this area is not controlled but owners are advised not to move horses from areas currently experiencing an outbreak, due to the risk placed on other horses when a possibly subclinical horse is moved into a new area. Reporting of ALL suspected AHS disease and sampling to confirm disease is vital to improve knowledge of the circulating AHS strains and spread of AHS naturally.

All horses living in the infected area must be vaccinated against AHS as for the AHS protection zone according to the Animal Diseases Act.

There has never been an outbreak of AHS in the AHS Free zone. The AHS Controlled area was established in 1997, and enjoyed several years of direct export, before outbreaks in the surveillance zone were reported, which created embargoes on direct exports, more recently there was a very severe AHS outbreak in Mamre in 2011.



The OIE has the following set of requirements for the official recognition of an AHS Free Zone within an AHS infected country:

- an effective surveillance programme has demonstrated either historical freedom or no cases of AHSV within the free zone in the last two years;

- systematic vaccination for AHSV is prohibited (as in only those horses that are coming and going out of the zones should be vaccinated);
- movements into the zone comply with the *Code (OIE)*;
- regular reporting, documentation of surveillance and regulatory measures is recorded;
- a surveillance system is in place within the zone, under the control of the competent authority and includes (among other requirements):
    - systems for detection and investigation of outbreaks;
    - methods for early detection of **clinical cases** and for demonstrating absence of AHSV **infection** in the absence of clinical signs of disease;
    - procedures for rapid collection and transport of samples from suspected cases to a laboratory for diagnosis
    - systems for recording, managing and analysis of diagnostic, epidemiological and surveillance data;
    - All suspected cases of AHS within the zone should be investigated immediately and samples should be taken and submitted to a laboratory. This requires that sampling kits and other equipment are available for those responsible for surveillance;
    - surveillance for both early detection of clinical cases and for demonstrating absence of circulating AHSV in the absence of clinical signs;
    - consideration of the epidemiology of the disease in the country/area and the potential role of wildlife.

At the very least, South Africa needs to comply with these requirements, which won't only benefit the horse export industry, but, if managed effectively, will greatly assist in the understanding, and hence control of the disease throughout the country. The lifting of the embargo on direct exports to the EU and other countries will greatly benefit the SA economy.

The Equine Research Centre still needs sentinel horses in the surveillance zone for monthly surveillance. If you have horses/donkeys that fulfil the following requirements, please contact camillaweyer@gmail.com .

1. Adult horses/donkeys resident in the surveillance zone, that are either unvaccinated, or have not been vaccinated against AHS in the past 2 years or longer;
2. Foals resident in the surveillance zone, between 4-6 months as at January 2014.

For movement enquiries, contact Danielle Pienaar on 082 936 3604 or danielle.pienaar@gmail.com.

SUMMARISED PUBLICATIONS

PROGRESS IN DEVELOPMENT OF AFRICAN HORSE SICKNESS VACCINE

The Equine Research Centre (headed by Prof Alan Guthrie), in collaboration with various international experts successfully developed a preliminary recombinant canarypox virus vectored (ALVAC®) vaccine for protective immunisation of equines against African horse sickness virus (AHSV) infection. The vaccine was tested on eight horses, which were immunised with ALVAC®-AHSV, incorporating specific genes of the AHSV type 4 into the canarypox vector virus. These horses showed complete resistance to infection with a virulent strain of AHSV-4. In contrast, a horse immunized with a commercial canarypox virus vectored flu vaccine depicted typical "dikkop" or cardiac form of African horse sickness following infection with virulent AHSV-4 type virus.

This study confirmed that the ALVAC®-AHSV will be useful for the protective immunization of equines against African horse sickness, and it also avoids many of the problems inherent to live-attenuated AHSV vaccines.

Results from this study caused scientists to suspect that there could be a participation of cell mediated immunity (CMI) in protection against AHSV4. CMI is an immune response that involves cells that engulf foreign objects (such as viruses) rather than the use of antibodies, which identify, bind and neutralise specific foreign objects. A further study was then conducted aimed at characterising the CMI induced by the experimental ALVAC®-AHSV4 vaccine. It was found that ALVAC®-AHSV4 vaccinations induced significant CMI specifically against VP2 and VP5 proteins in AHSV4.

Publication 1 : Vaccine 27 (2009) 4434-4438
Research Team
Alan J Guthrie, Melvyn Quan, Carina W Lourens – Equine Research Centre, University of Pretoria, South Africa;
Jean-Christophe Audonnet, Jules M Minke – Merial S.A.S., R&D, France;
Jiansheng Yao, Ling He – SanofiPasteur, Connaught Campus, Canada; Robert Nordgren – Merial Limited, USA;
Ian A Gardner – Dept of Medicine and Epidemiology, School of Veterinary Medicine, University of California, USA;
N. James MacLachlan – Equine Viral Disease Laboratory, Dept of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, USA.
Publication 2 : Veterinary Immunology and Immunopathology 149 (2012) 76-85
Research Team
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A Guthrie – Equine Research Centre, University of Pretoria, South Africa
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TRANSFER AND DETERIORATION RATE OF MATERNAL ANTIBODY AGAINST AHS

A study was conducted to determine the antibodies to the 9 known serotypes of African horse sickness virus (AHSV) in a group of 15 brood mares which had been regularly vaccinated with the modified live AHSV vaccine (currently widely used to control AHS in Southern Africa), and to measure the passive transfer of mother to foal, and deterioration rate, of maternal antibody to the individual virus serotypes in their foals. The study was conducted on a Stud farm in the AHS surveillance zone. No AHS cases had been reported within a 30km radius since at least 1996.

Serum was collected from the 15 mares before foaling and from their foals after foaling and monthly thereafter for 6 months. Antibodies to each of the 9 AHSV serotypes were determined by serum neutralisation testing.

The findings were that there were varied antibody responses of the mares to the individual serotypes, with higher responses to 1, 4, 6 and 9, and notably weaker to 8 and 5. It was also shown that individual mares had differing responses to the different serotype, when compared with each other. Some mares' antibody responses were low in general, despite the fact that they had been repeatedly vaccinated. This explains reported field observations of horses that have been well-vaccinated contracting severe AHS.

Antibodies are passed from the mare via the colostrum to the foal. The absent or low antibody levels in some mares would also restrict the protection passed on to their foals. The antibody levels passed from mare to foal also seemed to differ among serotypes as well as the duration of the transferred immunity, raising concern over the belief that foals born from vaccinated mares receive maternal immunity to AHS and therefore only need to be vaccinated between 6 and 9 months of age.

This study will assist researchers in further determining the effective utilisation of live-attenuated AHSV vaccines. Further studies will be required on a larger population to determine the optimal time for vaccinating foals.

Research Team
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N J MacLachlan – Dept of Pathology, Microbiology and Immunology, University of California, USA

RESEARCH INTO THE DISSEMINATION AND REPLICATION OF THE AFRICAN HORSE SICKNESS VIRUS IN THE VIRUS CARRYING MIDGE

Despite its important role in the carrying of the African Horse Sickness Virus (AHSV), little information is available on how this virus is spread in the midge *(Culicoides [Avaritia] imicola* Keiffer*).* The Equine Research Centre, together with Onderstepoort Veterinary

Institute, the Department for Veterinary Tropical Diseases, University of Pretoria and the Institute for Virology, Germany, recently conducted research on the detection of AHSV in dissected midges.

Researchers used the reverse transcription quantitative polymerase chain reaction (RT-qPCR) method to conduct this study. It has been shown previously for Bluetongue virus that viral infection of the Culicoides salivary glands is essential for the transmission of the virus to another host. This prerequisite is assumed to be the same for AHSV. In this research a total of 96 midges were fed on AHSV-infected blood, after which one test group was dissected into head/thorax (to test the salivary glands) and abdomen (to test the gut and therefore an indication of virus intake) segments immediately after feeding and the other only after 10 days of incubation.

In the first test group, it was found that there was no significant difference between the virus concentration in the heads/thoraxes and the abdomens immediately after feeding.

In the second test group, which was dissected after 10 days of incubation, the virus was found in 51% of the midges and it was confined to the abdomen in the majority of these cases. Only four of this group showed a presence of the virus in the head/thorax, which suggests in these cases that there was a barrier between the head/thorax and the abdomen preventing the virus from migrating from the abdomen (gut) to the head (salivary glands). Replication in the salivary glands was not shown. An increase of the virus concentration in the abdomen after incubation indicates localised viral replication.

The real-time RT-qPCR research protocol has been shown to be a very sensitive method for investigating AHSV viral load differences in different body parts of the *Culicoides* midges and is recommended for further studies investigating the replication and dissemination of AHSV in *Culicoides* midges. Future studies should include investigations of AHSV viral load in salivary glands and/or saliva.

The RT-qPCR protocol is capable of detecting AHSV viraemia prior to the development of clinical signs and has been adapted for the detection of other diseases, e.g. Equine encephalosis virus.

Publication : Onderstepoort Journal of Veterinary Research 78(1), Art. #344
Research Team
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Christopher Joone, Alan J Guthrie – Equine Research Centre, University of Pretoria;
Nikolaus Osterrieder – Institute for Virology, Germany.

Note : The research team is 75% on the way to having the RT-qPCR protocol officially accredited by the World Organisation for Animal Health (OIE). The next step is to test the protocol in other labs around the world to ensure that the same results are achieved as in South Africa.

YOUR QUESTIONS ANSWERED

Question : What is the maximum interval between AHS I and AHS II vaccines?

Answer : There is no specific scientific information regarding the maximum period between the interval of administering bottle I and bottle II for AHS.

However, due to the cross- reactivity of the serotypes in bottle I and II, bottle II should be regarded as a "booster" vaccine (with the serotypes in bottle II providing an enhanced immune response to their respective cross reacting serotypes in bottle I)– and thus follow the similar principle and interval period for booster vaccines. Hence the recommended interval of 21 to 28 days. The same principal applies if bottle II is administered first.

Question : How effective is the current vaccine?

Answer : It has been shown that vaccinated horses can still become infected with the AHS virus and even show clinical symptoms (See article "Immunised horses may still contract African horse sickness in the field" in Issue 2 of the ERC Newsletter). However it seems that vaccination of a population of horses will dramatically decrease the number of cases seen and therefore the number of deaths. This can be evidenced in the report from the Mamre outbreak in 2011 (See article "Illegal movement of horses could result in huge knock-on effect" in Issue 1 of the ERC Newsletter). In this outbreak a dramatic decrease in the number of cases seen per week was observed two weeks after the equine population in the outbreak areas were vaccinated.

Question : Can horses be worked normally during AHS vaccination?

Answer : OBP recommends a "resting" period of at least 2 weeks after the second vaccination for horses that have had previous sets of vaccination i.e. recent history of vaccination. For horses receiving their first vaccination they should be given rest for at least 21 days. Remember it is a live vaccine which puts stress on the immune system, and intense exercise/racing will further stress the animal which could impact the immune system.

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