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Dynamics of African horse sickness virus nucleic acid and antibody in horses following immunization with a commercial polyvalent live attenuated vaccine

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ABSTRACT

African horse sickness (AHS) is a fatal disease of equids relevant to the global equine industry. Detection of AHS virus (AHSV) during outbreaks has become more rapid and efficient with the advent of group specific reverse transcriptase quantitative polymerase chain reaction (GS RT-qPCR) assays to detect AHSV nucleic acid. Use of GS RT-qPCR together with recently described type specific (TS RT-qPCR) assays cannot only expedite diagnosis of AHS but also facilitate further evaluation of the dynamics of AHSV infection in the equine host. A potential limitation to the application of these assays is that they detect viral nucleic acid originating from any AHS live attenuated vaccine (LAV), which is the vaccine type routinely administered to horses in South Africa. The aim of this study was to contrast the dynamics and duration of the RNAaemia to the serological responses of horses following immunization with a commercial polyvalent AHSV-LAV using GS and TS RT-qPCR assays and serum neutralisation tests. The results of the study showed extended RNAemia in vaccinated horses, and that more horses tested positive on GS RT-qPCR with lower Cq values after receiving the AHSV-LAV containing types 1, 3 and 4 prior to the vaccine containing types 2, 6, 7 and 8, rather than when the vaccine combinations were reversed. Furthermore, lower Cq values were obtained when vaccines were administered 4 weeks apart as compared with a longer interval or 12 weeks apart. These findings are of particular relevance in regions where AHSV-LAVs are used as the use of these vaccines may complicate the accurate interpretation of diagnostic testing results.

1. Introduction

African horse sickness (AHS) is an infectious, non-contagious, arthropod-borne disease of equids caused by African horse sickness virus (AHSV) (genus Orbivirus, family Reoviridae). The AHSV genome includes 10 segments of double-stranded RNA [1–3]. AHSV infection in horses results in mortality rates of up to 90% [4]. Foals born to immune mares acquire passive immunity by colostrum ingestion, and maternally derived antibody generally declines to undetectable levels by four to six months of age [5]. AHS is widespread in sub-Saharan Africa with all 9 types of AHSV occurring regularly in southern and eastern Africa.

Annual immunization with polyvalent live attenuated vaccine (LAV) is currently the mainstay for control in endemic areas of Africa. In South Africa, a commercial polyvalent AHSV-LAV is supplied by Onderstepoort Biological Products as two separate vials: Comb1 and Comb2, which are administered at least three weeks apart. Comb1 includes AHSV types 1, 3 and 4 (AHSV-1, 3, and 4). Comb2 includes AHSV-2, 6, 7 and 8. Whole genome sequences of strains included in these vaccines are published [6,7]. The current formulation, which does not include either AHSV-5 or 9, was introduced into use in 1994 [8]. Serological cross-reaction reportedly occurs between certain types: AHSV-1 with AHSV-2, AHSV-3 with AHSV-7, AHSV-6 with AHSV-9, AHSV-8 with AHSV-5, whereas AHSV-4 does not exhibit cross-reaction with other types. Different types are allocated to the two combinations based on these cross reactions [8,9]. Immune response of horses to immunization with AHSV-LAVs has been investigated [8,10,11], however the viral kinetics following immunization have not been characterized.

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The objectives of this study were to determine the occurrence and duration of RNAaemia detected by GS RT-qPCR following immunization with a commercial polyvalent AHSV-LAV of: (1) weanlings following primary immunization, (2) yearlings following secondary immunization, and (3) adult mares with multiple previous immunizations. Furthermore, dynamics of the various AHSV-LAV types following immunization were characterized using type-specific RT-qPCR (TS RT-qPCR) assays, which were also compared to type-specific serological responses as determined using serum neutralisation tests (SNT's).

2. Materials and methods

2.1. Study population

Thoroughbred weanlings ($n = 44$), 7 to 10 months old at the start of the study, and mares ($n = 22$) were used in this study. Horses were resident on a breeding farm within the AHS Controlled Area (CA) of the Western Cape Province of South Africa [12]. No cases of AHS had been detected within at least a 30 km radius of this farm since the inception of the AHS CA in 1997 up to and including the period of this study. The weanlings were subjected to AHSV group-specific indirect ELISA (iELISA) tests [13] in January and June 2012, prior to commencement of the study. Ethical approval for the sampling strategy and use of these animals was granted by the Animal Use and Care Committee of the University of Pretoria.

2.2. Study design

Weanling foals in this study were randomly assigned to four groups ($n = 11$ per group), and received primary immunizations (with either Comb1 [AHSV 1,3,4] or Comb2 [AHSV 2,6,7,8]) at either 4 or 12 week intervals with the AHSV-LAV according to the schedule detailed in Supplemental Fig. S1. The weanlings in groups I and II were also sampled as yearlings after their secondary immunizations, which occurred 16 weeks after initial immunization.

Samples were collected from mares ($n = 22$) that had previously received multiple AHSV-LAV immunizations. Mares were immunized with Comb1 initially and Comb2 4 weeks later.

Whole blood (EDTA) samples were collected on the day of primary immunization (week 0) from all weanlings and the brood mares, and then weekly as detailed in Supplemental Fig. S1. Serum samples were collected from weanlings on the day of primary immunization (week 0) and weeks 4, 8, 12 and 16 after initial immunization.

2.3. Nucleic acid detection

2.3.1. Group specific PCR

A GS RT-qPCR assay with defined diagnostic sensitivity and specificity was used to quantify AHSV RNAaemia of whole blood samples as previously described [14]. Samples were classified as AHSV positive if the normalised fluorescence for the AHSV assay exceeded a 0.1 threshold within 37 PCR cycles [14].

2.3.2. Type specific PCR

TS RT-qPCR assays were applied to samples that tested positive by the GS RT-qPCR with a Cq value < 33, as previously described [15]. Samples were classified as positive for a specific AHSV type if the normalised fluorescence for the specific TS RT-qPCR exceeded a 0.1 threshold within 40 PCR cycles.

The gene encoding VP2 of the AHSV-7 vaccine strain (AHSV-7Vacc) is truncated [7] and the primers and probe of the AHSV-7 TS RT-qPCR assay targets the deleted region [15]. Therefore, primers and a probe (Table 1) were designed to detect the truncated VP2 gene of the AHSV-7Vacc strain, as well as that of other field and laboratory strains of AHSV-7 available on Genbank. Sequences of the AHSV-7Vacc primers and probe were evaluated *in silico* to ensure there were no cross-reactions with other AHSV types.

2.3.3. AHSV-LAV virus detection

LAV viruses contained in Comb1 and Comb2 preparations were processed by extraction and GS RT-qPCR followed by TS RT-qPCR in the same manner as the EDTA blood samples.

2.4. Group and type specific serology

Group specific antibodies were detected using an iELISA previously described [13]. The iELISA was initially used in order to confirm a seronegative status at the start of the study by comparing paired sera collected in January and June as mentioned previously. Further serological processing was done assessing type specific serology.

Type specific antibodies were detected using SNT assays as previously described [5,10]. Antibody titres are recorded as the reciprocal of the highest final dilution of serum that provided at least 50% protection of the Vero cell monolayer. A titre > 10 indicated positive results for that AHSV type. A four-fold increase in paired sample titres or a change from seronegative to seropositive indicated seroconversion [16].

2.5. Statistical analysis

GS RT-qPCR median Cq value distributions for the different weanling groups over time were compared using the Wilcoxon rank-sum test. A $p\text{-value} < 0.05$ was considered significant. The number of foals that seroconverted to each of the 9 types on SNT was compared between the different weanling groups using a two-way ANOVA test in R.[1] A $p\text{-value} < 0.05$ was considered significant.

3. Results

3.1. Weanling foals

3.1.1. iELISA results

In January 2012, 32/44 (73%) of the weanlings were seropositive by iELISA. The ages of the positive weanlings ranged from 56 to 149 days. All weanlings were seronegative by June 2012, when the foals were all >6 months of age.

3.1.2. GS RT-qPCR results

Kinetics of RNAemia as detected by GS RT-qPCR in immunized weanlings are provided in Figs. 1 and 2 and Supplemental Tables S1–S4. Fig. 1 summarises the number of weanlings positive on GS RT-qPCR per week for each group. Fig. 2 depicts the Cq value distributions of GS RT-qPCR positive weanlings in each group. Group I had a minimum median Cq value of 29.3 at week 3 after vaccination with Comb1 (AHSV 1,3,4). This was lower than the minimum median Cq values of the other groups. In group I there was an individual weanling that was positive on GS RT-qPCR with a Cq value of 22.3 at week 4. This was also lower than the minimum Cq value of any of the weanlings in any other group.

[1] R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

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