SEROLOGICAL RESPONSE OF FOALS TO POLYVALENT AND MONOVALENT LIVE-
ATTENUATED AFRICAN HORSE SICKNESS VIRUS VACCINES

J. E. Crafford¹*, C.W. Lourens², T.K. Smit³, I. A. Gardner⁴, N. J. MacLachlan¹,⁵, A. J. Guthrie²

¹ Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, Gauteng, South Africa

² Equine Research Centre, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, Gauteng, South Africa, email alan.guthrie@up.ac.za

³ Onderstepoort Biological Products SOC Ltd, Private Bag X07, Onderstepoort, 0110 Gauteng, South Africa, email theresa@obpvaccines.co.za

⁴ Department of Health Management, University of Prince Edward Island Atlantic Veterinary College, Charlottetown, PE Canada C1A 4P3, email iagardner@upei.ca

⁵ Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, 5325 VetMed 3A, Davis, CA 95616,USA, email njmaclachlan@ucdavis.edu

*Corresponding author email jannie.crafford@up.ac.za, Tel +27 012 529 8229, Fax +27 012 529 8312

Abstract

African horse sickness (AHS¹) is typically a highly fatal disease in susceptible horses and vaccination is currently used to prevent the occurrence of disease in endemic areas. Similarly, vaccination has been central to the control of incursions of African horse sickness virus (AHSV²) into previously unaffected areas and will likely play a significant role in any future incursions. Horses in the AHSV-infected area in South Africa are vaccinated annually with a live-attenuated (modified-live virus [MLV³]) vaccine, which includes a cocktail of serotypes 1, 3, 4 (bottle 1) and 2, 6, 7, 8 (bottle 2) delivered in two separate doses at least 21 days apart. In this study, the neutralising antibody response of foals immunized with this polyvalent MLV AHSV vaccine was evaluated and compared

¹ African horse sickness
² African horse sickness virus
³ modified-live virus
to the response elicited to monovalent MLV AHSV serotypes. Naïve foals were immunized with either the polyvalent MLV AHSV vaccine, or a combination of monovalent MLV vaccines containing individual AHSV serotypes 1, 4, 7 or 8. There was a marked and consistent difference in the immunogenicity of individual virus serotypes contained in the MLV vaccines. Specifically, foals most consistently seroconverted to AHSV-1 and responses to other serotypes were highly variable, and often weak or not detected. The serotype-specific responses of foals given the monovalent MLV vaccines were similar to those of foals given the polyvalent MLV preparation suggesting that there is no obvious enhanced immune response through the administration of a monovalent vaccine as opposed to the polyvalent vaccine.

Key words:
African horse sickness virus; modified-live virus vaccine; vaccination; neutralizing antibody

1. Introduction

African horse sickness (AHS\textsuperscript{4}) is an insect-transmitted, non-contagious disease of equids caused by African horse sickness virus (AHSV\textsuperscript{5}) [1]. AHSV is transmitted to horses by haematophagous Culicoides midges that serve as biological vectors of the virus [2]. AHSV is a member of the family Reoviridae genus Orbivirus [3], of which nine serotypes have been described [4;5]. AHSV is listed by the World Organisation for Animal Health (OIE\textsuperscript{6}) as important to the international trade and movement of horses and disease freedom of a country or zone is officially recognised by the OIE [6]. AHSV is widespread in extensive portions of sub-Saharan Africa All nine serotypes of AHSV occur in South Africa [7] [8].

AHS is typically a highly fatal disease in susceptible horses so vaccination is currently used to prevent the occurrence of disease in endemic areas [8]. Similarly, vaccination will be central to the control of any future incursions of AHSV into previously unaffected areas [9]. Several AHSV vaccines have been previously developed and used including: a polyvalent live-attenuated (modified-live virus

\textsuperscript{4} African horse sickness
\textsuperscript{5} African horse sickness virus
\textsuperscript{6} World Organisation for Animal Health
[MLV\textsuperscript{7}) vaccine of adult mouse brain origin, a polyvalent cell culture-adapted MLV vaccine, and inactivated AHSV vaccines. These vaccines and their potential limitations have been reviewed previously [10;11]. Although new generation vaccines have been described recently [12-15], only MLV AHSV vaccines are currently commercially available in southern Africa [16-18], Senegal [19;20] and Ethiopia [21].

The MLV AHSV vaccine that is currently used widely in southern Africa was first introduced in the 1960s [18] and has since undergone significant modifications. AHSV-9 is not included in the vaccine, and serotype 5 was removed from the original vaccine due to severe adverse reactions in some immunized horses [16]. However, cross-protection between AHSV serotypes 5 and 8 was recently reported in horses immunized with this polyvalent MLV vaccine, as well as between serotypes 6 and 9 [17]. The MLV vaccine currently used in southern Africa includes a cocktail of serotypes 1, 3, 4 (bottle 1) and 2, 6, 7, 8 (bottle 2) delivered in two separate doses at least 21 days apart.

Few published studies have evaluated seroconversion and serotype-specific antibody titres among horses vaccinated in the field with the commercially-available South African polyvalent MLV AHSV vaccine. The goal of the present study, therefore, was to evaluate the humoral immune (antibody) response of foals immunized with the polyvalent MLV AHSV vaccine, and to compare it with that of foals immunized with selected individual monovalent MLV AHSV serotypes contained in the vaccine. Findings from the study are relevant to the control of AHS in endemic areas, as well as regions and countries at risk of future incursions.

2. Materials and methods

2.1. Study population

Three groups of Thoroughbred foals (n=46) 7 to 10 months of age and confirmed to be seronegative by an AHSV-specific ELISA [22] were used to evaluate neutralizing antibody responses to MLV polyvalent and monovalent AHSV vaccines. Ethical approval was granted by the Animal Use and Care Committee of the University of Pretoria (protocol V052/07). The foals were all resident on a commercial stud farm located near Wellington in the Western Cape, South Africa. This farm is located in an area that is subject to an active AHSV surveillance programme that includes clinical surveillance

\textsuperscript{7} modified-live virus
and the monitoring of seronegative sentinel horses. No cases of AHS have been detected within a radius of at least 30 km of this farm since the inception of the surveillance programme in 1996. The foals were segregated into groups by gender, and individual foals remained within the same group throughout the study. All foals were identified with a microchip and given the same routine veterinary care, including immunization against equine influenza, tetanus and botulism.

2.2. Study design

The 46 foals were randomly assigned to three groups (Group I, II and III) and immunized at specific intervals with either polyvalent or monovalent (AHSV 1, 4, 7, 8) MLV AHS vaccines according to the schedule listed in Table 1. For preparation of the monovalent MLV AHSV vaccines, AHSV serotypes 1 and 4 were selected as representatives of the MLV strains contained in bottle 1 of the polyvalent MLV preparation, and serotypes 7 and 8 as representatives from bottle 2. As the foals included in this

<table>
<thead>
<tr>
<th>Table 1: Treatment groups, vaccination intervals, and AHSV vaccine serotypes included in the vaccines administered to the foals in the different groups included in this study.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AHSV serotype(s) and day of vaccination</strong></td>
</tr>
<tr>
<td>Group</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>III</td>
</tr>
</tbody>
</table>

\(^{†}\) Two foals were removed from the respective groups after Day 56.

\(^{††}\) One foal was removed from the group after Day 56.
study were from a commercial stud, they were all required to be vaccinated according to the regular MLV polyvalent vaccine regimen prior to the next AHS season to fulfil statutory requirements. It was, therefore, not possible to administer a second immunization of monovalent MLV vaccine to foals in groups II and III. Blood for harvesting of serum was collected from each foal and a blinded approach was used to determine the neutralising antibody titres to all nine AHSV serotypes on days, 56, 112, 196 and 238 after first vaccination.

2.3. Vaccine

Foals of Group I were vaccinated subcutaneously with 2 ml of the commercially available polyvalent MLV cell culture-adapted AHSV vaccine (Onderstepoort Biological Products SOC Ltd, Onderstepoort, South Africa) given as two doses 28 days apart. Foals of Group II were initially vaccinated with monovalent MLV AHSV serotype 1 followed by monovalent MLV AHSV serotype 8, 28 days later. Similarly, foals of Group III were vaccinated at a 28 day interval, initially with monovalent MLV AHSV serotype 4 and 28 days later with serotype 7. The monovalent MLV AHSV serotypes 1, 4, 7 and 8 were prepared and supplied by the manufacturer to contain the same viral load and seed stock viruses as those used in the commercial polyvalent MLV vaccine (Table 1) and contained a minimum immunizing dose of $1 \times 10^3$ pfu/ml for each serotype and a minimum infectivity titre of the final product of $1 \times 10^3$ pfu/ml [6].

All foals were vaccinated with an equine influenza-tetanus combination (ProteqFlu-Te® MERIAL; batch L209556) consisting of 2 equine influenza constructs in a canarypox vector and a tetanus toxoid and formalised aluminium hydroxide gel adsorbed toxoid of Clostridium botulinum types C and D (Onderstepoort Biological Products SOC Ltd; batch 472) as separate vaccinations on Day 0 and repeated on Day 28. All vaccines were administered intramuscularly in the neck using a 0.8 x 38 mm needle.

2.4. Serum-virus microneutralisation test

The serum neutralising antibody titre of individual foal sera to each of the nine reference strains of AHSV [4;5] was determined by the serum neutralisation test (SNT<sup>8</sup>) as previously described [22]. Briefly, each serum sample was inactivated at 56 °C for 30 min and serum dilutions were made in

---

<sup>8</sup> serum neutralisation test
Minimum Essential Medium (Highveld Biological, Modderfontein, Gauteng, South Africa) with 2 g/l NaHCO₃ (Merck, Wadeville, Gauteng, South Africa), 0.05 mg/ml gentamycin sulphate (Genta 50, Virbac Animal Health, Centurion, Gauteng, South Africa), and 5% foetal calf serum (Sigma-Aldrich, Johannesburg, Gauteng, South Africa). An estimated 100 TCID₅₀ of cell-culture-adapted prototype strains of each AHSV serotype (courtesy of the Agricultural Research Council’s Onderstepoort Veterinary Institute (ARC-OVI), Gauteng, South Africa) were added to duplicate serial 2-fold serum dilutions (1:10 to 1:320), and plates were incubated for one hour at 37 °C prior to addition of a suspension of Vero (CCL81) (African green monkey kidney) cells containing an estimated 4.8 x 10⁵ cells/ml. The development of any cytopathic effect was monitored daily for 4 to 5 days. Titres were determined as the reciprocal of the highest serum dilution that provided >50% protection of the cell monolayers. Titres of ≥10 were considered as positive.

2.5. Statistical analyses

Data was captured in Microsoft Access® database and statistical analysis was performed with Microsoft Excel® (Microsoft) and SigmaPlot® (Systat Software) software packages. The SNT titre distributions to the nine AHSV serotypes were statistically described with box-and-whisker plots. The median titre for all foals within each of the treatment groups was used to describe the antibody kinetics to individual serotypes of AHSV and the Wilcoxon rank-sum test was used to compare the groups. A p value <0.05 was considered significant.

3. Results

3.1. Treatment group I (n=16)

Foals were vaccinated with polyvalent MLV AHSV serotypes 1, 3, 4 (bottle 1) and serotypes 2, 6, 7, 8 (bottle 2) on Day 0 and 28 respectively. By day 56, 11 foals had seroconverted (SNT titres > 10) to AHSV serotype 1 with a median titre for the group of 120 (range for positive foals 40–320), 5 foals seroconverted to serotype 2 (group median <10, range for positive foals 10–56), 8 foals seroconverted to serotype 3 (group median <10, range for positive foals 10–320), 7 foals seroconverted to serotype 7 (group median <10, range for positive foals 10–80) and none of the foals seroconverted to AHSV serotypes 4, 5, 6, 8 and 9. Two foals were removed from the cohort at this
time for reasons unrelated to the study. The remaining 14 foals were re-vaccinated with the same vaccine (and in the same order) on Day 112 (bottle 1) and Day 168 (bottle 2).

Twenty-eight days after the last vaccination (Day 196) 13/14 foals were seropositive to AHSV serotype 1 with a median titre for the group of 320 (range for positive foals 10–320), 9 foals were seropositive to serotype 2 (group median =12, range for positive foals 10–160), 9 foals were seropositive to serotype 3 (group median =24, range for positive foals 10–80), 4 foals were seropositive to serotype 4 (group median <10, range for positive foals 10–224), 1 foal was seropositive to serotype 5 (titre 80), 10 foals were seropositive to serotype 6 (group median =14, range for positive foals 14–160), 9 foals were seropositive to serotype 7 (group median =14, range for positive foals 14–56), 4 foals were seropositive to serotype 8 (group median <10, range for positive foals 20–320) and 8 foals were seropositive to serotype 9 (group median =10, range for positive foals 10–80).

Forty-two days later (Day 238) 12/14 foals were seropositive to serotype 1 with a median titre for the group of 160 (range for positive foals 10–320), 4 foals were seropositive to serotype 2 (group median <10, range for positive foals 10–56), 9 foals were seropositive to serotype 3 (group median =20, range for positive foals 10–56), 3 foals were seropositive to serotype 4 (group median <10, range for positive foals 20–320), all foals were seropositive to serotype 5, 6 foals were seropositive to serotype 6 (group median <10, range for positive foals 10–56), 4 foals were seropositive to serotype 7 (group median <10, range for positive foals 10–28), 3 foals were seropositive to serotype 8 (group median <10, range for positive foals 14–224) and 2 foals were seropositive to serotype 9 (group median <10, range for positive foals 10–14) (Figure 1).

A single foal in Group I developed an SNT titre (10) to AHSV-5 only at Day 112, however this same foal had a titre of 226 to AHSV-8 at the same time. Similarly, although the foals were all seronegative to AHSV-9 at Day 112, 8/14 foals were seropositive by Day 196 when 10/14 were also seropositive to AHSV-6.
Figure 1: Box-and-whisker plots describing the SNT titre distributions of seropositive foals in Group I to the 9 AHSV serotypes after vaccination with the polyvalent MLV AHSV vaccine (Onderstepoort Biological Products SOC, Ltd, Onderstepoort, RSA). The dotted line represents the mean titres. (a) Represents the titres on days 56 and 112 after first vaccination with “bottle 1” and “bottle 2” including, respectively, serotypes 1, 3, 4 and 2, 6, 7, 8. (b) Represents the titres after re-immunisation with the same vaccine preparations used in (a). The time-line for the various vaccinations and the respective vaccine serotypes used are presented below the graphs.

Figure 2a represents the cumulative number of foals that were seropositive (SNT titres >10) at days 56, 112, 196 and 238 after immunisation with the polyvalent MLV AHSV vaccine, whereas Figure 2b depicts the percentage of additional seroconversions (titre ≥10) on Day 238, 70 days after the second round of vaccination. Ten and 8 foals respectively first seroconverted to AHSV serotypes 6 and 9 after the second round of vaccination, when, depending on serotype, either 3 or 4 foals first seroconverted to AHSV serotypes 1, 2, 4 and 8. Only 2 foals seroconverted after the second vaccination for each of AHSV serotypes 3 and 7, and no additional foals seroconverted to AHSV-5.
Figure 2: Serological response of foals in Group I following re-immunisation with AHSV serotypes 1, 3, 4 (bottle 1 at Day 112) and AHSV serotypes 2, 6, 7, 8 (bottle 2 at Day 168). Figure (a) represents the cumulative number of foals that seroconverted (SNT titres ≥ 10) at days 56, 112, 196 and 238 after immunisation. Figure (b) represents the percentage of foals that seroconverted 70 days (Day 238) after the second vaccination (re-immunisation)

3.2. Treatment group II (n=15)

Foals were vaccinated with monovalent MLV AHSV serotype 1 and serotype 8 on Day 0 and 28 respectively. By Day 56, 11/15 foals had seroconverted to AHSV serotype 1 with a median titre of 160 (range for positive foals 40–320), 5 foals seroconverted to serotype 2 (group median <10, range for positive foals 10–112), 1 foal seroconverted to serotype 8 (titre of 20), and all the foals remained seronegative (titres <10) to serotypes 3, 4, 5, 6, 7 and 9. Two foals were removed from the cohort after Day 56 for reasons unrelated to the study. By Day 112, the median SNT titres to AHSV-1 increased to 224 with 9/13 foals seropositive (range for positive foals 112–320) and 6 foals were seropositive to AHSV-2 (group median <10, range 10–80). At this time, all foals were seronegative to AHSV serotypes 3–9 (Figure 3a).
**Figure 3:** Box-and-whisker plots that describe the distribution of SNT titres against the 9 serotypes of AHSV at 56 and 112 days after vaccination of foals in groups II and III with monovalent MLV AHSV serotypes. Figure (a) shows titres of foals in Group II after vaccination with monovalent MLV AHSV-1 (Day 0) and serotype 8 (Day 28), and figure (b) shows titres of foals in Group III after vaccination with monovalent MLV AHSV4 (Day 0) and serotype 7 (Day 28). The dotted lines represent mean SNT titres. The time-line for the various vaccinations and the respective AHSV vaccine serotypes used are represented below the graphs.

### 3.3. Treatment group III (n=15)

The majority (12/15, 80%) foals vaccinated with monovalent MLV AHSV serotype 4 and serotype 7 on Day 0 and 28, respectively, remained seronegative to all 9 serotypes of AHSV at Day 56 (Figure 3b). One foal was removed from the cohort after Day 56 for reasons unrelated to the study. One foal seroconverted to AHSV serotypes 2, 4 and 8 (titres of 10, 320 and 28 respectively), another foal seroconverted to AHSV serotypes 1, 2, 3, 7 (titres of 160, 10, 20 and 14 respectively), and a third foal seroconverted to only AHSV-4 (titre 14).
3.4 SNT titres of foals that received polyvalent vaccine (n=16) compared to those that received monovalent vaccines (n=30)

There were no significant differences between SNT titres on Day 56 to AHSV-1 (p=0.852), AHSV-4 (p=0.150) and AHSV-8 (p=0.333) respectively, when comparing foals that received the polyvalent MLV vaccine to those that received the monovalent MLV components. Foals that received the polyvalent vaccine had a significantly greater response to AHSV-7 (p=0.021) than those that received serotype 7 as a monovalent MLV vaccine.

4. Discussion

The objective of this study was to compare the neutralising antibody responses of foals to each of the nine serotypes of AHSV following vaccination with a commercial polyvalent MLV AHS vaccine with that to immunization with selected individual AHSV serotypes contained in the polyvalent MLV preparation. There was a marked and consistent difference in the immunogenicity of individual virus serotypes contained in the vaccine, thus foals more consistently seroconverted to AHSV-1 than any other virus serotype. Responses to other serotypes were highly variable, and often weak or not detected. Furthermore, the presence of neutralising antibody to individual AHSV serotypes was often transient, and quickly waned after vaccination. Of potential significance is the fact that a second round of vaccination generally did not increase (> 4-fold) the antibody titres persisting from the initial immunization, indicating that there was a poor anamnestic response to re-vaccination. However, some animals that were seronegative after initial immunization did transiently seroconvert after re-vaccination. Unpublished data by the manufacturer, (Smit TK Personal communication) confirm a similar pattern in that a detectable antibody response is not evident in all animals to all AHSV serotypes and increased antibody titres to most or all virus serotypes develop only after repeated vaccination.

To assess whether or not there was interference between the individual virus serotypes contained in the polyvalent MLV vaccine, we also immunized foals with individual MLV serotypes (those contained in bottles 1 and 2 of the commercial vaccine). These data confirm that the serotype-specific responses of foals that received the monovalent MLV vaccines were similar to those of foals that received the polyvalent preparation, with the notable exception of AHSV-7 where the foals that received the polyvalent vaccine had significantly higher titres than those receiving the monovalent
vaccine. This finding was somewhat paradoxical in that it could be speculated that without interference between viruses contained in the polyvalent preparation, monovalent vaccines would be expected to induce higher antibody titres. However, the results do confirm that there is no obviously enhanced immune response through the administration of a monovalent vaccine as opposed to the polyvalent vaccine. In fact, the polyvalent vaccine offers advantages in terms of production lead time, production cost, and ease of use for the consumer with less “resting time” required post vaccination.

Data from the current study are consistent with results of a recent study on passive transfer of AHSV neutralising antibody from repeatedly vaccinated mares to their foals [23], where neutralizing antibody responses to individual AHSV serotypes were highly variable, even after repeated immunisation of foals. Similar data were also reported by other investigators using the same MLV AHSV vaccine, however in contrast to the present study where AHSV-1 was most immunogenic, data from the prior study indicated that AHSV-4 was most immunogenic [17]. The possibility of vaccine batch variation was not investigated.

This and other studies also indicate variability in responses of individual foals/horses to AHSV vaccination. Although only a limited or poor neutralising antibody response is elicited by the polyvalent vaccine, which could suggest limited immunogenicity of some of the serotypes, horses might still be immunologically primed so that they could respond more quickly following field exposure to virulent AHSV. This is consistent with the recent identification of subclinical AHSV infection in previously vaccinated horses that became viraemic after natural infection but did not develop fulminant disease [24]. The major immune response after AHSV vaccination has been recently confirmed as a humoral response [25], but a significant CD8+ response was also described in horses vaccinated with MLV AHSV-4. This is consistent with the immune response of sheep to Bluetongue virus [26-28].

Studies were not conducted to assess the level of protection afforded to foals by the different MLV vaccine regimens against homologous challenge with virulent AHSV. Although a cut-off titre of ≥10 was assumed as evidence of seropositivity, the actual SNT titre required for protection of individual horses is likely to be variable. The manufacturer relies on a minimum titre of 16 as a protective dose, based on extensive internal testing and establishment of the minimum quality requirements of the vaccine. Thus, data from the current study would suggest that the polyvalent vaccine is unlikely to
provide strong protection against all AHSV serotypes, however, other potential correlates of protective immunity were not assessed in the immunized foals, notably cellular immune mechanisms.

It is uncertain why most virus strains contained in the MLV polyvalent AHSV vaccine were so poorly immunogenic, at least as assessed by their ability to induce seroconversion in naïve and previously vaccinated foals. Possible explanations could include: i) antigenic competition between the multiple serotypes included in the polyvalent MLV preparations, although data from foals immunized in this study with monovalent vaccines suggests this is not the case; ii) epitope masking by circulating antibody when a second vaccination is administered; iii) direct effects of AHSV on immune cells, or the effect on virus replication by vaccine-induced antiviral mediators such as interferon; and iv) possible attenuation of antigenic determinants of outer capsid proteins VP2 and VP5, or even scaffolding proteins such as VP7, that affect binding of neutralising antibodies. Existing antibody can certainly suppress or enhance markedly the antibody response of an individual to a specific antigen, notably when antigens are administered as physiological preparations that are linked to large particles like red blood cells [29;30]. We were also not able to determine to what extent, if any, the concurrent vaccination with equine influenza, tetanus and botulism influenced the immune response in the foals to AHSV.

MLV AHSV vaccines continue to play a vital role in the control of AHSV infection of horses in endemic regions and have been used successfully in incursional areas. Whilst the basis of the efficacy of these vaccines has not been fully described, the results of our studies emphasize the need for on-going studies to enhance the understanding and knowledge of the immune response to AHSV. Such information will contribute significantly to the development of more effective or new generation AHSV vaccines.

Conflict of interest

None declared

Acknowledgements

These studies were supported by funding from Racing South Africa (Pty) Ltd, The Thoroughbred Racing Trust and the Equine Research Centre, University of Pretoria. Vaccines used in this study
were provided by Onderstepoort Biological Products SOC Ltd. We thank Mr Craig Carey and staff of ARC-EN-CEIL Stud for making his foals and facilities available and for support during the vaccination and blood collection from foals.

References


