Serological Evidence on the Role of the Onderstepoort Attenuated African Horse Sickness Vaccine by Induction of Antibody Responses to a Nine Serotype Containing CVRL Inactivated African Horse Sickness Vaccine

Wernery U[1*], Joseph S[1], Raghavan R[1], Spendrup S[2], and Dyer B[2]

[1]Central Veterinary Research Laboratory, P.O. Box 597, Dubai, UAE
[2]Kisima Farm, Nanyuki 10400, Kenya

Abstract: Although the frequency and severity of African horse sickness (AHS) outbreaks have declined significantly in Southern Africa, studies have shown that vaccinating horses only with the Onderstepoort attenuated vaccine did not always give full protection against AHS. The objective of this study was, therefore, to investigate whether an inactivated AHS vaccine containing all 9 serotypes would increase the serological response of horses that had been previously immunized with the Ondestepoort attenuated AHS vaccine. For this experiment, twenty-seven horses were selected from AHS endemic area in northern Kenya. The study horses were simultaneously vaccinated against all 9 AHS serotypes in 2 injections and their seroconversion was recorded. Sixteen horses, which were regularly immunized for many years with the Onderstepoort attenuated vaccine (Onderstepoort Biological Products, OBP) until 2013 and then annually 6 times until 2019 with the inactivated CVRL vaccine, developed high ELISA and virus neutralising antibodies (VN). Eleven horses which never received the OBP vaccine, but since 2015 six times the CVRL vaccine also developed ELISA and VN antibodies, which however were significantly lower than in the first group. The result of the current experiment confirmed the already known fact that multiple vaccinations against all 9 serotypes are important for the development of high antibody levels. Furthermore, it suggests the possible induction of higher levels of antibodies by the horses vaccinated with an inactivated vaccine which had been previously immunized with an attenuated vaccine compared to the ones which were not immunized with the latter vaccine. In conclusion, alternate immunization with an inactivated and an attenuated vaccine may provide better protection against AHS.

Keywords: *African horse sickness, CVRL inactivated vaccine, Horse, Onderstepoort attenuated vacine, Seroconversion*

Introduction

African horse sickness is an insect-borne viral disease of equids that is endemic to sub-Saharan African countries (Coetzer and Guthrie, 2004; Zientara, 2010). It is caused by the AHS virus (AHSV) of the genus *Orbivirus* in the family *Reoviridae*. *Culicoides* (*C.*) spp midges are the principal vectors of all 9 serotypes and *C. imicola* is the most important midge for the AHSV transmission (Guthrie and Ouan, 2009). There is no treatment for AHS available, but prevention can be achieved by vector control and vaccination. Vaccination is the most effective way of bringing the disease under control and vaccination was used successfully during the Spain outbreak between 1987 and 1991 (Rodriguez *et al*., 1992). First attempts to control AHS by vaccination dates back to the beginning of the last century by using commercially live, attenuated vaccines even until today, which provide strong humoral and cell immunity. Neutralising antibodies reflect the horses' immunity (van Dijk, 1999) and protection against AHS is serotype specific, which means that horses must be immune to all 9 serotypes. However, to ensure a polyvalent immunity against all 9 serotypes, horses need at least 3 to 4 annual vaccinations (van Dijk, 1999; Zientara *et al*., 2015).

There is increasing concern regarding the use of attenuated vaccines because of their potential reversion to virulence by re-assortment of their gene segments with other vaccine and field serotypes, which were reported by Weyer (2016) and Weyer *et al.* (2016). Similar drawbacks of attenuated *Orbivirus* vaccines are known from attenuated bluetongue (BT) vaccines, which even may cause abortion and congenital malformations when pregnant ewes are vaccinated. It was also discussed that clinical signs may be caused in some sheep breeds by the vaccine virus itself with a viraemia in the vaccinates. These vaccine viruses may then consequently be transmitted in the field by midges meeting field strains and then reassort to produce new virus strains. Consequently, the widespread use of such attenuated vaccines against BT was not recommended and the recent BTV-8 outbreak in Europe was controlled using inactivated vaccines (European Medicines Agency, 2009; Lefèvre *et al*., 2010).

The aim of this vaccination experiment was to evaluate the serological response of 27 Kenyan horses from the field with a different vaccination history. This study was conducted in Laikipia area, north of Nairobi where AHS is endemic and where vaccinations are therefore regularly performed.

*Corresponding Author. E-mail: cvrl@cvrl.ae

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Materials and Methods

Horses

Twenty-seven horses which were kept in the Laikipia area of Kenya were selected for this AHS vaccination experiment. During the day all horses were grazing together in open undulating Savannah and during the night they were kept near a homestead in open fenced areas under acacia trees. The 27 horses belonged to a group of 50 horses from a single owner who regularly use them for tourist purposes. From these 50 horses, a clear vaccination history was available for 27 horses, so they were selected for this trial. Sixteen of these 27 horses (1-16) had been regularly vaccinated with the OBP vaccine, until 2013. Thereafter, until 2019, these 16 horses received only the inactivated CVRL vaccine as an annual vaccination, every October of each year, being a total of 6 times. The remaining 11 horses (17-27) never received the OBP vaccine, and only the CVRL vaccine, again 6 times every October of each year between 2014 to 2019.

Vaccines

Over a period of 17 years all 9 AHS serotypes were isolated at Central Veterinary Research Laboratory from horse fatalities in Kenya. These serotypes were chemically inactivated by BEI and formalin, ultra-filtrated and used for this vaccine experiment which is described by Wernery *et al.* (2016). The inactivated vaccine is prepared in 2 injections which are simultaneously and subcutaneously (sc) injected into the left and right side of the horses' necks. Shot 1 contains serotypes 1,4,7,8,9 and shot two 2,3,5,6.

Serology

Four weeks after the last annual booster in October 2019, blood was withdrawn from the jugular vein of all 27 horses and tested for AHS antibodies with the competitive ELISA and the virus neutralization test (VNT) which are both described in details by the OIE (2018). Serum samples showing blocking percentage (Percentage Inhibition, PI) values lower than 45% were considered negative, whereas samples above 50% were considered positive. If the ELISA value ranges between 45% and 50%, the result is inconclusive and the test has to be repeated (OIE, 2018). Serotype specific antibodies which were tested with the VNT were according to the Spearman-Karber method expressed as negative $\log_{10}$. A similar study was performed by Lelli *et al.* (2013) who elicited a neutralizing antibody response of an inactivated AHS V9 vaccine in guinea pigs and horses and by Molini *et al.* (2015) who evaluated the serological response with the VNT of OBP vaccinated horses in Namibia. The difference between the mean results of both groups was calculated with the T test.

Results

Results of vaccination by OBP and CVRL vaccines on the induction of antibodies in horses are shown in Tables 1 and 2. Group 1 consisting of 16 horses (1-16) received the OBP vaccine until 2013 and then annually the CVRL vaccine 6 times until 2019. Horses had developed high ELISA and VN antibodies, also against serotypes 5 and 9 which were not in the OBP vaccine. The mean ELISA antibody result of these horses was 93 Percentage Inhibition (PI), whereas the mean VN antibody result were between 3 and 4 $\log_{10}$.

Table 1. ELISA and VN antibodies of 16 Kenyan horses vaccinated until 2013 with the attenuated OBP vaccine and 6 times until 2019 with the inactivated CVRL AHS vaccine containing all 9 serotypes

Sl. No.	Name	ELISA* Jul-19	VNT** (Serotypes)								
			1	2	3	4	5	6	7	8	9
1	Bali	94	2.5	2.5	4.75	4.25	2.3	3	3.75	2.5	5.5
2	Bilisa	94	3.25	2.75	3	3.5	3	3.3	3	4	3.5
3	Boo	94	3.5	3.5	4.25	3.75	3.5	3.3	2.5	4	4
4	Comanche	93	3.75	3.75	3.25	3.5	2.5	3.8	3	3	3.5
5	Diane	93	2.5	3	4	3.25	3	3	2.75	3.8	3.75
6	Joker	93	2.5	2.5	4	5	3	3.8	3.75	3	4.5
7	Kalahari	93	4	3.5	4	4	3.8	3.8	3	4	4
8	Punches Town	94	4	3.75	4	2	3	2	3	4	5
9	Starlight	93	3	3.25	3.25	3.75	2.8	3	1.75	3.8	2.75
10	Tiva	93	2	3	4.5	4.5	3.3	3.8	3.5	3.3	5.25
11	Masala	94	3.25	2.5	4.75	4.75	3	3	3	4	4.5
12	Suerte	93	2.25	2.75	4.5	4	4	4.3	3.75	3.3	6
13	Marion	94	2.5	2.75	4	4.5	2.3	3.8	3	2.5	4.25
14	Sandpiper	91	2	2.25	3.75	3.75	3	3.3	3.5	2.3	5
15	Picassa	93	4.25	3.75	4.25	5.5	3.5	3.8	4	4	5
16	Douglas	93	2	2.25	3	2.5	2.3	2.3	4.5	2.3	5.25
	Mean	**93**	**3**	**3**	**4**	**4**	**3**	**3**	**3**	**3**	**4**

** ELISA is expressed as Percentage Inhibition (PI%) and cut-off value for ELISA ≥ 50% are shown in green colour; ** VNT results are expressed in log10 and titres ≥ 1 are shown in yellow colour.*

Horses in group 2 comprising of 11 horses (17-27) which had received only the CVRL vaccine 6 times until 2019 also developed ELISA and VN antibodies which were, however significantly lower. The difference between the mean of both groups shown in Tables 1 and 2 was statistically significant with a P value of 0.00026.

Table 2. ELISA and VN antibodies of 11 Kenyan horses vaccinated 6 times with the inactivated CVRL AHS vaccine containing all 9 serotypes

Sl. No.	Name	ELISA* Jul-19	VNT** (Serotypes)								
			1	2	3	4	5	6	7	8	9
17	Scorpio	90	2.25	2.25	2	2	1.8	2.75	0.25	2	3.25
18	Piper	75	2.25	2.75	1.75	2	1.8	3	1.25	3	2.25
19	Hidalgo	93	3.25	2.75	4	3.5	2.5	3.25	1.5	3.75	4
20	Hubblebubble	80	2.25	2.75	2	5.5	3.5	2.5	1.5	2	2.5
21	Chelsea	91	1.25	0.25	2.5	2	2.3	2.25	0.25	2	5.25
22	Kerry	89	2	2	2	2	2.3	2.25	0.25	1.25	3.75
23	Alison	62	2.25	3.5	2.5	2.25	1.8	2.25	1	3	2
24	Suni	87	2.25	2.75	2.25	2	1.3	2	2	3	1.75
25	Kibiriti	90	1	0.5	2.75	2	2.3	2.25	0.25	1.5	3.5
26	Makaa	92	2	2	2.25	3.5	1.8	2.25	1	2.75	2
27	Toby	88	1.5	1.25	2.5	3	2.3	2.5	0.25	1.75	3.25
	Mean	**85**	**2**	**2**	**2**	**3**	**2**	**2**	**1**	**2**	**3**

** ELISA is expressed as Percentage Inhibition (PI%) and cut-off value for ELISA ≥ 50% are shown in green colour; ** VNT results are expressed in log10 and titres ≥ 1 are shown in yellow colour.*

Discussion

Inactivated vaccines for the protection of horses from AHS were previously successfully used to control an AHS serotype 9 outbreak in Spain (Lubroth, 1988) and in a vaccination and challenge experiment with serotype 4 by House *et al.* (1992). However, to the knowledge of the authors, so far no vaccination experiment had been conducted with an inactivated vaccine containing all 9 serotypes in 2 injections. Vaccination trials are currently carried out in Dubai with an inactivated vaccine containing all 9 AHS serotypes in one injection.

The results of this study show that multiple vaccinations against all 9 serotypes are essential for the development of high ELISA and VN antibodies. Horses showing high VN antibodies between 1 and 2 $\log_{10}$ and above seem to be immune against an AHS infection (Lelli *et al.*, 2013), but the absence of detectable VN antibodies to one or more serotypes may not always necessarily be suggestive of lack of protection against AHS, as these animals might appear to be resistant to a challenge which also may depend on a cell mediated immunity (Hamblin *et al.*, 1991).

Eleven horses in the second group which did not receive the OBP vaccine, but 6 times the inactivated CVRL vaccine like the first group developed significantly lower ELISA and VN titres. This seems to indicate that it is an advantage to vaccinate horses with inactivated AHS vaccines which had been immunized with an attenuated vaccine.

Conclusion

Multiple vaccinations against all 9 AHS serotypes are essential for the development of high and protective antibodies against AHS. It may be an advantage to immunize horses with both, the attenuated OBP and the CVRL inactivated vaccines alternately, as this combination induces significantly higher level of antibody production compared to a single vaccination, confirmed by serological evidence.

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Conflict of Interests

The authors declare that they have no competing interests.

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