The immunological & serological studies of Equine Infectious Anemia in Egyptian horses

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Abstract:

Equine infection anemia (EIA) occurs world-wide. The infection, formerly known as swamp fever, is limited to equids.The disease is characterized by recurrent febrile episodes, thrombocytopenia and anemia

We collected 10 cc. of aseptically collected whole blood samples from 180 horses at different localities in Egypt suffering from fever, illness, anemia and infertility. Samples may be submitted as serum or whole blood should be collected in vacutainers without anti-coagulant using disposable needles and sterile syringes. Samples must be properly identified, carefully packed, and sent as soon as possible to diagnostic lab. Clinical diagnosiswas done by applied Agar gel immunodiffusion and ELISA tests.

In this study recorded that **33 (18.33%)** positive EIA by using ELISA test but was **24 (13.33%)** by AGID test. The highest incidence was in Kaliobia Gov.**8/35** samples **22.85%** followed by Cairo **9/50** samples **18%,** then Giza **11/65** samples **16.92%** and Fayoum **5/30** samples **16.66%** by ELISA test. The same results were inKaliobia Gov. **5/35** samples **14.29%** followed by Cairo **7/50** samples **14%** then **9/65** samples **13.85%** in Giza and **3/30** samples **10%** in Fayoum by using AGID test.

Finally, we concluded that combining the specificity of AGID test with higher sensitivity, such as the ELISA test to enhance the accuracy of EIA diagnosis.

Introduction:

Equine infectious anemia (EIA) is an infectious viral disease of equidae caused by retrovirus that is related to the lentiviruses in the family Retroviridae, characterized by a variety of symptoms related to anemia that accompany either an acute, subacute or chronic illness that may terminate by death. The disease may be subclinical in some individuals ***(Leroux, et al, 2004)***.

Horses, ponies, mules and donkeys are the only known natural hosts of EIA virus. The virus was found in blood, milk, saliva, feces and semen of diseased horses. Clinically sick horses have higher titer of their blood and tissue than apparent carriers foals born from infected dams may carry maternal antibody

titer for 2-6 months after birth and mother's milk can transfer the infection , if the foal is infected they remain viramic carrier for life ***(OIE, 2008& 2013)*** . Most naturally occurring outbreaks of this disease develop during the late summer and early fall months ***(Yang et al, 2011).*** This coincides with the peak of the biting insect population, especially blood sucking horseflies. Transmission of infected blood by vectors is an important cause of natural outbreaks. Transmission via contaminated hypodermic needles or other instruments can produce new infections. All horses positive for EIA are potential spreaders of EIA infection ***(Craigo &Montelaro, 2013).***

The incubation period for EIA following the subcutaneous inoculation of infective blood into a susceptible horse is usually about 14 days up to several months. The initial illness is commonly acute or subacute with definite clinical signs, and lasts from 3 to 20 days followed by cycles of febrile periods and death or apparent recovery.During an acute attack, a severe anemia develops due to a destruction of red blood cells with leucopenia then death related to the severity of the anemia or due to intravascular clotting of blood and thrombus formation. In chronic form most infected horses are unable to perform hard work and may be unsatisfactory breeding ***(Albrecht, et al, 2000)*** and The virus titre is higher in horses with clinical signs and the risk of transmission is higher from these animals than carrier animals with a lower virus titre.Agar gel Immunodiffusion (AGID) tests ***(Coggins et al, 1972)*** and enzyme-linked immunosorbent assays(ELISAs) ***(Suzuki et al, 1982)*** are accurate, reliable tests for the detection of EIA in horses.

Materials and methods;

Samples:

Ten cc. whole blood samples were collected aseptically from 180 horses at different localities in Egypt suffering from fever, illness,anemia and infertility. Samples was submitted as serum or whole blood collected in vacutainers without anti-coagulant using disposable needles and sterile syringes. Samples were properly identified, carefully packed, and sent as soon as possible to diagnostic lab.Foals under six months of age who are nursing their dam are excluded from testing. Clinical laboratory diagnosis was done by applied Agar gel immunodiffusion and ELISA tests.

AGIDTEST: (coggins test)

The AGID was performed according to the instructions of the OIEmanual (2008) using 1% agarose (Invitrogen) in borate buffer. The positive OIE standard anti-EIAV serum was tested 2×concentrated, undiluted and 2×diluted. In addition to this standard serum, the

EIAV positive serum from the commercial IDEXX AGID EIA Ab

Test was also used and the anti-EIAV positive serum from IDEXX Lab
(USA). The tests were performed with 20 L of both reagents (antigen and serum). The optimal rp26 antigen concentration was determined as 2×diluted.

ELISA TEST:
A 96-wells ELISA microplate (IDEXX cELISA EIA Test,
USA) was coated with 100 L of the EIAVrp26 antigen. The optimum antigen concentration (3.75×diluted)determined by titration against OIE standard serum was used in0.05 M carbonate-bicarbonate buffer, pH 9.6 and incubated for 2 hin a humid chamber at 37 ◦C. The blocking step was performed with200 L of 4% non-fat dry milk solution and incubated for 1 h. Threewashings stages were carried out with 200 L of 0.1% PBS-Tween20 (PBS-T). The sera were diluted in a solution containing 0.1%PBS-T, 2% non-fat dry milk and 10 mM EDTA, added in the wells,incubated at 37 ◦C for 1 h and washed. After this, 100 L of conjugate(protein G horseradish peroxidase conjugated) was added anddiluted 1:90,000 in a solution containing 0.1% PBS-T and 2% defattened dry milk. After being incubated again for 1 h at 37 ◦C, the plates were washed 5 times. Next, 100 L of chromogenic substrate 0.1 Mcitrate-phosphate buffer (pH 5.0) was used. Finally, the plates were incubated at room temperature for 15 min and 100 L of 2 N sulfuricacid was added. The plates were read using an ELISA reader(Thermo Fisher Scientific, USA) at a wavelength of450 nm.

Results:
Agar gel immunodiffusion (AGID) test, also known as the Coggins test detects antibodies from equine blood against the EIA virus followed by the competitive enzyme-linked immunosorbent assay (cELISA) test, commonly used for EIA (Table, 1).

Table 1: *Serological results of serum samples from equids tested by ELISA test and AGID test.*

Localities	No. of horses	ELISAs test*	AGID test
- Cairo Governorate	50	9(18%)	7(14%)
- Giza Governorate	65	11(16.92%)	9(13.85%)
- Kaliobia Governorate	35	8(22.85%)	5(14.29%)
- Fayoum Governorate	30	5(16.66%)	3(10%)
Total	180	33 18.33%	24 13.33%

- US kits

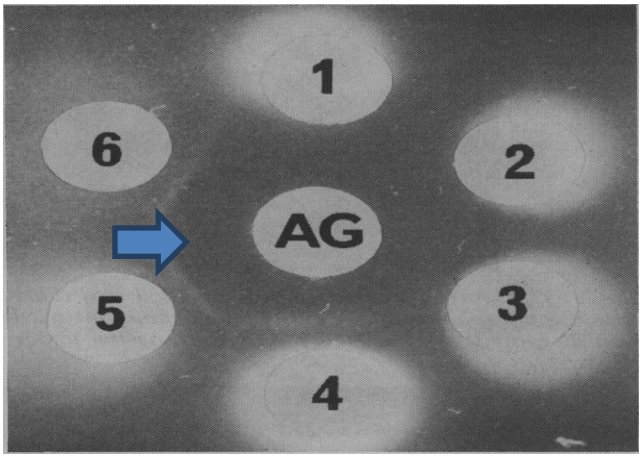

Fig 1: *Shows the positive reaction to AGID test.*

Discussion:

The horse industry is vitally important to economy and the veterinarian has a statutory responsibility to take all reasonable steps to protect it, the strong support of industry leadersand devised a regulatory program to control EIA in the country ***(Quinlivan et al, 2013)***

Equine Infectious Anemia is a viral disease for which there is no vaccine and no cure. Though most horses succumb rapidly to EIA a percentage of infected horses appear to recover. However they still harbor the virus and during times of stress may become ill again. It is because of these healthy appearing carriers that we test horses ***(Robert & Oglesby, 2007).***

In this study recorded that **33 (18.33%)** positive EIA by using ELISA test but was **24 (13.33%)** by AGID test (**Table, 1 & Fig. 1**). These results are consistent with results recorded by ***Cappelli et al (2011),*** who showed that AGID test checks for Equine Infectious Anemia antibodies in the horse's blood must be accompanied with ELISA test. The highest incidence was in Kaliobia Gov. **22.85%** followed by Cairo **18%,** then Giza **16.92%** and Fayoum **19.66%** by ELISA test. The same results were inKaliobia Gov.**14.29%** followed by Cairo **14%** then **13.85%** in Giza and **10%** in Fayoum by using AGID test (**Table, 1**).

An animal that tests positive on one occasion will do so for the rest of its life. The cELISA test is accepted for movement throughout countries. The current testing program has gone a long way toward reducing this disease. In the 1970's this disease killed many thousands of horses.It is important that your horse's pasture mates are as healthy appearing as your own horse. Board your horse only where a negative Coggins test is required of all horses before they come on the premises ***(Robert & Oglesby, 2007 and Scicluna et al, 2013).***

Infected equids become lifelong carriers, and must be permanently

isolated from other susceptible animals. Many countries have control programs requiring equids to be tested for equine infectious anemia. Most programs require one or more tests, particularly before entry of the horse into the herd, participation in organized activities and/or sale of the horse. Regular voluntary testing of the equids on a farm, as well as testing of new animals before introduction, is helpful in maintaining an EIA-free herd. No vaccine is available now ***(Brangan, et al, 2008 and Jiansen,et al, 2014).***

Finally, we concluded that combining the specificity of AGID test with higher sensitivity, such as the ELISA test to enhance the accuracy of EIA diagnosis and applied these recommendations:

- If a positive test is confirmed, the animal and its herd mates will be quarantined.
- Isolate all EIA animals at least one-quarter mile from all other non-infected equine.
- Isolate the EIA animals in an insect-free enclosure.
- Equines being entered into exhibitions or competitive events must be tested for EIA to ensure that all participating equines are test-negative.
- All equines that moved must be tested for EIA with a negative result within 12 months prior to movement and must be signed by an accredited veterinarian.
- A negative EIA test is required for all equines prior to sale.
- Repeat testing and removal of reactors when a reactor is detected in a herd and removed, testing for EIA must be repeated until all remaining equines on the premises test negative.The remaining animals in the herd must be retested at 30- to 60-day intervals, until no new cases are found. Once the remaining equines in the herd have negative test results for a minimum of 60 days, the quarantine may be lifted.
- A successful EIA control program should include an educational program directed toward equine owners in the industry.

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