Recommendations for African horse sickness vaccines for use in nonendemic areas

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HOUSE (J.A.). Recommandations pour l'utilisation de vaccins contre la peste équine dans des régions non endémiques. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 77-81

La peste équine (PE) est causée par des orbivirus et transmise par des *Culicoides*; elle détermine une mortalité jusqu'à 95 p. 100. Le but d'un programme de lutte et d'éradication est d'empêcher la propagation du virus par le vecteur biologique. Les mesures de lutte comprennent l'abattage des animaux infectés, la mise en étable étanche aux insectes des animaux suspects d'infection, et la vaccination. La vaccination a joué un rôle clef dans l'éradication lorsque la PE est apparue en dehors de l'Afrique. Des vaccins vivants modifiés ainsi que des vaccins inactivés ont été utilisés pour la lutte contre la PE. Pour être acceptable un vaccin doit être : sans danger, efficace et disponible. Le vaccin ne doit causer ni maladie, ni virémie, et le virus vaccinal ne doit pas redevenir virulent lors de passages sur des équidés sensibles. Il doit protéger contre la mort et contre les signes cliniques et, très important, doit prévenir une virémie chez les équidés vaccinés exposés au virus virulent. La méthode pour éprouver l'immunité à la PE par inoculation d'un virus virulent est commentée. Le vaccin doit être facilement disponible, soit par une production régulière dans des installations répondant aux normes internationales, soit dans une banque de vaccin. Des banques de stocks de vaccins vivants modifiés ou de vaccins inactivés concentrés permettent de disposer d'un vaccin contre la PE lors d'épizooties futures. Un test diagnostique a été développé récemment pour distinguer les animaux vaccinés d'animaux infectés naturellement, et fournit de l'information utile aux services officiels pour le contrôle de la PE.

Mots-clés : Peste équine - Contrôle de maladies - Vaccin inactivé - Vaccin vivant modifié - *Culicoides* - Vecteur de maladie - Abattage d'animaux.

THE DISEASE

African horse sickness (AHS) is an arthropod-borne, noncontagious, viral disease of Equidae that causes high mortality in horses, with decreasing mortality in mules, donkeys, and zebras. There are nine serotypes of AHS virus (AHSV) which form a subgroup in the genus *Orbivirus* in the family Reoviridae. African horse sickness is endemic in subsaharan Africa, but the disease has occurred outside of Africa on several occasions. In infected animals, AHSV develops to greatest concentrations in the spleen, lungs, and lymph nodes. Pathological changes in the lungs, heart, and blood vessels account for the clinical and necropsy findings. There are classically four clinical forms of AHS :

- The pulmonary or acute form has a clinical course of 5 to 7 days. Mortality can reach 95 %. Affected animals show rapid, distressed respiration within a few hours of death and die by literally drowning in their own fluids. Necropsy lesions include severe pulmonary edema and hydrothorax.

- The cardiac or subacute form has a course of 5 to 15 days. Mortality ranges from 50 to 90 % and affected animals may have edema of the supraorbital fossa (considered pathognomonic) and edema of the eyelids, conjunctiva, and/or subcutaneous tissues. Lesions observed at necropsy include edema of subcutaneous tissues, edema along the ligamentum nuchae, edema of the intermuscular fascia (particularly in the neck), hydropericardium, and occasional necrosis of the myocardium, especially of the papillary muscles.

- The mixed form, the most common form of the disease, is characterized by combination of the clinical signs and pathological lesions of the pulmonary and cardiac forms. Mortality ranges from 50 to 90 %.

- The horse sickness fever form is a mild form of the disease. By definition, animals recover from this form of the disease following a febrile period of approximately 5 days.

The prevalence of the disease is dependent on the competence and concentration of the vectors. *Culicoides* have been shown to be the most significant biological vectors in nature, but mosquitoes have biologically transmitted the disease under experimental conditions. Control measures include slaughter of infected animals, vaccination, and housing animals suspected to be infected in insect-proof stalls.

REVIEW OF VACCINES

The first vaccines developed for AHS were modified live vaccines (MLV) of adult mouse brain (AMB) origin. These vaccines have been and are used extensively throughout Africa. They were used to help control the AHS pandemic in the Middle East during 1958-63. However, during that outbreak, vaccine related cases of encephalitis in donkeys and horses were reported in Israel and India (11,

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13). The AMB vaccines caused encephalitis in guinea pigs inoculated intranasally (3). Recently, the AMB vaccines have been associated with encephalitis and chorioretinitis in production workers exposed to aerosols of these vaccine viruses (14). The AMB original vaccine for AHSV serotype 4 (AHSV-4) had poor immunogenicity and was eliminated from more recent polyvalent AHS AMB vaccines (4). Due to the outbreak of AHS in Spain, we performed studies to evaluate the safety and efficacy of the AMB AHS vaccine seeds that were available at the Foreign Animal Disease Diagnostic Laboratory. These seeds were tested because diplomatic relations with South Africa were unfortunately not favorable, making the availability of more current vaccines questionable. There was a spectrum of response to the AHS AMB vaccines ranging from a failure to induce any antibody to induction of solid protective immunity (table I). It is especially interesting to note that ponies inoculated with vaccines for serotypes 6 and 7 did not develop any neutralizing antibody after vaccination. Following challenge inoculation, these ponies developed a primary response and survived challenge inoculation. This may indicate that cell-mediated immunity plays a role in protection from AHS. It is also of interest that these ponies had a long febrile period after vaccination (7 to 14 days). Although data on viremias following vaccination are not available, it is likely that there would be enough virus present to infect vectors. Such viremic Equidae infected simultaneously with a second strain or serotype of AHSV could foster genomic reassortment.

TABLE I Responses of ponies vaccinated with AHS AMB vaccines.[a]

Vaccine by serotype number (#ponies)	Days of fever following vaccination	Virus neutralizing antibody after vaccination	Immune response after challenge	Result of challenge
1(1)	3	No	Primary	Died 26 DPC[b]
4(1)	2			Died 21 DPC
5(2)	4, 7			Died 9 DPC
6(2)	7, 12	No	Primary	Survived
7(1)	14			
2(1)	2	No	Anamnestic	Survived
8(1)	3			
3(1)	3	Yes	None	Survived
9(1)	9			

[a] *Information is extracted from reference 6.*
[b] *Days post challenge.*

In 1978, ERASMUS reported the development of cell culture origin MLV vaccines for AHS derived from nonvirulent large plaque variants of AHSV (4). Currently, vaccines for 8 of the 9 serotypes of AHSV are produced by this procedure and used in South Africa. The AHSV-6 vaccine is considered to induce adequate cross-protective immunity to AHSV-9, so no vaccine for AHSV-9 is used. The vaccines are administered as 2 quadravalent vaccines consisting of serotypes 1, 3, 4, and 5 and 2, 6, 7, and 8. A monovalent vaccine for AHSV-4 was produced at the Onderstepoort facility for use in the Spanish outbreaks of AHS 1987 through 1990. Information on the safety and efficacy of these vaccines is not available from the literature.

Following a request from Spanish veterinary officials, we inoculated 3 ponies with the South African monovalent AHSV-4 MLV vaccine (5). None of the 3 ponies had any clinical reaction to the vaccine; and, as noted in table II, following challenge inoculation, none developed any clinical sign attributable to AHS. The virus detected in the blood of pony 2 following intravenous challenge inoculation with AHSV-4 Spain 87 is likely residual challenge virus from the intravenous inoculation of challenge virus. Pony 3 had a prolonged and substantial viremia ; the levels (up to $10^{4.8}$ $TCID_{50}$/ml blood) would likely be adequate to infect insect vectors. It is estimated that a viremia of 10^4 infectious particles/ml blood is adequate to infect a significant proportion of vectors with bluetongue virus (10). Since the number of ponies examined was small, it is not possible to predict the incidence of this viremia under field conditions.

TABLE II Viremia in ponies vaccinated with AHS 4 MLV vaccine (tissue culture origin) following challenge inoculation[a].

Pony identity	Viremia following challenge inoculation
1	None
2	$10^{2.8}$ $TCID_{50}$ per ml of blood on day 3
3	$10^{3.1}$ to $10^{4.8}$ $TCID_{50}$ per ml of blood from days 3 to 11

[a] *Information is extracted from reference 5.*

Backpassage information on commercially produced AHS MLVs is not available. A small plaque AHSV-4, isolated from wild AHSV, did not cause clinical signs of AHS when 10^8 $TCID_{50}$ were inoculated intravenously in 4 ponies (5). No virus was isolated from blood samples collected daily following inoculation. The inoculation induced antibody in all 4 vaccinated ponies and protective immunity in the 2 ponies that were challenge inoculated with AHSV-4 Spain 87. In a backpassage study, daily heparinized blood samples from 2

ponies inoculated with the small plaque virus were respectively pooled; and each pool was inoculated into 1 pony. One of the backpassage ponies died of AHS 35 days after inoculation with the pooled blood. It is difficult to extrapolate the implications of this study to field conditions since the likelihood of vectors becoming infected from an animal without a detectable viremia is quite remote. However, the apparent return to virulence of the backpassaged virus raises a valid question about the stability and safety of MLV AHS vaccine viruses.

Recently, an inactivated AHSV-4 vaccine was commercially produced. Information on the production method and efficacy was reported by DUBOURGET (2). This vaccine has not had extensive use under field conditions to date. Laboratory studies were conducted with one and two doses of vaccine (7,8). Table III summarizes the responses of vaccinated ponies. After challenge inoculation viremia occurred in one vaccinate that received one dose of vaccine. The virus level in the blood was 10^3 infectious particles per ml of blood. This was about 10^1 $TCID_{50}$ below the level considered to be the threshold level (10^4 infectious particles/ml blood) estimated to infect a significant number of *Culicoides* with bluetongue viruses (10).

CONSIDERATIONS FOR FUTURE GUIDELINES FOR AHS VACCINE PRODUCTION

An acceptable vaccine should be : pure, safe, potent, efficacious and available. This paper will address all of these but purity (freedom from contaminants). Guidelines and procedures for AHS cell culture origin MLVs have been presented by the Office International des Epizooties (OIE) (12).

There are several areas to consider for future guidelines for AHS vaccine development, production, and testing. A more complete set of requirements for vaccine production and control could be based upon the master seed principle for virus stocks and cell cultures (1). In addition, good manufacturing procedures for containment and prevention of cross contamination of products could be included (1). However, the development and implementation of these guidelines and test systems would require a considerable investment. It is unlikely that commercial firms would spend the funds to validate such tests because of the small market share of these vaccines in nonendemic areas.

Information on backpassage for the tissue culture-derived MLVs is not available. Backpassage information on MLVs is useful to regulatory officials deciding on the use of vaccines in non-endemic areas. The febrile response of ponies following vaccination with some of the AHS AMB vaccines is of concern because it indicates that these vaccines may produce notable viremias. The lack of clinical response and of detectable viremia following vaccination with cell culture MLVs makes it unlikely that backpassaging via vector infection would occur under field conditions. However, it would be useful to determine whether the vaccine viruses reverted to virulence under controlled experimental backpassage conditions.

Following challenge inoculation, an ideal vaccine for arthropod-borne diseases should prevent a viremia capable of significant vector infection. For AHS, this thre-

TABLE III Responses of ponies vaccinated with commercial inactivated African horse sickness vaccine[a].

Pony response	1 Dose regime[b]		2 Dose regime[c]	
	Vaccinates	Controls	Vaccinates	Controls
Immune response after vaccination	8/9	NA[d]	5/5	NA
Anamnestic response after challenge inoculation	7/9	NA	0/5	NA
Viremia $\geq 10^{2.5}$ MLD_{50} per ml of blood following challenge inoculation	1/9	3/3	0/5	2/2
Clinical signs from challenge inoculation	3/9	3/3	0/5	2/2
Fever from challenge inoculation	3/9	3/3	0/5	2/2
Survived challenge inoculation	9/9	0/3	5/5	0/2

[a] *Equipest™ Rhône Mérieux, Lyon, France.*
[b] *Information is extracted from reference 8.*
[c] *Information is extracted from reference 7.*
[d] *= Not applicable.*
[e] *= Mouse lethal doses 50%.*

shold value has yet to be determined and promises to be difficult to estimate. Work with wild vectors is extremely complex ; colonized insects may provide a means of estimating the level.

The experimental challenge system for arthropod-borne viruses is often not representative of a natural challenge. An intravenous challenge is usually given for AHS, while the natural route of infection is by superficial bites of *Culicoides*. The subcutaneous route of inoculation of challenge virus results in a 2 to 3 day longer incubation before clinical signs appear. Information on the intradermal route of inoculation of AHSV is not available even though this route is closest to the natural route of infection. Regarding the source of the virus, challenge using infected *Culicoides* is not feasible on a routine basis due to the expense of maintaining infected *Culicoides*. As well, there would likely be a wide range of variability in the percentage of insects infected and in the actual dosage of challenge virus administered. One advantage of the *Culicoides* propagated virus that cannot be duplicated by virus produced in cell culture or suckling mouse brain virus, is that it probably represents naturally occurring populations of virus. Challenge virus derived from the blood of viremic horses may also closely resemble populations of virus occurring in nature. Viremic horse blood is preferable for challenge to viruses derived from cell culture or suckling mouse brain as these may represent selected populations of AHSV. Unfortunately, tissue culture or suckling mouse brain viruses are the most easily obtained and the most commonly used challenge viruses.

The master seed principle for vaccine production (1) centers around the development of master seed stocks for production such as virus, bacterial or cell culture master seeds. Once certified for production use, these seeds represent the starting point for production of vaccines. The highest certified passage from the master seed represents the highest passage that can be used for production. Generally for MLV vaccines, the immunogenic potential of a vaccine is determined by performing an immunogenicity test to certify the minimal immunizing dose of vaccine virus able to protect 19 of 20 vaccinated animals following exposure to challenge virus. To release a vaccine for distribution, the vaccine must have adequate virus to account for losses during shipment and storage. The "release titer" (that accounts for potency) for a vaccine virus is normally determined by adding $10^{1.5}$ to $10^{2.0}$ $TCID_{50}$ of virus to the protective dose validated in the immunogenicity test. The release titer for AHS MLVs, based upon the master seed principle has not been determined, although a recommended release titer for tissue culture MLVs is stated as 10^5 plaque forming units of virus per dose (12).

Currently, there is no standardized laboratory potency test for inactivated AHS vaccines. A sucrose gradient method of estimating the amount of AHSV particles, similar to that used for the estimation of intact viral particles in foot-and-mouth disease vaccines, has been used (2). Inoculation of laboratory animals (mice or guinea pigs) followed by an assessment of the serological response is a potential biological assay. These potency tests would have to be correlated with immunity induced in vaccinated Equidae.

African horse sickness vaccine must be available for emergency use; and it is preferable that it be produced in a laboratory operating under international standards. The OIE is striving to develop and improve standards for diagnostic techniques and vaccine production (12). International implementation of such standards could allow the use of vaccines in AHS free areas under emergency conditions. Standards for these laboratories are not yet accepted internationally, but as more diseases are controlled and eradicated, the need for these standards and facilities becomes increasingly apparent.

Cryopreservation of MLVs or inactivated vaccines in a vaccine bank provides previously safety and potency tested vaccines for rapid finishing and shipment. A commercial inactivated vaccine concentrate for AHS-4 is currently cryopreserved by Rhône Mérieux (Dr. M. LOMBARD, personal communication).

The ability to differentiate AHS vaccinated from naturally infected animals is important, particularly for officials conducting a control and eradication program. The commercial inactivated vaccine does not induce antibodies to nonstructural proteins 2 and 3 (NS2 and NS3). This characteristic allows a Western blotting procedure to be used to differentiate antibodies induced with inactivated vaccines from those induced with MLV vaccines or field virus (9).

CONCLUSIONS

Vaccines for protecting animals against AHS should be safe, efficacious, and available. Safety of MLV's can be shown by backpassage studies. Efficacy of vaccines can be demonstrated by protection against clinical disease but more importantly by reducing viremia in challenged animals to a point below that necessary to infect insect vectors. A means of continuous availability must be developed from internationally approved laboratories. A recently developed diagnostic test for AHS using Western blotting can differentiate animals vaccinated with a commercial inactivated AHS vaccine from ones naturally infected or given MLV vaccine. This provides regulatory officials with useful information for the control of AHS.

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HOUSE (J.A.). Recommendations for African horse sickness vaccines for use in nonendemic areas. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 77-81

African horse sickness (AHS), which causes mortality up to 95 %, is caused by orbiviruses and is transmitted by *Culicoides*. The goal of a control and eradication program for AHS is to prevent the spread of the virus via the biological vector. Control measures include slaughter of infected animals, housing of suspected infected animals in insect-proof stalls, and vaccination. Vaccination has played a key role in eradication when AHS occurred outside of Africa. Both modified live vaccines (MLV) and inactivated vaccines have been used to control AHS. An acceptable vaccine should be : safe, efficacious, and available. The vaccine should not cause disease or viremia, and the vaccine virus should not revert to a virulent virus upon backpassage in susceptible Equidae. The vaccine should protect against death and clinical signs and, most importantly, should prevent viremia in vaccinated Equidae following exposure to virulent AHS virus. The challenge inoculation system for assessing immunity to AHS is discussed. The vaccine should be readily available, implying that it is either in routine production in facilities that meet internationally accepted guidelines for biological production facilities or in a vaccine bank. Banking of cryopreserved stocks of MLV or concentrates of inactivated vaccines is a means of having AHS vaccine available for future epizootics. A recently developed diagnostic test to differentiate vaccinated from naturally infected animals provides regulatory officials with useful information for the control of AHS.

Key words : African horse sickness - Disease eradication - Inactivated vaccine - Modified live vaccine - *Culicoides* - Vector - Slaughtering.

HOUSE (J.A.). Recomendaciones para el uso de vacunas contra peste equina en zonas no endémicas. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 77-81

La peste equina (AHS) presenta tasas de mortalidad de hasta 95 %. Es producida por un orbivirus y transmitida por un *Culicoides*. La finalidad de toda campaña de erradicación y de control de AHS es la prevención de la dispersión del virus mediante el vector biológico. Las medidas de control incluyen el sacrificio, la estabulación de los animales sospechosos en establos adaptados contra la protección de insectos y la vacunación. La vacunación ha jugado un papel importante en la erradicación contra la peste equina, cuando ésta se ha presentado fuera del continente africano. Para el control se han utilizado tanto vacunas vivas (MLV), como inactivadas. La vacuna adecuada debe ser : segura, eficaz y disponible. No debe provocar ni la enfermedad, ni viremia y el virus vaccinal no debe adoptar una forma virulenta durante el pasaje por un equino susceptible. La vacuna debe proteger contra la muerte y los síntomas clínicos, pero sobre todo, debe prevenir la viremia en los equinos vacunados después de una exposición a la forma virulenta del virus del AHS. Se discute el método para probar el sistema de inoculación para asegurar la inmunidad. La vacuna debe ser facilmente accesible, lo cual implica que debe de encontrarse en los bancos de vacunas, o que las facilidades de producción rutinaria deben seguir las normas aceptadas a nivel internacional para la producción biológica. Una forma de asegurar la disponibilidad de la vacuna contra AHS, es la crio-conservación de stocks de MLV o de concentrados de vacunas inactivadas. El desarrollo reciente de una prueba diagnóstica que permite la diferenciación entre una forma de infección vaccinal de la forma natural, permite la determinación de patrones reguladores, de gran utilidad para el control de AHS.

Palabras claves : Peste equina - Erradicación de enfermedad - Vacuna inactivada - Vacuna viva - *Culicoides* - Vector - Sacrificio.