Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 2, 301-304

# Overview of African horse sickness virus (AHSV) situation in Egypt from 2017 to 2022

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**ARTICLE INFO**

Recieved: 02 August 2023

Accepted: 15 October 2023

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Keywords:

African horse sickness virus (AHSV)
Egypt
ELISA
Serosurveillance
PCR

**ABSTRACT**

African horse sickness (AHS) is a non-contagious arthropod-borne infectious disease of Equidae. Because of its severity and quick spread, It is cosidered as a notifiable disease. The current study intended to look into the current suitation of the vector-borne African horse sickness virus (AHSV) in Egypt, determine viral seroprevelance, and assess the associated risk factors. In this context, 2739 sera and 150 spleen samples were collected from different Egyptian governorates and tested for AHSV screening. The sera were investigated for presence of antibodies against AHSV whilst spleen samples were tested for AHSV Ag and RNA detection. The obtained results revealed that all 2739 sera samples tested negative for AHSV antibodies. Furthermore, using ELISA and conventional reverse-transcription polymerase chain reaction (RT-PCR), to identify AHSV Ag and nucleic acid, the 150 tested spleen samples gave negative results with both assays. In conclusion, the recorded results indicated the absence of antibodies, antigen, and viral nucleic acid of AHSV in all tested samples which proved that there is no circulating virus in the investigated Egyptian governorates in the period from 2017 to 2022. Evenually, the effective control programs are recommended by carrying out further epidemiological investigations to understand the current situation of arboviruses in the country.

## Introduction

African horse sickness (AHS) is a non-contagious viral disease that affects all Equidae members, including horses, mules, donkeys, and zebras (Mellor and Hamblin, 2004). The World Organization for Animal Health has classified AHS as a notifiable disease because of its severity and the potential risk of rapid worldwide transmission (OIE, 2019).

The impact of AHS extends beyond its direct effects on equine health. It has significant economic consequences, particularly in areas where the equine industry plays a vital role in agriculture, transportation, and leisure activities. Outbreaks of AHS can lead to quarantine measures, movement restrictions, and the disruption of international trade in horses and related products (Carpenter *et al*., 2017).

The African Horse Sickness Virus (AHSV) mostly infects equids, with fatality rates of up to 90% in horses, 50% in mules, and 10% in donkeys (Zientara *et al*., 2015). AHSV is transmitted by *Culicoides* species (biting midges), particularly *Culicoides imicola* and *Culicoides abolition*, while additional arthropod vectors have been involved in disease transmission (Carpenter *et al*., 2017). Clinical manifestations of AHS in horses include serous effusion and bleeding in multiple organs and tissues as a result of harm to the circulatory and respiratory systems. Based on the degree and severity of the clinicopathological aspects, the disease has been categorized into three types: subacute or cardiac form, cardio-pulmonary or mixed form, and peracute or pulmonary form (Fernandez and White, 2010; Zientara, 2010). The disease is characterized by a range of clinical signs, including fever, respiratory distress, swelling, and hemorrhaging in various organs. Depending on the strain of the virus and the individual horse's immune response, the disease can progress rapidly, leading to death within a matter of days (Fernandez and White, 2010).

AHSV is a member of the *Reoviridae* family and the genus *Orbivirus*. The virus has a linear double stranded RNA genome. This genome is made up of ten linear ds RNA segments that encode seven structural proteins (VP1, VP2, VP3, VP4, VP5, VP6, and VP7) and five non-structural proteins (NS1, NS2, NS3, NS3A, and NS4) (Zwart *et al*., 2015). The virion is an isometric, non-enveloped particle made up of three concentric protein layers. The outside capsid proteins include VP2 and VP5, while the inner capsid proteins are VP3 and VP7. Minor inner capsid proteins include VP1, VP4, and VP6 (Guthrie *et al*., 2015). According to the virus capsid protein VP2, AHSV is classified into nine serotypes. Serotypes 1–8 have only been documented in sub-Saharan Africa, whereas serotype 9 is more prevalent and caused epidemics outside Africa. serotype 4 caused the outbreaks in Spain and Portugal (Aklilu *et al*., 2014; Bunpapong *et al*., 2021).

AHSV is found in tropical and subtropical regions of Africa, south of the Sahara, from Senegal through Ethiopia, Somalia, and South Africa. The virus then spread on a regular basis, causing severe epidemics and outbreaks in newly impacted regions such as Egypt, Syria, Jordan, Lebanon, and Palestine from 1943 to 1944. The virus expanded throughout the Middle East and South-West Asia in 1959 and 1960, causing outbreaks in Cyprus, Turkey, Lebanon, Iran, Iraq, Syria, Jordan, Palestine, Pakistan, and India. AHSV was first detected in Morocco in 1965, then expanded to Algeria and Tunisia before crossing the Strait of Gibraltar into Spain in 1966 (Fall *et al*., 2015; Liebenberg *et al*., 2015; Zientara *et al*., 2015; Sanchez-Matamoros *et al*., 2016). Egypt recorded the virus in 1928, 1943, 1953, 1958, and 1971. AHSV was also isolated from roaming dogs in Aswan, Egypt, and the authors concluded that the dog may play a role in AHSV epizootiology (Salama *et al*., 1981).

AHSV different serotypes mostly share the immunogenic antigen VP7, which is found in the inner capsid and is frequently used in rapid diagnostic assays such as ELISA, complement fixation tests, and quantitative reverse-transcription polymerase chain reaction (RT-qPCR); howev-

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er, serotype-specific antigens are found in the outer capsid and produce neutralizing antibodies (Bunpapong *et al*., 2021). Vaccination programs, vector control measures, and disease surveillance systems arerecommended to prevent and control AHS. Efforts are being made to prevent infected animal movement, protect horses from midge bites, and maintain tight biosecurity protocols in affected areas (Dennis *et al*., 2019).

Due to the lack of information on the epidemiological state of AHSV in Egypt's equid population, and the monitoring system is seen as crucial for providing knowledge on the epidemiology and factors leading to disease spread in countries. Therefore, serological and molecular diagnosis were carried out to evaluate the current situation of vector-borne AHSV in Egypt, as well as to establish seroprevalence and analyze disease risk factors.

## Materials and methods

*Samples collection and preparation*

Sera samples

A total number of 2739 sera samples were collected from equid species during the serosurveillance performed in Egypt during the last six years from 2017 to 2022 (Table 1). The 2739 serosurveillance samples were collected during 2017 (n=312), 2018 (n=402), 2019 (n=397), 2020 (n=468), 2021 (n=785) and 2022 (n=375) from equid species (horses and donkeys 1:4) and both sex (males and females) of different ages (less than 5 years, between 5 to10 years and more than 10 years' animal ages). The samples were collected from different Egyptian governorates Egyptian Governorates including Cairo, Giza, El Mnofiya, El Sharkia, El Dakahleya, El Gharbia, El Qalubia, Beni Suif, Assuit, El-Menia, Sohag, Ismailia, Alexandria, Luxor, Quena, Aswan, New Valley and Hurghada. Blood was collected from the jugular vein in clean dry centrifuge tubes, allowed to clot, then centrifuged at 1500x g for 20 minutes to separate serum, which was stored at - 20°C until utilized in the detection of antibodies against AHSV.

Spleen samples

A total of 150 spleen samples were collected from recently dead animals from different Egyptian Governorates during the last six years; 2017 (n=24), 2018 (n=41), 2019 (n=33), 2020 (n=10), 2021 (n=28) and 2022 (n=14) (Table 2). Spleen samples were prepared to be used for AHSV Ag detection using ELISA and for the molecular detection of the virus nucleic acid using conventional RT-PCR assay. For the Ag detection ELISA, 1/2 dilution must be done (W/V) using the diluent supplied in the kit (1g of sample in 2 ml of diluent). The sample was homogenized using a pestle. After that, the supernatant was taken after centrifugation at 1000 rpm for 10 minutes to be used in the ELISA test.

For RT-PCR, the collected spleen samples were processed by sterile sterile scissors and forceps and ground to a 10% final concentration (W/V) in a mortar with sterile sand and an equal volume of sterile phosphate-buffered saline (PBS) containing sodium penicillin (1000 IU/mL), streptomycin (1 mg/mL), and mycostatin (100 IU/mL). The homogenized suspension was centrifuged for 10 minutes at 1000 rpm, and the supernatant was stored at - 80°C for further analysis.

*AHSV antibodies detection ELISA kit*

INGEZIM® AHSV COMPAC PLUS ELISA kit (Madrid, Spain) was used in the current study according to the manufacturer's instructions. This kit is based on a blocking enzymatic immunoassay. The supplied plates are coated with antigen which is fixed in a solid support (polystyrene plate). The kit is based on VP7 recombinant protein from the AHSV (serotype 4) obtained using the baculovirus expression system to investigate AHSV specific IgG antibodies in the tested sera samples.

*AHSV Ag detection ELISA kit*

INGEZIM® PEA DAS ELISA kit (14.PEA.K2) (Madrid, Spain) was used in this study according to the manufacturer's instructions. The kit is based on a double antibody sandwich enzymatic immunoassay or capture Elisa. The plate is coated with anti-AHSV-VP7 antibodies.

*Molecular detection of AHSV*

RNA extraction

The QIAamp viral RNA Mini kit (Qiagen, Germany, GmbH) was used to extract RNA from the prepared spleen tissue. In brief, 140 µl of the sample suspension was incubated at room temperature for 10 minutes with 560 µl of AVL lysis solution and 5.6 µl of carrier RNA. 560 µl of 100% ethanol was added to the lysate after incubation. The sample was then washed and centrifuged according to the manufacturer's instructions. The nucleic acid was eluted by 60 µl of the kit's elution buffer.

PCR amplification

The current work used Oligonucleotide Primers from (Metabion Germany) to amplify an 1179 bp fragment of the AHSV VP7 gene (OIE, 2019). A 25 µl reaction was set up. 12.5 µl Quantitect probe RT-PCR buffer (Qiagen, Gmbh), 1 µl forward primer (20 pmol concentration) (5′- GTTA-AAATTCGGTTAGGATG -3′), 1 µl reverse primer (20 pmol concentration) (5′- GTAAGTGTATTCGGTATTGA -3′), 0.25 µl RT-enzyme, 5.25 µl water, and 5 µl template were used in the reaction. A Biometra thermal cycler was used to carry out the process. Reverse transcription was performed at 50°C for 30 minutes, a primary denaturation step was done at 95° C for 5 minutes, followed, followed by 35 cycles of 94°C for 30 seconds, 55°C for 1 minute, and 72°C for 2 minutes. A final extension step of 10 minutes was performed at 72°C.

Analysis of the PCR Products

The PCR products were separated using gradients of 5V/cm electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature. For gel analysis, 15 µl of the products was loaded in each gel slot. The fragment sizes were determined using a gel pilot 100 bp plus ladder (Qiagen, gmbh, Germany). A gel documentation system (Alpha Innotech, Biometra) photographed the gel, and the data was analyzed using computer software.

## Results

*AHSV antibodies detection using ELISA*

All the 2739 sera samples tested negative for AHSV antibodies as shown in Table 1.

*AHSV Ag detection using ELISA*

The ELISA results revealed that the tested 150 spleen samples were negative for AHSV Ag as shown in Table 2.

*Molecular detection of AHSV using RT-PCR*

Using the RT-PCR to detect AHSV nucleic acid, all spleen samples gave negative results comparing to the positive and negative control used in this assay.

Table 1. Results of 2739 equid sera samples collected from 2017 to 2022 and tested for the detection of antibodies against African horse sickness virus (AHSV) using Elisa.

| Governorate | 2017 | | 2018 | | 2019 | | 2020 | | 2021 | | 2022 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number | Result | Number | Result | Number | Result | Number | Result | Number | Result | Number | Result |
| Cairo | | | 30 | Negative | 45 | Negative | 45 | Negative | 60 | Negative | | |
| Giza | | | 60 | Negative | 100 | Negative | 85 | Negative | 85 | Negative | | |
| El-Menia | | | 81 | Negative | 150 | Negative | 169 | Negative | 126 | Negative | 30 | Negative |
| Alexandria | | | 26 | Negative | 27 | Negative | 25 | Negative | 25 | Negative | | |
| El Mnofiya | 130 | Negative | 117 | Negative | 75 | Negative | 109 | Negative | 75 | Negative | 20 | Negative |
| El Qalubia | | | | | | | 15 | Negative | 15 | Negative | | |
| Quena | | | | | | | | | 60 | Negative | 25 | Negative |
| Aswan | | | | | | | | | 60 | Negative | 30 | Negative |
| New Valley | 10 | Negative | | | | | | | 20 | Negative | 15 | Negative |
| Sohag | | | | | | | | | 120 | Negative | 5 | Negative |
| El Gharbia | 63 | Negative | | | | | | | | | 33 | Negative |
| El Sharkia | 39 | Negative | | | | | | | | | 35 | Negative |
| Ismailia | 25 | Negative | | | | | | | | | 10 | Negative |
| Beni Suif | 45 | Negative | | | | | | | | | 32 | Negative |
| Luxor | | | | | | | | | 40 | Negative | 35 | Negative |
| El Dakahleya | | | | | | | | | | | 50 | Negative |
| Assiut | | | 88 | Negative | | | | | 79 | Negative | 55 | Negative |
| Hurghada | | | | | | | 20 | Negative | 20 | Negative | | |
| Total | 312 | 312 | 402 | 402 | 397 | 397 | 468 | 468 | 785 | 785 | 375 | 375 |

Table 2. Results of 150 equid spleen samples collected from 2017 to 2022 and tested for the detection of African horse sickness virus (AHSV) antigen and nucleic acid using Elisa and RT-PCR assays.

| Governorate | 2017 | | 2018 | | 2019 | | 2020 | | 2021 | | 2022 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number | Results | Number | Results | Number | Results | Number | Results | Number | Result | Number | Result |
| Cairo | 9 | Negative | 23 | Negative | 17 | Negative | 2 | Negative | 6 | Negative | 1 | Negative |
| Giza | 4 | Negative | 7 | Negative | 2 | Negative | 1 | Negative | | | 5 | Negative |
| El-Menia | 2 | Negative | | | | | 1 | Negative | | | 3 | Negative |
| Alexandria | 5 | Negative | 2 | Negative | 4 | Negative | 1 | Negative | 4 | Negative | 3 | Negative |
| El Mnofiya | 1 | Negative | | | 3 | Negative | | | | | 1 | Negative |
| El Qalubia | | | 1 | Negative | 1 | Negative | 1 | Negative | 3 | Negative | | |
| Quena | | | 1 | Negative | | | | | 2 | Negative | | |
| Aswan | 1 | Negative | | | 1 | Negative | 1 | Negative | | | | |
| New Valley | | | 1 | Negative | | | | | | | | |
| Sohag | | | | | 1 | Negative | | | 2 | Negative | | |
| El Gharbia | 1 | Negative | | | | | | | 3 | Negative | | |
| El Sharkia | 1 | Negative | 1 | Negative | | | | | | | | |
| Ismailia | | | | | 2 | Negative | 1 | Negative | 1 | Negative | | |
| Beni Suif | | | 1 | Negative | 1 | Negative | | | 3 | Negative | | |
| Luxor | | | | | | | | | 2 | Negative | 1 | Negative |
| El Dakahleya | | | 2 | Negative | 1 | Negative | 1 | Negative | 1 | Negative | | |
| Assiut | | | 1 | Negative | | | | | 1 | Negative | | |
| Hurghada | | | 1 | Negative | | | 1 | Negative | | | | |
| Total | 24 | 24 | 41 | 41 | 33 | 33 | 10 | 10 | 28 | 28 | 14 | 14 |

## Discussion

AHS mostly infects horses and other equids (donkeys, mules, and zebras) and causes a deadly, acute, or subacute disease characterized by respiratory and/or circulatory symptoms. Many factors influence AHS distribution, including the effectiveness of control measures, the availability of reservoirs, vertebrate hosts, vectors, and climate (Wellby *et al*., 1996). Many African countries have high temperatures, warm to humid weather, damp to muddy soils, and plant coverings that give hygrometric factors that allow vectors to live all year (Dennis *et al*., 2019).

The epidemiology of AHSV in the Middle East and East Central Africa, particularly Egypt, is poorly documented. This cross-sectional study was conducted to estimate the prevalence of AHSV in Egypt and identify the possible risk factors related to the disease among the equid population for a better understanding of the current situation of the disease in the country.

In the present study, a total number of 2739 sera samples were collected from different Egyptian governorates during the survey performed from 2017 to 2022. The obtained data showed negative results for the detection of specific IgG antibodies against AHSV using the ELISA technique. These results came in agreement with the study of Soliman and his co-workers who showed the absence of neutralizing antibodies in 90% of non-vaccinated provinces and low titers of AHS antibodies in the rest of the provinces (Soliman *et al*., 1996). Similar to our results, the horse Population in the Eastern and central regions of Saudi Arabia was AHSV-free in the period 2014-2016 (Hemida *et al*., 2017). On the other hand, our results disagreed with the results of serosurveys carried out in other African countries. AHSV is endemic in other Sub-Saharan Africa, with estimated sero-prevalence of antibodies in horses of 86.6% in Nigeria (Ehizibolo *et al*., 2014), 81.0% in Gambia (Staeuber *et al*., 1993), 46.0% in Ethiopia (Ende *et al*., 2015), 58.93% in Cameroon (Ndebé *et al*., 2022a) and 85.9% in Sudan (Karamalla *et al*., 2018). Also, prevalence of Anti-AHSV antibodies in donkeys was reported to be 24.6%, 73%, 72.6%, 75%, 82.33% and 98 % in Ethiopia (Tesfaye *et al*., 2012), Uganda (Nakayima *et al*., 2017), Burkina Faso (Savadogo *et al*., 2018), Zimbabwe (Gordon *et al*., 2017), Cameroon (Ndebé *et al*., 2022b) and Sudan (Abu-El-zein *et al*., 1989), respectively. These discrepancies might be explained by variations in the climatic conditions, agro-ecological zones, and control strategies in each country, which have an impact on the survival and reproduction of insects as well as the spread of AHSV (Ndebé *et al*., 2022b).

The AHSV antigen can be detected in blood samples at the beginning of the febrile phase and within 90 days of exposure to the virus, however, the viral genome is detected up to 120 to 145 days post-infection (Latimer *et al*., 2003; Weyer, 2010). The equid spleen has been recommended as a suitable sample for AHSV routine diagnosis since it contains the highest amount of the virus (Tessler, 1972; Wohlsein *et al*., 1997; OIE, 2019)

Due to its great sensitivity and ability for rapid testing of large number of samples, ELISAs are frequently employed for the detection of viral infections. Moreover, the results are obtained in a short period of time much less than time needed for viral isolation. This is particularly crucial for disease like AHS, where a prompt diagnosis is required to implement control measures and prevent the spread of the disease (Laviada *et al*., 1992; Rubio *et al*., 1998).

A number of indirect or competitive ELISAs for the detection of AHSV group antigens have been developed over the last years. They are being used routinely in diagnostic laboratories and appear to have adequate sensitivity, with some of them commercially available. Polyclonal or monoclonal antibodies are used in these tests (Zientara *et al*., 2015). A sandwich ELISA was employed in this study to identify AHSV in infected spleens. The plates were coated with two monoclonal antibodies (MAbs) that recognize two non-overlapping epitopes of the major core protein (VP7) to coat solid phase and one labelled with biotin as the secondary antibody. When compared to viral isolation in cell culture, this ELISA has a sensitivity of 97.4% and a specificity of 100%, and it can identify nine AHSV serotypes (Laviada *et al*., 1992). Our findings showed that AHSV antigen detection in spleen samples using ELISA was negative in all examined samples. PCR has been a critical tool in the epidemiology, surveillance, and control-eradication programs of emerging and significant diseases such as AHSV due to its speed, sensitivity, and specificity (Fernández-Pinero *et al*., 2009). RT-PCR was carried out in this study, and all spleen samples were tested negative for viral RNA detection. These negative results ensure the results of Ag detection ELISA.

## Conclusion

Antibodies, antigens, and viral nucleic acid of AHSV are absent in all tested samples which proved that there is no circulating virus in our governorates in the period from 2017 to 2022.

## Conflict of interest

The authors declare that they have no conflict of interest.

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