## Q & A from previous meeting

THAILAND, DLD-AHS TEAM

Q: For the outbreak in Sep 2020, when the infected zebra was imported and tested in ELISA?

A: Zebras (60 heads) were imported from South Africa on 28 January 2020. All zebras were tested in private laboratory of South Africa. Tested date was on 15 January 2020.

Q: Currently what method DLD used for AHS detection and confirmation. RT-PCR or real-time PCR:

A: For laboratory test, we use real-time RT-PCR (OIE: Guthrie et al., 2013) in suspicious animals.

Q: After getting outbreak of AHS, do you change your SPS measures for importing horses from abroad? If it is changed, what is the current quarantine measures for importing horses to Thailand?

A: We do not change any import requirement of horses. In fact, our requirements for importation of equines did contain the quarantine measure. Equines are required to be quarantined prior the exportation for 30 days and upon arrival for 30 days.

However, before March 2020, zebra is not under authority of the DLD. It can be imported with no restrictions. Currently, zebra is under authority of the DLD so we apply these requirements to zebra as well.

Please see the requirements (next slide).



#### **Import requirements**

#### REQUIREMENTS FOR THE IMPORTATION OF HORSES AND OTHER EQUINES

#### INTO THE KINGDOM OF THAILAND

- A health certificate in English signed by a full time authorized veterinary official of the government of the exporting country stating:-
  - 1.1) number and species of the animals,
  - 1.2) breed, sex, age and color,
  - 1.3) animal identification,
  - 1.4) name and address of the owner/exporter and identification of the premises of origin,
  - 1.5) certification of condition items (3) to (12).

Before March 2020, zebra is not required to follow this protocol. Currently, this requirement apply to all equids.

- 2) Stud-book, silhouette or other pedigree certificate shall accompany the animals on arrival.
- 3) The animals must come from an area where animal diseases are under control. They must be healthy, free from signs of any infectious and contagious diseases including ectoparasitism at the time of export and fit to travel.
- 4) The country/region/zone of origin has been free from African Horse Sickness, Equine Encephalomyelitis (Eastern, Western and Venezuelan Encephalomyelitis and Borna Disease), Vesicular Stomatitis, Dourine and Epizootic Lymphangitis.
- 5) The animals must originate from the premises accommodated the animals, where no clinical signs or other evidence of Anthrax, Equine Infectious Anemia, Equine Viral Arteritis, Equine Viral Rhinopneumonitis, Rabies, Equine Influenza, Equine Herpes Virus (abortion and neurological disease), Trypanosomiasis (T.evansi), and Horse Pox has been found during the past 12 (twelve) months prior to export.
- 6) During the 2 (two) years prior to export, no clinical signs or other evidence of all diseases as mentioned in the condition item (5) has been found on any premises accommodated animals

- 7) During the 12 (twelve) months prior to export no clinical signs or other evidence of Glanders, Strangle, Equine Paratyphoid (<u>S.abortus equi</u> infection), Equine Coital Exanthema, Equine Piroplasmosis, Surra, Contagious Equine Metritis (CEM), Epizootic Lymphangitis and Mange has been found at any premises where the animals have been resident.
- —8) On examination by an authorized veterinary official, the animals must show no evidence of obvious genetic defects as pigeon toed, undershot jaw, etc.
  - 9) All animals to be exported to Thailand are subjected to quarantine in the approved premises and under the control of a duly authorized veterinary official of the government of the exporting country during the 30 (thirty) days prior to export.
    Quarantine
  - 10) Each individual animal must be submitted to tests with negative results using test methods or other methods recommended by the Office International des Epizooties (OIE). The tests must be carried out during the 30 (thirty) days prior to export for the following diseases:-
    - 10.1) Equine Infectious Anemia
    - 10.2) Equine Viral Arteritis
    - 10.3) Contagious Equine Metritis
    - 10.4) Glanders
    - 10.5) Equine Piroplasmosis
    - 10.6) Dourine
    - 10.7) Equine Trypanosomiasis (T.evansi)

**Import requirements** 

- 11) The animals must be treated with an approval broad spectrum anthelmintic and external parasite shortly prior to export.
- 12) The vehicles and containers used for transporting the export animals must be thoroughly cleaned, disinfected, and vacanted prior to loading which is compliant with the relevant criteria laid down in the OIE International Animal Health Code.

# Import requirements

- 13) The accommodation provided for the animals during shipping must be designed to avoid any risk of injury and unnecessary suffering which is compliant with the relevant criteria laid down in the OIE International Animal Health Code.
- 14) The ship/aircraft or other vehicles carrying the animals may only call at approved intermediate ports after leaving the country of origin. While in transit, the animals must not come in contact with other animals not similar health status. Unloading or movement of the horses may only take place under the approval and supervision of a veterinary authority of the transit country.
- 15) No other animals not similarly health status, animal feed or fodder allow to be taken on board the ship/aircraft after they leave the country of origin.
- 16) The animals are subjected to quarantine at an approved premises for a period of not less than 30 (thirty) days upon arrival, during which they shall be submitted to tests and/or treatments deemed necessary. The importer/owner shall be fully charged for the incurred expenses.
- 17) Failure to follow the import procedures may result in returning the animals to the country of origin or destroying without compensation.

Quarantine

Q: Do the animal movement restriction mean all kind of animals or only equids?

A: In general, all animals (under Epidemic Act) are under animal movement control measures. Different species may have different movement restriction. The movement restriction presented (in AHS presentation) last week is applied to only equids.

Q: Mild clinical sign is caused by vaccination itself or by the re-infection with virulent strains?

A: Both are possible. If the clinical sign is caused by vaccination, the clinical sign could be seen within 7-10 days after vaccination. Vaccinated horses could show mild symptom of AHS infection: fever, in-appetite, swelling at supraorbital fossa, eyelid and face. They would recover within a few week.

(continue next slide)

Q: Mild clinical sign is caused by vaccination itself or by the re-infection with virulent strains?

A: In the presentation case, there is no strong evidence to prove that recurrent infection of virulent AHS viruses occur in this case.

**More details:** Clinical signs were seen after 60 days post vaccination. Some vaccinated horses had a swelling at supraorbital fossa and face, while some showed generalized edema and swelling. But those horses still alert, normal appetite and body temperature. We collected whole blood and sera from horse farms which owners claimed that they had swelling signs. Laboratory results showed positive detection of AHS serotype 1, 3 or 4 with high Ct value (Ct 30-38). The results did not relate to the disease history of swelling. The AHSV could not be cultured and isolated from the collected specimens. However, all horses had antibodies against AHS virus using ELISA. Approximately 80% of vaccinated horses had high antibody titers against AHSV serotype 1 -field strain (at antibody titer more than 1:40). Hypothetically, recurrent infection of virulent AHS viruses might occur in the horse farms especially within the outbreak area. Due to the fact that vaccinated horses have neutralizing antibodies to fight the virulent AHS viruses so that AHSV causes less clinical symptom in vaccinated horses. Secondly, after viral infection, an overwhelming immune response has been found that generalized edema and swelling are associated with high cytokine storm. We found some horses with high SNT titer (>1:56) associated with the history of swelling. All horses had no blood parasites (Trypanosoma spp., Babesia spp., and Theileria spp.) and slightly increased leukocytes.

Q: Are all horses vaccinated microchipped?

A: Yes.

More details: Our protocol for vaccination is

- 1) Animals must have animal ID RFID (microchipped)
- 2) Animals must be kept in netting stable or vector prove stable. Farms must have a vector
- 3) Animal must be tested with AHS negative prior the vaccination (collect blood to screen AHS prior vaccination).
- 4) After vaccination, all vaccinated horses must be kept in netting stable for at least 40 days. Thus, if any vaccinated horse shows clinical signs, they already be contained in the netting stable. In addition, the DLD also applied the movement control measures.

Q: Are you use any pesticides? I mean by spraying on equines or by pour on pesticides. If yes what kind of pesticides you used?

A: We use several pesticide and repellent to control vector. Spraying: alphacypermethrin and pyrethroid. Spot on: Etofenprox.

Q: Have you seen any evidence of infection in donkeys/mules?

A: No. No death cases reported to the DLD.

Q: are zebra moved to vector protected facility. Otherwise further re-circulation of the virus may happen

A: Yes, most zebras are also kept in netting stable. However, this measure could not be applied to all facilities. For example, there is a place that keep animals mimic to wildlife habitat (safari). The netting stable is not applicable to this facility. The DLD also applies other control measures to prevent re-circulation of the virus as well.

Q: How long the viremia of a horse lasted after vaccinated with attenuated AHSV vaccine?

A: Our lab results indicate that LAV vaccine isolates can prolong in blood about 1-2 weeks (active stage, VI and PCR). However, we can detect AHS RNA genome more than 156 days.

Q: If there are movement restrictions how was disease passed to Malaysia?

A: By illegal movement. Although the DLD implement the movement restriction, we still need collaboration from all sectors. Private sectors and horse associations could be our eyes and ears to strengthen our law enforcement.