

## Communication

**BIDJEH (K.), GANDA (K.), DIGUIMBAYE (C.), IDRIS (A.).** Outbreaks of goat-pox in Chad. *Revue Élev. Méd. vét. Pays trop.*, 1990, **43** (1) : 31-33.

Several outbreaks of goat-pox were observed from 1985 to 1989 in different villages of the N'Djamena area. The virus strain circulating in this region seemed to be host-specific for goats since sheep kept in contact with goats did not suffer from the disease. Nevertheless, it has to be studied further on whether the virus isolated from sick goats could be pathogenic for sheep. *Key words* : Sheep - Goat - Virus - Strain - Goat-pox - Chad.

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## Detection of African horsesickness (AHS) in recently vaccinated horses with inactivated vaccine in Qatar

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**HASSANAIN (M.M.), AL-AFALEQ (A.I.), SOLIMAN (I.M.A.), ABDULLAH (S.K.).** Détection de la peste équine africaine au Qatar sur des chevaux récemment vaccinés avec un vaccin inactivé. *Revue Élev. Méd. vét. Pays trop.*, 1990, **43** (1) : 33-35.

Deux chevaux de course arabes âgés de 7 ans ont montré des symptômes typiques de la peste équine africaine (PEA) au Qatar et sont morts peu après. Les chevaux avaient été vaccinés avec un vaccin inactivé au formol environ 10 jours avant le début de la maladie. Des prélèvements de sang ont permis d'isoler le virus de la PEA sur un seul prélèvement après inoculation intracérébrale sur des souriceaux nouveaux-nés. L'identité du virus a été confirmée par le test de fixation du complément à partir de l'antigène viral et du sérum hyperimmune de référence du virus de type 9 de la PEA. Le sérotype du virus isolé a été identifié par le test de neutralisation du sérum à l'aide des sérotypes de référence du virus de la PEA. Deux étiologies possibles sont suggérées : soit une resurgence endémique naturelle d'un virus dans le pays, soit la présence d'un virus infectant résiduel dans le vaccin inactivé. *Mots clés* : Cheval - Peste équine africaine - Vaccin inactivé - Qatar.

## Introduction

African horsesickness (AHS) is a disease affecting horses with a well defined geographical distribution and seasonal occurrence (9). The disease is caused by nine antigenically different serotypes of viscerotropic viruses (11). The last two serotypes to be isolated were types 8 and 9. Type 8 was isolated in both Central and South Africa and type 9 was isolated during outbreaks of the disease in different countries of the Near and Middle East (8). However, isolation of AHS virus has not yet been reported in Qatar or in most of the neighbouring states including Saudi Arabia.

On a small scale, the disease is controlled by a killed vaccine using formol-treated infected horse tissue emulsion (4), whereas a formol inactivated aluminium gel adsorbed vaccine is more widely used. However, data are now available suggesting that neither formol-

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treated nor aluminium gel vaccines are sufficiently reliable (6). This paper investigates recently vaccinated racing horses showing typical AHS symptoms in Qatar.

### Materials and methods

Two recently vaccinated 7-year old Arabian racing mares were involved in this investigation. A formol inactivated aluminium gel adsorbed vaccine was used, which was obtained from a commercial supplier. Ten days after vaccination, the horses showed typical AHS symptoms and died shortly after. Blood and serum samples from both horses were collected.

Monkey kidney cell line (MS) was used in this investigation. A detailed description of the cell culture technique has been previously reported (12).

Ten per cent of each blood sample was inoculated intracerebrally (i.c.), according to ALEXANDER (1935), into two litters ( a litter for each sample) of suckling mice (12 mice per litter) with a dose of 0,025 ml per mouse. An additional litter was inoculated i.c. with a similar dose, using ten per cent of normal horse blood as uninfected control. Clinical signs and mortality of these mice were recorded. Selected mice which exhibited severe symptoms were used for further passages. Brain suspensions of the mentioned mice were used as inoculum for the next passage. At the fifth passage, brain suspensions were lyophilized, titrated in suckling mice and kept at -20C until used.

Neurotropic tissue culture adapted strains of all nine types of AHS virus were used to serotype the isolated virus.

Rabbit hyperimmune serum against the isolated virus was prepared as previously described (12). Using a similar method of preparation, rabbit hyperimmune serum against type 9 reference strain of AHS virus was obtained from Serum and Vaccine Institute, Cairo, Egypt.

### Serological identification of the isolated virus

Complement fixation test (CFT) : The isolated virus antigen was prepared in mouse brains according to Casals method (1949). The test was performed as previously described (10) using type 9 rabbit antisera.

Serum neutralization test (SNT) : Serum neutralization tests to serotype the isolated virus were conducted in MS cell culture, according to HAZRATI and OZAWA (7), using the nine reference strains of AHS virus and prepared rabbit hyperimmune serum against the isolated virus (Serum and Vaccine Institute, Cairo, Egypt).

### Results

Virus isolation was based on the observation of nervous manifestations (tremors, incoordination and circling) and mortality recorded from inoculated suckling mice. Mice mortality rates were recorded and virus titre reached 5,6LD<sub>50</sub> at the fifth passage in mouse brains. Virus detection was confined to horse 1 as the other horse was negative up to the third blind passage in mouse brains.

Serum samples of both affected horses reacted positively to type 9 reference virus antigen in the CFT. The antibody titres were 0,9 and 1,8 log<sub>10</sub> in horse 1 and horse 2, respectively (Table I). On the other hand, the isolated virus antigen, which was prepared from mouse brains showed a positive reaction with type 9 reference hyperimmune serum (Table I).

TABLE I Virus isolation and complement fixation tests.

Horse n°	Virus isolation	Virus antigen titre*	Antibody titre of the sera sample**
1	+	1.2***	0.9
2	-	-	1.8

\* The antigen was prepared in suckling mice brains and tested against reference type 9 antiserum.

\*\* Antibody against AHS was tested using type 9 reference virus.

\*\*\* Titres were calculated in log<sub>10</sub>.

In the serum neutralization test (SNT), antibodies to type 9 reference strain were detected in the rabbit hyperimmune serum against the isolated virus. The remaining eight reference types of AHS virus were negatively neutralized by the same serum (Table II). The titres were calculated, using REED and MUENCH method (14).

TABLE II Serotyping of the isolated AHS virus using SN tests.

Serum sample	Types of reference AHS viruses								
	T1	T2	T3	T4	T5	T6	T7	T8	T9
Hyperimmune serum of the isolated virus	-	-	-	-	-	-	-	-	3.6*

\* Titres were calculated in log<sub>10</sub>.

### Discussion

In the present work, AHS virus isolation was attempted from blood samples of affected horses. When suckling mice were inoculated i.c. with horse 1 sample, they

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developed nervous manifestations. Similar symptoms were previously reported in mice infected i.c. with AHS virus (1). Sample inoculation also caused mice mortality and led to a titre of 5,6LD50 at the fifth passage. Further identification of the isolated virus was made using serological tests. In the CFT, the viral antigen prepared from mice brains reacted positively with anti-type 9 AHS virus reference hyperimmune serum. With the same test, serum samples from both affected horses showed anti-AHS virus antibodies. Because of the common CFT between all nine types of AHS virus (11), it was necessary to perform SNT, in order to identify the serotype of the isolated virus. In the SNT, the only reference virus being neutralized by the prepared hyperimmune serum against the isolated virus was type 9 AHS virus. This result indicated that the isolated virus was a type 9 AHS virus. The failure to isolate virus from horse 2 blood sample did not necessarily mean that the horse was not infected with the virus, especially as the horse exhibited typical symptoms of AHS infection (5). It is possible that the virus was not in the viraemic stage at the time of sample collection.

The source of this infection might be explained by two possibilities. The first one was that the virus had been introduced into the country through various means, or that the disease was already endemic. In some parts of the Middle East, the disease has been reported to be endemic (8). However, the disease has not yet been reported in Qatar or in most of the neighbouring countries, including Saudi Arabia.

It is noteworthy that the initial reason for vaccinating the two horses was simply for preventive purposes.

The second possible source of the infection was the presence of residual infectious virus in the inactivated vaccine, especially as the onset of the disease occurred approximately ten days after vaccination. This is within the normal range of AHS virus incubation period (5). This type of vaccine failure has been previously reported. GIRARD et al. (6) and SCHNEIDER (15) were able to detect infectious « foot and mouth disease » virus after intradermal inoculation into cattle with a formol inactivated vaccine incubated during a prolonged period with 0,05 per cent formol. BACHRACH and McKERCHER also reported that the curvilinear rate of inactivation implied the slowing of the inactivation reaction with increasing incubation time and thus deviated from first order kinetics. Hence, it was difficult to predict the time for complete virus inactivation, these types of vaccines should be more strictly controlled and thoroughly tested both in vivo and in vitro before being licensed. Further studies should be made in Qatar and in neighbouring countries to determine the epidemiology of the disease in these areas.

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Two 7-year old Arabian racing horses were reported to show typical AHS symptoms in Qatar and died shortly after. The horses had been vaccinated with formol inactivated vaccine approximately 10 days before the onset of the disease. Blood samples from these horses were collected and AHS virus isolated from one sample after intracerebral (i.c.) inoculation into suckling mice. The virus identity was confirmed by complement fixation test (CFT) using the virus antigen and reference type 9 of AHS virus hyperimmune serum. The serotype of the isolated virus was identified by serum neutralization test (SNT) using reference types of AHS virus. Two possibilities of the original source of this infection were suggested. The infection might be due first to the natural endemic occurrence of the virus in the country and secondly, to the presence of residual infectious virus in the inactivated vaccine. *Key words*: Horse - African horsesickness - Inactivated vaccine - Qatar.

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