



Equine Research Centre • Onderstepoort

Faculty of Veterinary Science

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FROM THE ERC TEAM



Our thoughts are with all the owners, breeders and veterinarians battling with Equine Encephalosis and African Horse Sickness. The widespread rain means it may be a particularly hard hit year, which emphasises the importance of vaccination, surveillance, and the development of a new improved vaccine.

HOT OFF THE PRESS

SA HORSE EXPORT STRATEGY

At the conclusion of the Strategy Workshop for the export of horses from South Africa, held at the Wits Health Consortium, Johannesburg on 15-16th April 2014 the attendees from Department of Agriculture, Forestry and Fisheries (DAFF), the Sport Horse and Horseracing Industry concluded a new way forward.

Export of horses provides South Africa with a foreign exchange income of approx. R250 million/annum, with a potential for growth to over R1 Billion/annum and significant job creation. Trade is currently hampered by severe quarantine restrictions and recent outbreaks of African horse sickness (AHS) in 2011, 2013 and 2014 in the AHS Controlled Area in the Western Cape which has led to the temporary suspension of exports to the EU and other trading partners. The meeting recognized that the existing strategy reliant on maintaining freedom of the AHS Controlled Area is fragile, with South Africa only being able to export for 50% of the time in the last 17 years.

“This important meeting has demonstrated the ability of all parties from National and Provincial Government, and those involved in the Sport Horse and Horseracing Industry to collaborate towards the objective of AHS disease control and export of horses”, said Prof. Ian Sanne who hosted the important workshop.



The approach recommended includes the following:

1. Export from the vector protected Kenilworth Quarantine Station which is situated in the AHS Free Zone in Cape Town with an upgrade of the facility to enable horses to be confined to the facility for the full duration of the quarantine period and to be transported to and loaded into the aircraft under continuous vector protected conditions. This strategy is combined with continued surveillance and movement control to enable risk mitigation, and to meet international import requirements. Participate in international laboratory testing to ensure the World Organisation for Animal Health (OIE) recognizes the RT-PCR molecular test for AHS – to shorten the pre-export quarantine period to a minimum of 14 days.
2. Export to Mauritius from a vector-protected facility compliant with OIE and international standards. Technical assistance will be offered to the Mauritian Veterinary Authorities to support OIE code conditions for AHS Country freedom.
3. Continue to export from South Africa to the USA with a 60 day post arrival quarantine. Under this model, which has been in existence for the last 65 years, the USA ensures the import requirements and testing is met under the supervision of the USDA.
4. Investigate the viability of establishing an AHS Free Zone in a part of South Africa where the risk of outbreaks of AHS is considerably reduced.

The meeting recommended continued investment in the coordination of surveillance, movement control and reporting of disease within South Africa and Internationally. Ongoing development of a recombinant vaccine, with related laboratory testing approaches will seek to replace the current live-attenuated vaccine.

Hon Mr. Lulu Johnson Member of Parliament, Chair of the Parliamentary Portfolio Committee of Agriculture, Forestry and Fisheries commented "Parliament has a constitutional mandate to oversee the spending of public funds." Mr Johnson addressed the workshop and stressed business and transformation are imperative to build the nation. He highlighted examples of where different Government Departments had worked together to achieve success. He suggested the relevant departments of Government: Department of Agriculture, Forestry and Fisheries; Department of Trade and Industry; Department of Science and Technology and Department of Sports and Recreation need to come together and form a committee to work towards solving the problems affecting the broader Equestrian Industry.

International experts from Australia, Italy, Singapore, and the USA participated in the workshop which was facilitated by Australian epidemiologist, Dr Evan Sergeant, of AusVet Animal Health Services.



SUPPORTIVE TREATMENT FOR AFRICAN HORSE SICKNESS

In February 2012 a workshop was conducted by the South African Equine Veterinary Association (SAEVA), which was attended by veterinarians from around the country. Following is a summary report arising out of the workshop. The issues facing SAEVA were :

- Perception of lack of consensus amongst veterinarians regarding supportive treatment of African horse sickness (AHS);
- Confusion amongst horse owners and trainers regarding recommended veterinary supportive treatment, which opens the way for acceptance and use of unproven, potentially unsafe remedies in horses;
- Whilst the freedom and rights of horse owners to select the health care of their choice is recognised, the irrational use of 'medications' without scientific evidence is not in the best interests of horse welfare.

The goal of the workshop was to discuss and document supportive treatment options used by veterinarians treating AHS cases, with focus on the scientific rationale for treatment, scientific evidence supporting efficacy and safety of the treatment, and reach agreement on whether the treatment was considered justified as veterinary supportive treatment for AHS or not.

Veterinary Supportive Treatment of African horse sickness

It was emphasised and agreed that there is no generic veterinary supportive treatment guideline for every AHS case and that veterinary treatment must be customised on a patient by patient basis. It further should be noted that treatments agreed with are supportive in basis and not curative. Many other factors, such as vaccination status and immunocompetence, are important in individual horse response to African horse sickness virus (AHSV) infection.

1. Rest

Whilst not a veterinary treatment *per se* participants agreed unanimously that strict rest is mandatory for horses suffering from AHS.

2. Nonsteroidal anti-inflammatories

- Participants agreed unanimously that treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) is justified as a veterinary supportive treatment option for horses suffering from AHS.
- The majority indicated preference for use of flunixin. Phenylbutazone is also used, while few participants use ketoprofen.
- The rationale for treatment is anti-inflammatory, analgesic and antipyretic effects, based on cyclooxygenase (COX) inhibition.
- There is strong scientific evidence supporting the rationale, efficacy and safety of NSAIDs such as flunixin and phenylbutazone in horses. It was agreed that hydration status must be considered with use, as NSAIDs have



potential to cause renal failure and be associated with gastrointestinal ulceration. However, participants note that horses suffering from AHS generally drink and eat well and renal failure and gastric ulceration is not commonly recorded in clinical cases in the field.

3. Corticosteroids

- The majority of participants agreed that judicious treatment with short to medium acting corticosteroids, selected on a case by case basis is justified as a veterinary supportive treatment for horses suffering from AHS.
- The rationale for treatment includes potent anti-inflammatory effects, stabilization of cell membranes and preservation of vascular membrane integrity.
- There is strong scientific evidence supporting the rationale, efficacy and safety of corticosteroids in horses.
- The majority of participants indicated use of dexamethasone in AHS cases, with a single initial treatment only or an alternate day regimen.
- None have recorded laminitis as a side effect with treatment of AHS cases. Concern over use in cases with a history of laminitis, equine metabolic syndrome or Cushing's disease was agreed with, and potential for immunosuppression with use of long acting preparations and high doses was noted.
- The duration of treatment by participants varied according to individual case response.

4. Antimicrobials

- Participants agreed that treatment with antimicrobials is justified as a veterinary supportive treatment for horses suffering from AHS, however, they are not used in AHS cases as a routine.
- The rationale for treatment is as prophylactic for secondary bacterial infection, particularly bacterial pneumonia as AHS cases with pulmonary oedema, leukopaenia and neutropaenia are considered at risk.
- There is strong scientific evidence supporting the rationale, efficacy and safety of specific antimicrobials in horses.
- Participants indicated use of penicillin or trimethoprim-sulfonamides.

5. Intravenous Crystalloids

- Participants agreed that treatment with crystalloid intravenous (IV) fluids is justified as a veterinary supportive treatment for selected cases suffering from AHS.
- Intravenous fluids are used in limited cases (e.g. those with hypovolaemic shock) in the field as it was indicated that most cases drink water voluntarily and maintain hydration.
- The rationale for treatment is to correct hypovolaemia and improve peripheral perfusion.
- Concern with risk of increased extravascular fluid leakage due to increased capillary permeability was noted by participants.
- There is strong scientific evidence supporting the rationale, efficacy and safety of crystalloid IV fluids in horses.



6. Intravenous Colloids

- Participants agreed that treatment with synthetic colloid IV fluids is justified as a veterinary supportive treatment for selected cases suffering from AHS.
- The rationale for treatment is to improve oncotic pressure and limit extravascular leakage of fluid.
- There is scientific evidence supporting the rationale, efficacy and safety of selected synthetic colloids in horses.
- The use of synthetic versus natural colloid (plasma) was discussed. It was postulated that plasma might provide additional factors beneficial to a case of AHS, but the high volume of plasma generally required and expense in an adult horse was recognised as a limitation to routine use.
- Private practitioners indicated that the expense of synthetic colloids restricted their routine use in the field. Use in a referral or veterinary academic hospital situation was indicated as more frequent.

7. Calcium

- Treatment with IV calcium has been utilized as a veterinary supportive treatment for AHS cases in the field.
- Participants did NOT agree that treatment with IV calcium is justified as a routine veterinary supportive treatment for horses suffering from AHS.
- A rationale for treatment was indicated as potential stabilizing effects on cell membranes.
- There is lack of evidence supporting ionized hypocalcaemia in AHS cases and evidence for efficacy in AHS cases is lacking.
- There is however evidence supporting the safety of recommended dosages of intravenous calcium in horses.

8. Diuretics

- Treatment with diuretics such as furosemide has been utilized as a veterinary supportive treatment for AHS cases in the field.
- Participants did NOT agree that treatment with furosemide is justified as a routine veterinary supportive treatment for horses suffering from AHS.
- The rationale for treatment is to decrease pulmonary intravascular pressure.
- There is scientific evidence supporting the rationale in other equine conditions, efficacy and safety of furosemide in horses.
- Concern was noted regarding safety and exacerbation of hypovolaemia in cases of AHS.

9. Dimethyl sulfoxide

- Whilst the majority of participants agreed that treatment with dimethyl sulfoxide (DMSO) is justified as a veterinary supportive treatment for cases suffering from AHS, concern was noted regarding the lack of strong scientific evidence in horses.
- The rationale for treatment is to scavenge free oxygen radicals released in damaged tissue.
- The scientific evidence supporting the rationale and efficacy of IV DMSO in horses is controversial.
- There is scientific evidence supporting safety at recommended dosages.



10. Antioxidants

- Treatment with antioxidants such as vitamin E has been utilized as a veterinary supportive treatment for AHS cases in the field.
- Participants did NOT agree that treatment with antioxidants is justified as a routine veterinary supportive treatment for horses suffering from AHS.
- The rationale for treatment is to limit oxidative injury; however there is lack of scientific evidence supporting the role of oxidative injury in AHS cases.
- There is scientific evidence supporting the efficacy and safety of selected antioxidants in horses.

11. Formalin

- Treatment with formalin IV has been utilized by some practitioners as a veterinary supportive treatment for AHS cases in the field.
- Participants did NOT agree that treatment with formalin IV is justified as a veterinary supportive treatment for horses suffering from AHS.
- The rationale for treatment is unspecified but may be to promote haemostasis.
- Limited scientific evidence does not support a beneficial effect on coagulation parameters in horses.
- There is some evidence for safety but higher doses are associated with adverse reactions.

12. Homeopathic remedies

- Treatment with homeopathic remedies has been utilized by some practitioners as a veterinary supportive treatment for AHS cases in the field.
- Participants did NOT agree that treatment with homeopathic remedies is justified as a veterinary supportive treatment for horses suffering from AHS.
- Some scientific evidence is available, however use in horses is controversial and strong evidence supporting a rationale, efficacy and safety in AHS cases is lacking.
- Practitioners noted that use in AHS cases promoted owner involvement in supportive care.

13. Sodium chlorite, colloidal silver, hydrogen peroxide, ozone

- Participants noted that there is NO scientific evidence to support use of the alternative remedies indicated above in horses and do NOT support their use as supportive treatment for horses suffering from AHS.
- As there is no recognised scientific efficacy or safety data in horses, horse owners and trainers are cautioned against use of these unregistered remedies in their horses.

This is a summary of a report compiled by Dr Patrick Page, who was the Workshop Chair.

Practical precautions against African horse sickness:

1. Ensure that your horses' vaccination status, with a registered vaccine against African horse sickness, is up to date.
2. To reduce contact with biting midges infected with African horse sickness virus, stable horses at night from 2 hours before sunset to 2 hours after sunrise.



3. Provide additional protection to stables to reduce midges entering by using 70% shade cloth (e.g. Alnet) to cover all stable openings (e.g. door and window openings) at night.
4. Spray stable walls and protective mesh with an insecticide effective against biting midges, e.g. Fendona 6 containing alphacypermethrin.
5. Apply insecticides registered for use on horses in the late afternoon and early morning, focussing on preferred midge biting sites such as the head, neck, back and belly, e.g. fly sprays containing cypermethrin or permethrin.
6. Apply insect repellent to horses in the late afternoon and early morning, focussing on preferred midge biting sites such as the head, neck, back and belly. (Note the effective repellent DEET is not registered for use on horses and horses should be monitored for adverse skin reaction).
7. Move horses away from midge breeding sites such as vleis and muddy areas.
8. Repair leaking water pipes and troughs to reduce midge breeding sites.

THE ANNUAL YEARLING SALES AND PIROPLASMOSIS (BILIARY)

It was at the Annual Yearling Sales in 1999, following the re-opening of horse exports from South Africa, that the prevalence of piroplasmosis (biliary) in South Africa presented an obstacle for the export of horses. It became a requirement for horses to be tested, particularly those going to the East, e.g. Hong Kong, Singapore, Malaysia, Australia, which, as well as the USA, all require horses to be negative for piroplasmosis for entry to these countries.

In 1999, all the horses at the National Yearling Sales were tested. Fifty seven per cent of these horses tested positive, and in many cases farmers had not treated their foals at all against this tick-borne virus. It became evident that controls needed to be put in place for the preventative treatment of foals. A strategy was developed whereby even if a mare was positive, they could still produce negative foals. Infected farms can implement control measures to reduce the incidence of piroplasmosis in their stock.

This strategy has resulted in a dramatic reduction over the years of the incidence of biliary in horses at the yearling sales. We asked Dr Ashley Parker, co-owner with his mother, Rose Parker of Ascot Stud, what impact this had on his farm, and this is the report he sent to us.



“In 1999, virtually all Ascot yearlings consigned to sales, were positive for piroplasmosis – *Theileria equi*.

With the possibility of exports it became important to try and manage exposure to ticks. We embarked on a programme of strict tick control which included weekly dipping with a synthetic pyrethroid and spot treatment with tick grease - under tails and in ears. Injectable doramectin then later moxidectin were included in the deworming programme and used specifically when the potential exposure to ticks was the highest. (It is important to use at double the cattle dose for effective internal and external parasite control in horses. These were used off label with no side effects.)

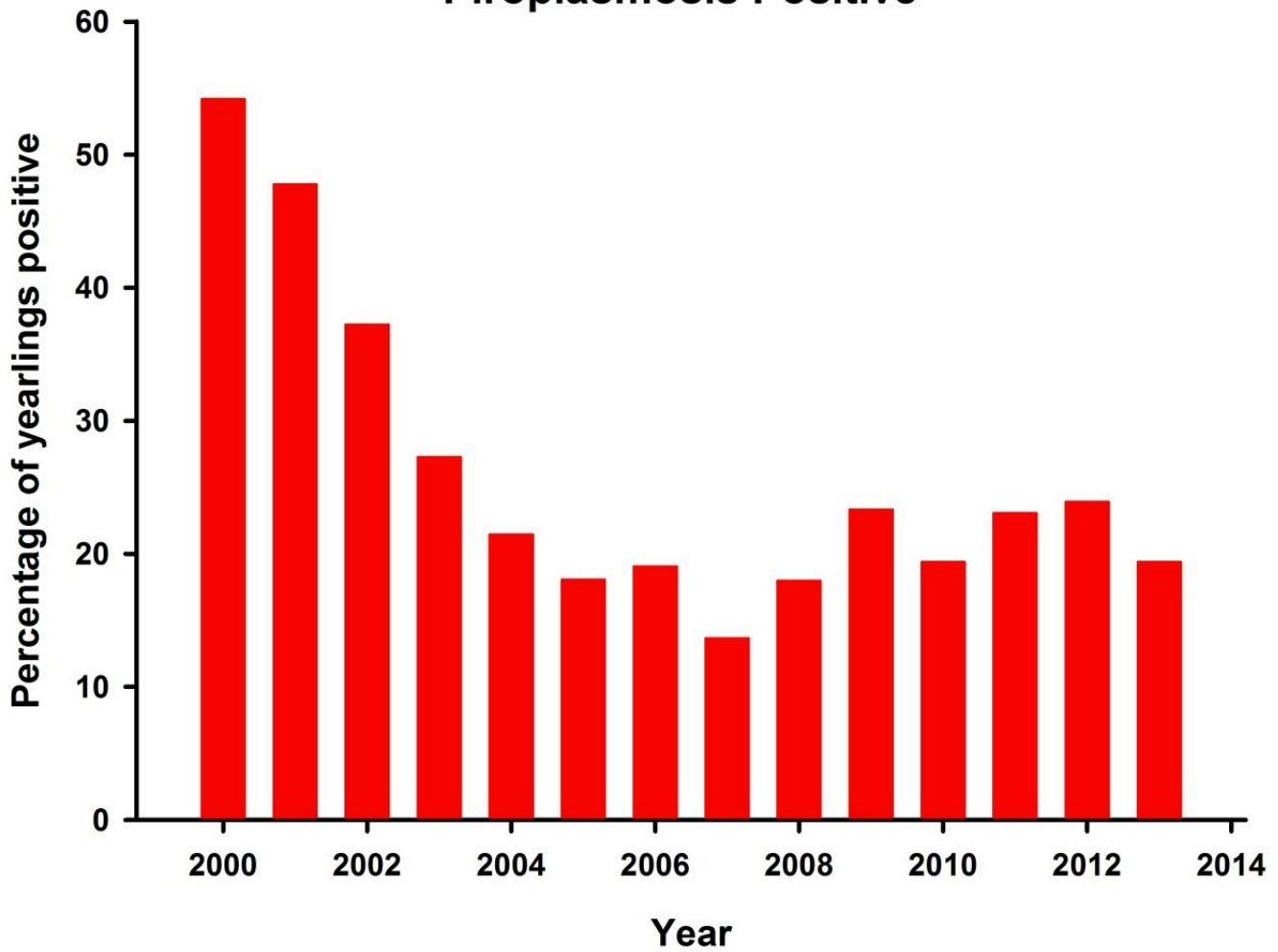


In the year 2000, 48% of our yearlings sent to sales were positive for Theileria equi; in 2001, 10%. Since then below 5% with most years 2%.

As new products became available, a topical flumethrin was tried but it burnt some horses. I now use a topical cyflumethrin, which is commercially available at half the strength of flumethrin, with good results and no burning or skin irritation. I use a sponge lightly dipped in the cyflumethrin (otherwise it drips off and is wasted) and gently wipe from behind the ear down either side of the neck, down each leg onto the hoof and one wipe on each side of the top line of the body and another wipe on each side of the thorax and abdomen of the body.

The farm has been "cleaned" of the red legged tick, Rhipicephalus evertsi, and we don't need to treat as regularly. injectable moxidectin twice a year as part of our deworming programme."

Piroplasmosis Positive





BILIARY IS HERE TO STAY – IT CAN BE CONTROLLED, BUT NEVER ERADICATED

Equine piroplasmosis, babesiosis or biliary fever is an infectious tick-borne disease of horses caused by two different protozoa : *Babesia (Theileeria) equi* and *Babesia caballi*. Once infected, and if left untreated, horses may remain life-long carriers of *B. equi* infections whereas with *B. caballi* infections, which are self-limiting, horses remain carriers for up to four years.

In South Africa the red-legged tick *Rhipicephalus evertsi evertsi* transmits both *B. equi* and *B. caballi*, while *Hyalomma truncatum* only transmits *B. caballi*.

Both these ticks are known as two host ticks, i.e. they feed on two separate hosts. The larvae of these tick species will hatch from the eggs in the grass, attach themselves to a host (can be equine) and feed. They will remain on this host animal, develop into nymphae, re-engage and feed once again. These nymphae when engorged will drop to the ground and develop into adults. These adults will attach to a second host and feed.



It is this life cycle that must be broken to minimise transmission of piroplasmosis between horses on farms.

Rhipicephalus evertsi evertsi

The larvae of this tick are babesia-free when they attach to a host. They normally attach deep in the ears of the hosts. If they attach to a carrier of *B. equi* or *B. caballi* or a horse with a current babesia infection they become infected. The infection remains with the ticks during their moult to adults and thus when the adults attach to new hosts, they will transmit the infection to susceptible horses.

Control

Immature *R. evertsi evertsi* attach deep in the ears of hosts. If weekly acaricidal treatment (tick grease) is used as a barrier against immature infestation in the ears of horses, the risk of ticks becoming infected from an already infected host is very low.

The adult ticks attach under the tails of equids. Again, weekly applications of tick grease , particularly in this area in all horses, will reduce the possibility of already infected adult ticks transmitting babesia to these host animals.



Hyalomma truncatum

This tick species is only able to transmit *B. caballi*. It is also a two host tick but the *B. caballi* organisms are able to pass from the adult of this tick through the egg, to the larval stages and through all the life stages to the next generation of adult tick. So, infected immature stages can transmit the protozoa, and will moult into infected adults that will infect susceptible horses. These ticks prefer to feed at the back of the pasterns, inner thighs, udder, prepuce and umbilical regions.



Control

The only way to keep horses piroplasmosis free is through good tick control. Stud farms that are in areas with a high prevalence of babesia may want to consider a modified approach to babesia free maiden mares returning to stud. The farm's veterinary advisor should be contacted regarding this approach.

Serological Tests

Two serological tests can be performed on serum samples to determine the babesia status of horses.

1. IFAT (Indirect Fluorescent Antibody Test): This is the most sensitive and reliable of the tests currently accepted for international trade. Horses are required to test negative using the IFAT while in pre-export quarantine in South Africa before shipment to Hong Kong, Malaysia and Singapore. In the case of horses destined for Australia (which currently does not allow direct import of horses from South Africa) negative IFAT's are required while resident in Europe or the USA prior to re-export.
2. CFT (Complement Fixation Test): This is a markedly less sensitive, less reliable and substantially more expensive test than the IFAT. This test is only accepted by the USA where it is used for post-arrival testing of horses.

It is not uncommon for a horse to test negative for piroplasmosis on CFT but positive on IFAT. It is therefore preferable that the IFAT is always done prior to export to ensure subsequent entry to Australia, Hong Kong, Malaysia and Singapore. It should be noted that piroplasmosis occurs in a number of EU countries and the UAE, and therefore testing for piroplasmosis is not required prior to export to these countries.

Professor Alan Guthrie and his team developed a Treatment and Sterilization Protocol, which is available only to veterinarians for safety reasons. This protocol has been successfully adopted in the United States of America.

See Summarised Publications re *Babesia caballi* and *Theileria equi* later in this Newsletter.

SUMMARISED PUBLICATIONS

VACUUMING HORSES PROVIDES USEFUL INFORMATION ABOUT MIDGES

It is widely understood that *Culicoides* biting midges carry various pathogens including African horse sickness virus (AHSV), which cause African horse sickness. The predominant species shown to be a carrier of the virus is the *Culicoides (Avaritia) imicola* Keiffer. However, it is essential as part of any risk assessment to monitor known carriers as well as potential species acting as carriers. To do this, researchers needed to find the most effective way of collecting midges for study. In a recent study conducted by the Equine Research Centre, in collaboration with various other local and international



experts (listed below) two methods of collection of *Culicoides* midges were compared – one being the more traditional overnight collections in the conventional Onderstepoort light trap, and the other being mechanical aspiration – or more simply put, ‘vacuuming’ midges directly off bait horses during the sunset period (15 minutes prior until 5 minutes after sunset).

The ‘vacuuming’ method proved to be a useful addition to conventional light trap collection, as while *Culicoides imicola* was confirmed the predominant species in both trapping methods, other species such as *Culicoides bolitinos* and *Culicoides gulbenkiani* were much more prevalent in the ‘vacuuming’ method than in the light trap method. This is an important finding, as *Culicoides bolitinos* has been shown to transmit AHSV and was the dominant vector in a previous AHS outbreak in the Eastern Cape. The high incidence of *Culicoides gulbenkiani* in the ‘vacuuming’ method indicates that this species may have previously been underestimated using the light trap method. Its potential role as a vector of AHSV merits further investigation.

Also of interest is the observation of a peak of *Culicoides* biting midges on the horses in May (using the ‘vacuuming’ method), along with the positive RT-qPCR pools that were identified through June, suggesting that AHSV transmission may occur even after the ‘traditional’ end of summer season.

Overall, the ‘vacuuming’ method proved to be the better choice when investigating the various *Culicoides* midge species.

Publication : *Veterinary Parasitology* 185 (2012) 265-273 – Comparison of two trapping methods for *Culicoides* biting midges and determination of African horse sickness virus prevalence in midge populations at Onderstepoort, South Africa.

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DEVELOPMENT OF REAL TIME PCR TESTS FOR QUANTITATIVE DETECTION OF *BABESIA CABALLI* AND *THEILERIA EQUI* INFECTIONS IN HORSES, AND THE OBSTACLES TO ACHIEVING THIS

This summary has been extracted from three publications, all of which are listed at the foot of this article.

Development and evaluation of real-time PCR tests for detection of *Babesia caballi* and *Theileria equi* infections in horses from South Africa



Babesia caballi and *Theileria equi* are parasites that cause equine piroplasmosis, more commonly known as Biliary. The disease is of worldwide importance and occurs throughout the tropical and subtropical parts of the world with its prevalence related to the distribution of tick vectors. Both these parasites cause

disease, which may be either acute or chronic with mortalities ranging from less than 10% up to 50%. The disease is generally characterised by fever and anaemia. The clinical signs are often variable and non-specific, making it easy to confuse the disease with other conditions, and therefore complicating diagnosis. It is also not possible to differentiate between *B. caballi* and *T. equi* infections based on clinical signs alone. Once infected, and if not treated, horses may remain life-long carriers of *T. equi* infections, whereas with *B. caballi* infections, horses can remain carriers for up to four years.

The global transport of horses has led to the spread of equine piroplasmosis from its endemic tropical and subtropical zones to more temperate regions. Stringent regulatory import restrictions are therefore in place in some countries to prevent the entrance of infected horses. Regulations often require the serological testing of horses in order to confirm seronegativity and to identify seropositive animals whose movement is restricted. There are various serological tests available, but they are restricted by antibody detection limits and cross-reactivity.

A quantitative real-time PCR (qPCR) test was developed for the detection of *B. caballi* and *T. equi* infections. These tests were shown to be efficient and specific, and more sensitive than the indirect fluorescent antibody test (IFAT) and the reverse-line blot (RLB) during the early onset of the disease.

The development of the qPCR test followed the findings of three other research projects that determined that there was sequence heterogeneity (diverse/not comparable) in the 18S rRNA gene, the equi merozoite antigen gene (*ema-1*) and the gene encoding the rhoptry-associated protein-1 (RAP-1). What this means is that, before a qPCR test can be used with confidence, scientists must first identify a group of like-genes or the test will be inaccurate.

Sequence heterogeneity in the 18S rRNA gene within *Theileria equi* and *Babesia caballi* from horses in South Africa

The outcome of a molecular epidemiological survey of 488 samples tested for *T. equi* and *B. caballi* suggested the presence of a novel species or genotype. Sequence variation was found in both *B. caballi* and *T. equi*, although more so in *B. caballi*. The extent of sequence heterogeneity detected within *T. equi* and *B. caballi* was unexpected since concerted evolution is thought to maintain homogeneity (similar/comparable) within repeated gene families, including rRNA genes. The findings reported here show that careful examination of variants of the 18S rRNA gene of *T. equi* and *B. caballi* is required prior to the development of molecular diagnostic tests to detect these parasites in horses. Species-specific probes must be designed in regions of the gene that are both conserved within and unique to each species.



Sequence heterogeneity in the equi merozoite antigen gene (*ema-1*) of *Theileria equi* and the development of an *ema-1*-specific test for the detection of *T. equi*

Although a qPCR test for the detection of *T. equi* has been developed and evaluated, it is possible that additional, as yet undetected 18S rRNA gene sequence variants may exist. A qPCR test targeting a different gene, used in conjunction with the *T. equi* 18S rRNA qPCR test, could assist in the detection of all *T. equi* genotypes in field samples. Tests on 107 South African field samples using both the IFAT and a *T. equi ema-1*-specific qPCR showed the qPCR test performed poorly in comparison with the IFAT test. Alignment of the sequences revealed extensive sequence variations in the target regions of the primers and probes, explaining the poor performance of the qPCR test. Based on these observations, a new qPCR test, targeting a more conserved region of the *ema-1* gene, was developed. This test was shown to be efficient and specific.

Publication : *Veterinary Parasitology* 168 (2010) 201-211 – *Development and evaluation of real-time PCR assays for the quantitative detection of Babesia caballi and Theileria equi infections in horses from South Africa.*

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Publication : *Veterinary Parasitology* 159 (2009) 112-120 – *Sequence heterogeneity in the 18S rRNA within Theileria equi and Babesia caballi from horses in South Africa.*

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Publication : *Veterinary Parasitology* 172 (2010) 33-45 - *Sequence heterogeneity in the equi merozoite antigen gene (*ema-1*) of Theileria equi and development of an *ema-1*-specific TaqMan MGB assay for the detection of *T. equi*.*

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