

## EDITORIAL

# African horse sickness in Thailand: Challenges of controlling an outbreak by vaccination

## 1 | A SHOCKING ALERT: AFRICAN HORSE SICKNESS REPORTED IN SOUTH-EAST ASIA

As the world continues to battle the COVID-19 pandemic and the pig farming sector is concerned with the relentless geographical spread of African Swine Fever, another serious veterinary epizootic, which has gone largely unnoticed by the general media, emerged in the early months of 2020. African horse sickness (AHS), perhaps the most feared equine infectious disease, appeared in South-East Asia and threatens the equine industry worldwide. Indeed, on 17 March 2020, the Thai veterinary authorities communicated to the OIE (World Organisation for Animal Health) a confirmed case of African horse sickness (AHS) in Pak Chong, Nakhon Ratchasima, Thailand.<sup>1,2</sup> The outbreak is thought to have started a few days earlier in February. Since then, despite the implementation of a range of control measures (including control of animal movements, use of insect netting, and vaccination with the live attenuated vaccine), the disease spread to other dispersed locations in the country. A total of 15 different outbreaks have been recorded so far, resulting in more than 500 horse deaths, bringing the mortality rates above 90%. This is the first ever outbreak of AHS in Thailand and the first occurrence of AHS in Asia in some 60 years. In the absence of AHS occurring in neighbouring countries in decades, the emergence of AHS thousands of miles away from endemic regions for the disease, it is likely that the Thai outbreak has its origins in the importation of AHS-infected animals.

Thailand is a non-endemic country for AHS and consequently its whole equine population is immunologically naive to the virus and therefore highly susceptible to the severe clinical manifestations of the disease. The hot and humid climate of Thailand, with the absence of cold winters that would interrupt the activity of *Culicoides spp* biting midges, the hematophagous insects that transmit African horse sickness virus (AHSV), make the continuous transmission of the disease in the region and its spread to other parts of Asia highly plausible. Some of these recent outbreaks occurred in provinces of Thailand that share borders with Myanmar, Cambodia, Malaysia and Laos, and if they are not brought under control, they will threaten the livelihoods of a large proportion of the human population of these countries, already battered by the

effects of the COVID-19 pandemic. Indeed, local Thai ponies play an important role in the agricultural systems in these communities, and given the high mortality rates and effects of AHS on international trade of horses, this outbreak can also have a devastating impact on the thriving thoroughbred industry of mainland China and Hong-Kong.

The evolution of historic outbreaks of AHS outside the African continent indicates that international and local veterinary bodies should monitor the Thailand epizootic very carefully. The Iberian Peninsula epizootic of 1987 – 1993<sup>3,4</sup> amply illustrates the need for caution. It started in central Spain in 1987 and then spread to the South of the country the following seasons. Despite all possible control measures that were put in place, both in Spain and neighbouring Portugal, the virus spread to Portugal in 1989.<sup>5</sup> It was only after vigorous and comprehensive control measures, which included widespread vaccination, control of animal movements, insect control and rigorous sero-surveillance, that this outbreak was finally eradicated. It is likely, that the mild and short winters in the South of the Iberian Peninsula contributed to the presence of AHS in that part of the world for consecutive seasons.

## 2 | AHS IS A NOTIFIABLE DISEASE TO THE OIE

AHS is a lethal disease that can spread rapidly within an equine population, has a high negative economic impact in countries where it occurs and affects dramatically international trade of horses. For all these reasons AHS is one of the listed diseases of the OIE (World Organisation for Animal Health)<sup>6</sup> and is the only equine infectious disease for which the OIE issues an official recognition of disease freedom for a country or parts thereof (OIE, Terrestrial Animal Health Code).<sup>7</sup> The loss of the AHS-freedom status is already affecting Thailand and the same will happen to neighbouring countries should AHS spread in the region. As a consequence, horse exports will be severely restricted, for example horse exports to the EU will be banned for 2 years.<sup>8</sup> In addition, regaining OIE AHS-freedom status and restoration of equine trade

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with the rest of the world will require sound active and passive disease surveillance strategies, using appropriate and validated diagnostic methods. This is a task that requires efficient coordination of the veterinary services and authorities both at national and international levels. The logical diversion of national funding efforts to combat the current COVID-19 pandemic will undoubtedly play a detrimental role in the capacity to curtail the expansion of AHS in the region.

### 3 | TO BE OR NOT TO BE (VACCINATED), THAT IS THE QUESTION

Vaccination is an essential pillar of any AHS control programme and it needs to be implemented in conjunction with other control measures such as rapid accurate diagnosis, disease surveillance, prevention of insect biting and control of the movement of horses between geographical regions. The latter, normally results in the implementation of regionalisation policies and the geographical demarcation of 'AHS-free', 'Protection', 'Surveillance' and 'Infected' Zones, following the general principles established by the OIE Terrestrial Code.<sup>9</sup> The application of these OIE guidelines have been translated into different pieces of legislation in the EU,<sup>10</sup> the UK<sup>11</sup> and the Republic of South Africa. Now, these policies and regionalisation strategy are being adopted in Thailand.

However, monitoring the spread of the disease and regulating the movement of horses between regions of different disease status is labour-intensive and very demanding for veterinary authorities since it often involves, amongst other measures, strict checks of the infectious status of individual horses prior to movement, and the application of protracted quarantine procedures. These procedures will become even more complicated when systematic vaccination of the horse population is performed.

Indeed, the only two types of vaccines that have ever been used in the field to combat AHS are: a) the whole virus attenuated AHSV vaccine (LAV), in use today; and b) the whole virus inactivated vaccine, used on a very limited scale in Iran in the 1960s and in Spain during the last year of the outbreak in 1993.<sup>3</sup> Both types of vaccine induce an antibody response to all virus antigens and therefore it is not possible to differentiate naturally infected from vaccinated animals. Systematic vaccination of an entire horse population with these vaccines would provide protection but at the same time would result in high levels of seroprevalence. Therefore, demonstrating AHS disease freedom and/or monitoring the spread of the disease on the basis of serological data would be very complicated. The use of RT-PCR testing for monitoring the spread of infection, aiming at detecting recent infections within a population vaccinated with the attenuated vaccine, would also be challenging since RT-PCR tests can detect viral RNA in the blood of vaccinated animals for up to 100 days post vaccination. Interpretation of RT-PCR test results in horse populations vaccinated with the inactivated vaccine would be easier but these vaccines are not available yet and their protective efficacy against

AHSV serotype 1, responsible for the current outbreak, is yet to be demonstrated.

Systematic use of vaccination of a horse population is therefore incompatible with the AHS disease-freedom status. This is reflected in the Article 12.1.2 of the OIE Terrestrial code, which states that '*...A country or zone may be considered free from AHS when infection with AHSV is notifiable in the whole country, systematic vaccination is prohibited, importation of equids and their semen, oocytes or embryos are carried out in accordance with this chapter, and either:.....'*<sup>9</sup> Therefore, the main dilemma veterinary authorities are confronted with during an AHS epizootic occurring in a non-endemic country, such as Thailand and other countries in the region, is whether to vaccinate or not the horse population or parts thereof. If vaccination is finally mandated, then further considerations need to be taken into account such as: a) when to vaccinate; b) in which parts of the country is the vaccine going to be used and what animals would be vaccinated (all equids? or only certain species or breeds?); c) how surveillance is going to be performed; and d) what is the strategy to eradicate the disease, i.e. when to stop vaccinations and how to regain the AHS-freedom status.

With regards to the current outbreak, these dilemmas are not only for Thailand to consider, but also for neighbouring countries that are threatened by the spread of AHS in their territories and are monitoring this outbreak. It is being reported that Cambodia and China are already testing horses for AHS.<sup>12</sup>

The pre-emptive use of current licensed vaccines in Thailand's neighbouring countries will inevitably result in loss of the AHS-freedom status and will result in economic losses due to trade restrictions. Surveillance of disease and infection using diagnostic tests validated according to international standards recommended by the OIE will play an essential role in the control of the current outbreak.

### 4 | THE IMPORTANCE OF DIAGNOSTICS

Rapid accurate diagnostic tests play a critical role in disease control and surveillance, health certification and international trade of horses. The last two decades have witnessed the development of both RT-PCR and serological AHS diagnostics. Also, significant advances have been made towards the harmonisation and standardisation of diagnostic procedures. The coordinated efforts of the main AHS stakeholders (research scientists, official diagnostic laboratories, the International equine Industry and the OIE) culminated in the demonstration that the most commonly used RT-PCR and ELISA diagnostic tests perform according to the stringent criteria specified in the OIE Diagnostic Manual.<sup>13-16</sup>

Management of the current outbreak in Thailand requires the implementation of the measures mentioned previously with special emphasis on surveillance, which has to be tailored to the specific epidemiological context of this particular outbreak. With the availability of only one type of vaccine (LAV) and the risks associated with the pre-emptive use of vaccination in AHS-free areas, detection of AHS cases and monitoring the spread of disease, using the

laboratory tests mentioned above, is going to play an absolutely critical role in controlling this outbreak.

However, the use of RT-PCR for surveillance will be hampered by the high sensitivity of test, capable of detecting traces of viral RNA in blood for long periods of time after infection,<sup>17</sup> and the impossibility of discerning whether a positive result is the consequence of vaccination or infection. Furthermore, viral RNA can be detected in blood of AHS convalescent animals that are no longer infectious and makes it more difficult to discern whether an active virus infection is circulating within a population. The complications of using RT-PCR for surveillance in vaccinated populations are well-known.<sup>18</sup>

The use of serology for surveillance is of high value as it would provide complementary information to the RT-PCR data and permits testing a large number of samples. However, serological data would only indicate whether AHS has been previously circulating in a horse population, unless serum samples are periodically collected from such population and the levels of antibodies are quantifiable by the serological test. In the latter case, a seroconversion (normally defined as four-fold increase in antibody titre) detected between two serum samples collected from the same animal within a period of 2 or more weeks, would indicate that an active infection occurred between the sample collection timepoints.

However, of the AHS serological tests recommended by the OIE,<sup>13</sup> only the SNT is suitable for quantifying serum antibody titres. However, the SNT is very laborious, slow, highly dependent of well-trained laboratory personnel, difficult to standardise and requires the use of high containment laboratories (up to Biosafety Level 3).

Considering that vaccination is currently being used in Thailand, the strategy for disease surveillance is going to play a vital role in controlling this outbreak but adaptation of existing tests to the current epidemiological context would be necessary for improved control.

## 5 | FUTURE PROSPECTS FOR IMPROVED CONTROL OF AHS: THE DIVA APPROACH

As discussed earlier, the decision to vaccinate against AHS in a non-endemic country in the face of an imminent or on-going outbreak comes at the expense of losing the AHS-freedom status. However, it is possible to develop control strategies based on vaccines that are compatible with disease-freedom status and surveillance operations. The development and use of vaccines that are based on a selected suite of viral protective antigens would enable conducting disease surveillance using complementary diagnostic tests based on antigens that are not part of the vaccine. Thus, the immune response of animals vaccinated with such vaccines could be differentiated from the immune response that results from a natural AHSV infection if the complementary diagnostic tests were used. These Differentiation of Infected from Vaccinated Animals (DIVA) vaccines could bring important benefits to the control of AHS before, during and after the outbreak occurs.

a. **Before the outbreak** starts it would be possible to pre-emptively vaccinate and protect a horse population in high risk areas or

countries. The equine population receiving the DIVA vaccine would develop antibodies against the vaccine antigen but not against those antigens that are not included in the vaccine composition. Detection of antibodies against viral antigens not present in the vaccine would be the basis for identifying horses that had experienced a natural AHSV infection. In this situation, the whole horse population would be protected and if an incursion of AHSV from a neighbouring country occurred, this would be detected by the DIVA test. The differentiation of vaccinated animals from those infected with AHSV would allow OIE certified disease-freedom status and consequent economic benefits would be maintained by a non-endemic country even when the equine population has acquired protective immunity via vaccination.

b. **During a new outbreak**, the use of a DIVA strategy would facilitate significantly the detection of infected animals in a population that would be otherwise protected by the DIVA vaccine. By contrast, use of whole virus vaccines following the emergence of AHS in a non-endemic country would trigger a series of measures to control the spread of the infection, including amongst others, the demarcation of an 'Infected Zone', a 'Protection Zone' surrounding it (normally extending 100 km from the infected zone); and a 'Surveillance Zone' surrounding the latter. The rest of the country would be classified as uninfected or disease-free. These demarcations can change with time depending on the spread of the disease. The transit of animals between zones has to be highly regulated and in some cases even prohibited. The primary objective of these regulations is to stop infected animals moving to other zones to prevent spreading the disease. Quarantine protocols, certification of the infection status of individual animals and disease surveillance within all these zones require the use of high-quality diagnostic tests. In these circumstances the use of the LAV would only be applied to animals within the 'Infected' and 'Protection' zones, but not to those in the 'Surveillance Zone'. The objective of not vaccinating animals in the 'Surveillance Zone' is to ensure that they remain seronegative for AHSV unless transmission of the outbreak virus occurs within that zone. If the virus spread beyond the Protection Zone, the geographical borders of all the zones would have to be re-defined, which would complicate and prolong the eradication of the disease. Zoning modifications of this kind occurred in Portugal during the 1989-1991 outbreak.<sup>5</sup>

c. **Declaration of the end of an AHS outbreak and regaining AHS-freedom status** would be facilitated significantly by the use of a DIVA strategy. In contrast, the very high AHS-specific antibody prevalence in a population vaccinated with the LAV would complicate the laboratory detection of new infections, especially any that are sub-clinical, which are expected to be relatively frequent in a vaccinated population. Currently it is not possible to use any antigen-detection or serological diagnostic test that enable the discrimination between infected and those that are simply vaccinated but not infected. Consequently, confirming the absence of new active AHSV infections in a vaccinated population at the end of an outbreak would require periodic

sampling of the horse population and testing for the lack of viral RNA in blood and/or lack of seroconversions. In contrast, it would be possible to discern whether AHSV transmission is occurring with an equine population vaccinated with a DIVA vaccine by using a DIVA RT-PCR or ELISA test. Any positive result obtained with a DIVA test from a horse (either vaccinated or not) would indicate that such particular animal has experienced an active viral infection.

All of the above indicates that an AHS DIVA vaccine would reduce costs of control policies when an outbreak occurs, improve safety of horse movements within endemic countries and finally would facilitate international trade of horses in both directions (from Thailand to the rest of the world and vice versa).

## 6 | ARE AHSV VACCINES FEASIBLE?

Due to the drawbacks of the LAV (especially, the risk of reversion to virulence and genome segment re-assortment between vaccine and field virus), AHS research has consistently focused on finding alternative vaccine strategies that are safer and more efficacious. Over the last three decades, a broad range of vaccine platforms for AHS have been explored, ranging from protein sub-unit vaccines, to genetically modified AHSV and viral vector vaccines (reviewed by Dennis et al.<sup>19</sup>). None have yet reached the market. The reasons for this are multiple, and include amongst others, the size of the equine vaccine market, high costs of vaccine development (need for high containment animal facilities), ethical constraints (use of horses for research) and costs of manufacturing. Despite this, some of these DIVA strategies are close to entering the clinical development phase.

Indeed, these new vaccines use selected viral protective antigen(s) enabling the development of complementary diagnostic tests based on the antigen and gene segments that are not included in the vaccine. The DISA (Disabled Infectious Single Animal),<sup>20</sup> DISC (Disabled Infectious Single Cycle),<sup>21</sup> VLP (virus-like particle)<sup>22-24</sup> approaches are all attractive options for further research and are theoretically DIVA compatible. Their disadvantage for DIVA is that on top of developing the vaccine, investment is needed to develop and validate the DIVA accompanying test. In the case of DISC and DISA approaches, the licensing pathway for these biologically-active genetically-modified AHS virus vaccines, seems more cumbersome than for other alternative technologies.

Other DIVA compatible vaccine strategies, however, have a long-track record of safety and efficacy, and can be used already in conjunction with differential diagnostic tests currently in use. Indeed, vaccines that are based only on AHSV-VP2 and/or VP5, the main targets of virus neutralising antibodies, are already DIVA compatible when used with AHSV-VP7-based diagnostic tests. These are routinely used in AHS-diagnostic laboratories. Furthermore, both RT-PCR tests based on the VP7 gene, and VP7-based ELISA procedures are recommended by the OIE<sup>13</sup> and some of these specific tests have been very rigorously tested and reached Stage III of the OIE validation pathway,<sup>14-16</sup>

In terms of efficacy, baculovirus expressed VP2/VP5 sub-unit vaccines have been demonstrated to induce virus neutralising antibodies and protection in laboratory rodents and also in horses,<sup>25,26</sup> It is believed that they did not reach the market due to the high costs of manufacturing in an industrial setting. However, recent studies demonstrated that vaccine production yields can be improved significantly and that at least in the mouse model, protection can be achieved with just one vaccine dose.<sup>27</sup> Canarypox-based vaccines expressing AHSV-VP2/VP5 have also shown promising results in horses, at least with AHSV serotype 4, demonstrating both cellular and humoral protective immune responses could be induced.<sup>28,29</sup> Likewise, AHSV-VP2 vaccines based on recombinant modified Vaccinia Ankara virus (MVA) were shown to be protective in small animal models and in horses and it was shown that the main protective mechanism was mediated by virus neutralising antibodies.<sup>30-35</sup> Furthermore, the potential to use this strategy as a polyvalent approach was also demonstrated.<sup>36</sup>

With regards to safety, in contrast with attenuated vaccines, the risk of developing an active AHSV infection in horses vaccinated with baculovirus-expressed VP2/VP5 sub-unit, Canarypox VP2/VP5 or MVA-VP2 vaccines is effectively nil. This is because these vaccines are based on non-replicative AHSV viral antigens. There are a large number of vaccines based on these technology platforms that have been developed and commercialised to prevent human and veterinary diseases. A few examples of these studies include vaccines preventing equine influenza, West Nile, human papillomavirus, MERS, Ebola, human influenza.<sup>28,29,37-42</sup> Therefore, claims that these technologies are not safe or not suitable for manufacturing AHS vaccines<sup>22</sup> are not supported by scientific evidence.

Thus, all three vaccine technology platforms are suitable for industrial production, have an excellent track record of safety and have proven efficacy in the field. Any of these three vaccine platforms represent a realistic prospect for development and commercialisation of a safe, efficacious and DIVA compatible AHS vaccine. They also present the advantage that they are compatible with one another since they are based on the same protective antigens (i.e. AHSV-VP2).

## 7 | CONCLUSION

AHS is a truly devastating disease from both an animal welfare perspective and in respect of the economic damage that it causes. The virus is endemic to Sub-Saharan Africa but historically has emerged periodically in Southern Europe, North Africa, the Arabian Peninsula, the Middle East, Iran and India. Today it is causing a serious outbreak in Thailand, threatening the whole region. The European experience with blue-tongue, a disease of ruminants that has become endemic in this continent and is caused by a virus closely related to AHSV and transmitted by the same insect vectors, is a serious reminder of what could potentially occur with AHS in South-East Asia. The spread of AHS and possible endemicity of AHSV in Asia is particularly worrying due to the lack of safe and effective vaccines able to control the disease without expensive and time-consuming additional disease management measures.

The control of the current outbreak in Thailand and limitation of its wider spread in Asia will be challenging. The use of the polyvalent live attenuated vaccine, which contains in its formulation attenuated viruses of several AHSV serotypes other than AHSV type 1, will require careful design of disease surveillance protocols. Besides the implementation of vaccination with the live attenuated vaccine, the International Horse Sports Confederation (IHSC) is focusing its support on the development of polyvalent whole virus inactivated vaccines,<sup>43</sup> which may prove sub-optimal due to the limited DIVA capability and unknown protective efficacy for AHSV-1. Therefore, at present, immediate control measures of AHS in Thailand need to focus strongly on disease surveillance, control of animal movements and re-adjusting of regionalisation policies, whilst improvements in diagnostics that facilitate surveillance procedures are developed. The Thailand outbreak of AHS is highlighting the problems that are associated with the use of whole virus attenuated vaccines in a non-endemic context and the lack of alternative safe and effective vaccines with DIVA capacity.

At the time of finishing writing this article, a total of 15 outbreaks have been reported from Thailand<sup>44</sup> and another outbreak of AHS has been reported in Malaysia, near the border with Thailand,<sup>45</sup> which reflects the seriousness of the present situation. It will be crucial to determine the serotype and nucleotide sequence of this virus in order to elucidate the origins of this new outbreak.

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## REFERENCES

1. [https://www.oie.int/wahis\\_2/public/wahid.php/Reviewreport/Review?page\\_refer=MapFullEventReport&reportid=33768](https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=33768); Accessed 12 August 2020
2. [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\\_data/file/878255/poa-ahs-thailand-2020.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/878255/poa-ahs-thailand-2020.pdf). Accessed 12 August 2020
3. Sánchez-Vizcaíno JM. Control and eradication of African horse sickness with vaccine. *Dev Biol (Basel)*. 2004;119:255–8.
4. Rodríguez M, Hooghuis H, Castaño M. African horse sickness in Spain. *Vet Microbiol*. 1992;33:129–42.
5. Portas M, Boinas FS, Sousa JOE, Rawlings P. African horse sickness in Portugal: A successful eradication programme. *Epidemiol Infect*. 1999;123(2):337–46.
6. <https://www.oie.int/animal-health-in-the-world/official-disease-status/african-horse-sickness/>. Accessed 12 August 2020
7. [https://www.oie.int/index.php?id=169&L=0&htmfile=chapitre\\_ahs.htm](https://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_ahs.htm). Accessed 12 August 2020
8. European Commission. Implementing Regulations (EU) 2020/485 of 2 April 2020 amending Annex I to Implementing Regulation EU 2018/659 as regards the entry into the Union of live equidae and of semen, ova and embryos of equidae from Thailand. <https://eur-lex.europa.eu/legal-cont>. Accessed 12 August 2020
9. <https://www.oie.int/standard-setting/terrestrial-code/access-online/>. Accessed 12 August 2020
10. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:01992L0035-20080903>. Accessed 12 August 2020
11. [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\\_data/file/244348/pb13831-ahs-control-strategy-20130923.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/244348/pb13831-ahs-control-strategy-20130923.pdf). Accessed 12 August 2020
12. <https://www.reuters.com/article/us-china-horses-disease/china-to-test-horses-in-border-areas-for-african-horse-sickness-idUSKCN2251CB>. Accessed 12 August 2020
13. [https://www.oie.int/fileadmin/Home/eng/Health\\_standards/tahm/3.05.01\\_AHS.pdf](https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.05.01_AHS.pdf). Accessed 12 August 2020
14. Agüero M, Gómez-Tejedor C, Cubillo MÁ, Rubio C, Romero E, Jiménez-Clavero MA. Real-time fluorogenic reverse transcription polymerase chain reaction assay for detection of African horse sickness virus. *J Vet Diagnostic Investig*. 2008;20(3):325–8.
15. Guthrie AJ, MacLachlan NJ, Joone C, Lourens CW, Weyer CT, Quan M, et al. Diagnostic accuracy of a duplex real-time reverse transcription quantitative PCR assay for detection of African horse sickness virus. *J Virol Methods*. 2013;189(1): <https://doi.org/10.1016/j.jviromet.2012.12.014>
16. Durán-Ferrer M, Agüero M, Zientara S, Beck C, Lecollinet S, Sailleau C, et al. Assessment of reproducibility of a VP7 Blocking ELISA diagnostic test for African horse sickness. *Transbound Emerg Dis*. 2019;66(1):83–90.
17. Weyer CT, Quan M, Joone C, Lourens CW, MacLachlan NJ, Guthrie AJ. African horse sickness in naturally infected, immunised horses. *Equine Vet J*. 2013;45(1):117–9.
18. Weyer CT, Grewar JD, Burger P, Joone C, Lourens C, MacLachlan NJ, et al. Dynamics of African horse sickness virus nucleic acid and antibody in horses following immunization with a commercial polyvalent live attenuated vaccine. *Vaccine*. 2017;35(18):2504–10.
19. Dennis SJ, Meyers AE, Hitzeroth II, Rybicki EP. African Horse Sickness: A Review of Current Understanding and Vaccine Development. *Viruses*. 2019;11(9):844.
20. van Rijn PA, Maris-Veldhuis MA, Potgieter CA, van Gennip RGP. African horse sickness virus (AHSV) with a deletion of 77 amino acids in NS3/NS3a protein is not virulent and a safe promising AHS Disabled Infectious Single Animal (DISA) vaccine platform. *Vaccine*. 2018;36(15):1925–33.
21. Lulla V, Losada A, Lecollinet S, Kerviel A, Lilin T, Sailleau C, et al. Protective efficacy of multivalent replication-abortive vaccine strains in horses against African horse sickness virus challenge. *Vaccine*. 2017;35(33):4262–9.
22. Dennis SJ, Meyers AE, Guthrie AJ, Hitzeroth II, Rybicki EP. Immunogenicity of plant-produced African horse sickness virus-like particles: implications for a novel vaccine. *Plant Biotechnol J*. 2018;16(2):442–50.
23. Rutkowska DA, Mokoena NB, Tsekoa TL, Dibakwane VS, O’Kennedy MM. Plant-produced chimeric virus-like particles - A

- new generation vaccine against African horse sickness. *BMC Vet Res.* 2019;15:432.
24. Maree S, Maree FF, Putterill JF, de Beer TAP, Huismans H, Theron J. Synthesis of empty African horse sickness virus particles. *Virus Res.* 2016;213:184–94.
  25. Scanlen M, Paweska JT, Verschoor JA, Van Dijk AA. The protective efficacy of a recombinant VP2-based African horsesickness subunit vaccine candidate is determined by adjuvant. *Vaccine.* 2002;20(7–8):1079–88.
  26. Kanai Y, van Rijn PA, Maris-Veldhuis M, Kaname Y, Athmaram TN, Roy P. Immunogenicity of recombinant VP2 proteins of all nine serotypes of African horse sickness virus. *Vaccine.* 2014;32(39):4932–7.
  27. Aksular M, Calvo-Pinilla E, Marín-López A, Ortego J, Chambers AC, King LA, et al. A single dose of African horse sickness virus (AHSV) VP2 based vaccines provides complete clinical protection in a mouse model. *Vaccine.* 2018;36(46):7003–10.
  28. Guthrie AJ, Quan M, Lourens CW, Audonnet JC, Minke JM, Yao J, et al. Protective immunization of horses with a recombinant canarypox virus vectored vaccine co-expressing genes encoding the outer capsid proteins of African horse sickness virus. *Vaccine.* 2009;27(33):4434–8.
  29. El Garch H, Crafford JE, Amouyal P, Durand PY, Edlund Toulemonde C, Lemaitre L, et al. An African horse sickness virus serotype 4 recombinant canarypox virus vaccine elicits specific cell-mediated immune responses in horses. *Vet Immunol Immunopathol.* 2012;149(1–2):76–85.
  30. Chiam R, Sharp E, Maan S, Rao S, Mertens P, Blacklaws B, et al. Induction of antibody responses to African horse sickness virus (AHSV) in ponies after vaccination with recombinant modified vaccinia Ankara (MVA). *PLoS One.* 2009. <https://doi.org/10.1371/journal.pone.0005997>
  31. Castillo-Olivares J, Calvo-Pinilla E, Casanova I, Bachanek-Bankowska K, Chiam R, Maan S, et al. A Modified vaccinia Ankara virus (MVA) vaccine expressing African horse sickness virus (AHSV) VP2 protects against AHSV challenge in an IFNAR 2/2 mouse model. *PLoS One.* 2011;6(1): <https://doi.org/10.1371/journal.pone.0016503>
  32. Alberca B, Bachanek-Bankowska K, Cabana M, Calvo-Pinilla E, Viaplana E, Frost L, et al. Vaccination of horses with a recombinant modified vaccinia Ankara virus (MVA) expressing African horse sickness (AHS) virus major capsid protein VP2 provides complete clinical protection against challenge. *Vaccine.* 2014;32(29):3670–4.
  33. Calvo-Pinilla E, de la Poza F, Gubbins S, Mertens PPCPPC, Ortego J, Castillo-Olivares J. Vaccination of mice with a modified Vaccinia Ankara (MVA) virus expressing the African horse sickness virus (AHSV) capsid protein VP2 induces virus neutralising antibodies that confer protection against AHSV upon passive immunisation. *Virus Res.* 2014;180:23–30.
  34. Calvo-Pinilla E, De La Poza F, Gubbins S, Mertens PPCPPC, Ortego J, Castillo-Olivares J. Antiserum from mice vaccinated with modified vaccinia Ankara virus expressing African horse sickness virus (AHSV) VP2 provides protection when it is administered 48 h before, or 48 h after challenge. *Antiviral Res.* 2015;116:27–33.
  35. Calvo-Pinilla E, Gubbins S, Mertens P, Ortego J, Castillo-Olivares J. The immunogenicity of recombinant vaccines based on modified Vaccinia Ankara (MVA) viruses expressing African horse sickness virus VP2 antigens depends on the levels of expressed VP2 protein delivered to the host. *Antiviral Res.* 2018;154:132–9.
  36. Manning NM, Bachanek-Bankowska K, Mertens PPC, Castillo-Olivares J. Vaccination with recombinant Modified Vaccinia Ankara (MVA) viruses expressing single African horse sickness virus VP2 antigens induced cross-reactive virus neutralising antibodies (VNAb) in horses when administered in combination. *Vaccine.* 2017;35(44):6024–9.
  37. Paillot R, El Hage CM. The use of a recombinant canarypox-based equine influenza vaccine during the 2007 Australian outbreak: A systematic review and summary. *Pathogens.* 2016;5(2):42.
  38. Monie A, Hung CF, Roden R, Cervarix™ WuTC. A vaccine for the prevention of HPV 16, 18-associated cervical cancer. *Biologics: Targets and Therapy.* 2008;2(1):107–13.
  39. Volz A, Sutter G. Modified Vaccinia Virus Ankara: History, Value in Basic Research, and Current Perspectives for Vaccine Development. *Adv Virus Res.* 2017;97:187–243.
  40. Mutua G, Anzala O, Luhn K, Robinson C, Bockstal V, Anumendem D, et al. Safety and Immunogenicity of a 2-Dose Heterologous Vaccine Regimen with Ad26.ZEBOV and MVA-BN-Filo Ebola Vaccines: 12-Month Data from a Phase 1 Randomized Clinical Trial in Nairobi, Kenya. *J Infect Dis.* 2019;220(1):57–67.
  41. Koch T, Dahlke C, Fathi A, Kupke A, Krähling V, Okba NMA, et al. Safety and immunogenicity of a modified vaccinia virus Ankara vector vaccine candidate for Middle East respiratory syndrome: an open-label, phase 1 trial. *Lancet Infect Dis.* 2020;20(7):827–38. <https://www.ema.europa.eu/en/news/new-vaccine-prevention-ebola-virus-disease-recommended-approval-european-union>. Accessed 12 August 2020.
  42. <https://oiebulletin.com/index.php?panorama=03-4-ahs-vaccine-en&edition=7913&pdf=panorama&article=9711>. Accessed 12 August 2020.
  43. [https://www.oie.int/wahis\\_2/public/wahid.php/Countryinformation/Countryreports](https://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Countryreports). Accessed 12 August 2020.
  44. [https://www.oie.int/wahis\\_2/public/wahid.php/Reviewreport/Review?page\\_refer=MapFullEventReport&reportid=35575](https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=35575). Accessed 12 August 2020.

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