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SEROEPIDEMILOGICAL STUDIES ON EQUINE INFLUENZA WITH SPECIAL REFERENCE TO BIOSECURITY APPLICATIONS IN EQUINE FARMS IN EGYPT

By

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ABSTRACT

Equine influenza virus (EIV) is caused by two main strains of influenza A (equi-1 and equi-2) and is considered the most important cause of epizootic respiratory disease of equine because it is highly contagious and has the potential to disrupt major equestrian events. Equine influenza (EI) can be controlled by vaccination but it has been demonstrated repeatedly in the field that antigenic drift impacts on vaccine efficacy so the bio risk regulations is urgently needed and appropriate bio risk management strategies should be developed established and implemented in the control programs to deal with this possibility. In this study a total number 357 of different equidea species were examined for the presence of clinical signs of Equine Influenza including fever, harsh, dry cough and mucopurulent discharge. Serum samples (n=357) were collected from the examined equidea from ten Egyptians governorates classified into three regions, Upper Egypt (Beni Suef - Fayoum - Qena- Luxor), Central region (Cairo - Giza) and Lower region (Alexandria - Kafr El-Sheikh - El Beheira - Gharbia). ELISA was conducted on the sera samples to detect antibodies against EIV, where58% of samples were tested positive for ELISA against EIV from 95% unvaccinated equidea. Biosecurity measures were applied on farm from the central region against another farm with no biosecurity measures application for a period from 2014 till 2016 and the result was decreasing the percentage of the clinically suspected cases (from 33% till 10%) in the farm which apply the biosecurity measures and the other one still show increasing in the new clinically suspected cases (from 46% to 50%).

Keywords:

Equine, Influenza, ELISA, HI test, Biosecurity, Respiratory.

INTRODUCTION

Equine influenza is considered the most economically important respiratory disease of the equine (horses, donkeys, mules and zebras) in countries with basic breeding and industries. Also, is considered the most important respiratory virus of horses because it is highly contagious and has the potential to disrupt major equestrian events **Cullinane *et al.,* (2010)**.Clinical signs include pyrexia, nasal discharge and a harsh dry cough; pneumonia in young foals and donkeys and encephalitis in horses have been described as rare events **(Daly *et al.,* 2006 and Gerber, 1970).** Equine influenza virus belongs to the Orthomyxoviridae family of the genus influenza A (a single stranded, negative-sense RNA virus). Influenza A viruses can be divided into subtypes on the basis of the antigenic reactivity of the surface glycoprotein's, the Hemagglutinin (H1 – H16) and Neuraminidase (N1 – N9) molecules **(Fouchier *et al.,* 2005).** There are two distinct subtypes, H7N7, formerly equi-1 and H3N8, formerly equi-2 **(Sovinova *et al.,* 1958; Wadell *et al.,* 1963 and Annon, 2006).** It is generally accepted that, the former is no longer in circulation as the last confirmed outbreak caused by this virus was in 1978 **(Webster, 1993 and OIE, 2015)**, however, outbreaks caused by H3N8 viruses occur annually. **Mumford *et al.,* (1998)** found that, the pathogen was responsible for two-thirds of equine viral respiratory infections in Colorado. Two major strains known to cause disease in equines are H7N7 (A/eq/Prague/56); first isolated in (Czechoslovakia) in 1956 and H3N8 (A/eq/Miami/2/63) first isolated in Miami in 1963 (**Webster, 1993)**. So, first recognized in 1963 as a cause of widespread epidemics and has subsequently become endemic in many countries, except for New Zealand and Iceland. China, Japan, and Australia experienced devastating epidemics of equine influenza affecting tens of thousands of horses in 2007. Equine influenza had not been reported in China since 1993, in Japan since 1972, and had never been reported in Australia **(NRCE, 2008).** It is crucial to have specific and sensitive diagnostic/screening virus detection method for rapid diagnosis of equine influenza. Where, the gold standard has been virus isolation using Embryonated hen eggs. Other diagnostic tests include virus culture in Madin Darby canine kidney cells or measurement of a rise in antibody titer in paired sera by haemagglutination inhibition assay **(OIE, 2000).** However, those techniques are laborious and time consuming. As well as, the sensitivity of virus isolation is dependent on the presence of infectious

particles and some virus strains are difficult to isolate **(OIE, 1996).** Serological techniques provide only retrospective data which make the quick intervention and management not easy. In recent years' enzyme-linked immunosorbent assays (ELISA) and reverse transcription (RT)-PCR have been used to provide more timely results although the sensitivity of ELISA has been quite variable **(Morley *et al.,* 1995; Quinlivan *et al.,* 2004 and Van Maanen *et al.,* 2003).**

MATERIAL AND METHODS

Animals:

A total of 357 of equidae spp. (horse, donkey and mules) from three regions, Central (Cairo - Giza), Lower Egypt (Alexandria - Kafr El-Sheikh – El Beheira - Gharbia) and Upper Egypt (Beni Suef - Fayoum – Qena- Luxor) as showed in (Table 1). These equidea from ten Egyptians governorate were examined in this study in the period from October 2014 till February 2016. The animals were subjected for clinical examination as well as all the epidemiological data were recorded (species, breeds, sex, age, season and localities). Also, the hygienic status, measures, previous medications and vaccination status were recorded. Also, 95% of these animals were unvaccinated. The examined animals were suffering from clinical respiratory signs appeared as Pyrexia, harsh dry cough and mucopurulent nasal discharge.

Samples:

A total of 357 sera samples were collected from rom 357 different equidea spp. (horse, donkey, mules) suspected of viral respiratory disease during a period from October 2014 till February 2016 for Enzyme Linked Immuno-Sorbent Assay (ELISA) and Hemagglutination Inhibition (HI) testing.

Serological tests:

ELISA (enzyme-linked immunosorbent assay).

ELISA (enzyme-linked immunosorbent assay), was used to determine the presence of antibodies (indirect ELISA) against the causative agents of EIV. The reaction was carried out according to all instructions by the producer of the ELISA kit - ***ID Screen® Influenza A Antibody Competition Multi-species ELISA KIT,*** **FRANCE**. The reaction was read on an ELISA reader at wavelength of 450 nm and adapted from the OIE Manual of Standards for Diagnostic Tests and Vaccines **OIE, (2000) and Kittelberger *et al.,* (2011)**.

Haemagglutination inhibition test (HI).

HI test was used to determine the presence of antibodies against the equine influenza virus. Also, The HI test targets cross-reactivity of hemagglutinin protein of avian H7 and H3 origin. The HI titer was taken as the highest dilution of serum with complete inhibition of agglutination in a twofold serial dilution of the original sera.

Table (1): Distribution of equidea according to their localities.

Governorate	*A. Horse*	*N. Horse*	*Donkey*	*Mule*	*Total*
Cairo	**57**	**0**	**0**	**0**	**57**
Giza	**17**	**0**	**0**	**0**	**17**
Alexandria	**0**	**11**	**0**	**1**	**12**
El Beheira	**10**	**0**	**0**	**0**	**10**
Gharbia	**20**	**4**	**25**	**3**	**52**
Beni Suef	**0**	**9**	**41**	**3**	**53**
Kafr El-Sheikh	**0**	**32**	**18**	**0**	**50**
Fayoum	**10**	**3**	**14**	**0**	**27**
Qena	**0**	**43**	**0**	**0**	**43**
Luxour	**15**	**6**	**4**	**11**	**36**
Total	**129**	**108**	**102**	**18**	**357**

A. Horse: Arabian horse breed, N. Horse: Native horse breed.

BIO SECURITY measures SOPs:

There are three principle steps for biosecurity: Physical segregation, Cleaning- for removing contamination and Disinfection- for killing any remaining virus.

RESULTS AND DISCUSSION

Three hundred and fifty-seven sera samples submitted from cases of suspected viral respiratory disease were tested for the presence of antibodies against equine influenza virus and the prevalence in different governorates (Table 2).

Table (2): Results of indirect ELISA of the sera samples.

Governorate	No. of sera samples	No of positive samples	Positivity %
Alexandria	12	0	0
Gharbia	52	37	11
Kafr El-Sheikh	50	25	7
El Beheira	10	0	0
Cairo	57	39	11
Giza	17	8	2
Beni Suef	53	46	13
Luxour	36	12	3
Fayoum	27	18	5
Qena	43	22	6
Total	357	207	58

The results revealed that 58 % (207 out of 357) of samples showed positive ELISA which means that those animals had antibodies specific for EIV in their blood, while 42% were ELISA negative as shown in Fig.(1).

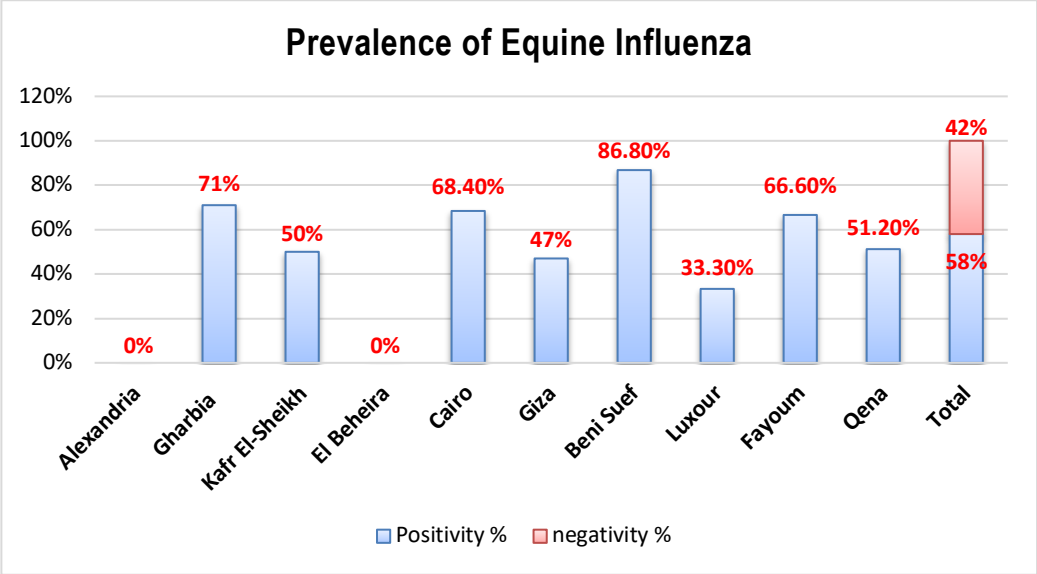

Fig. (1): Prevalence of equine influenza among equines (ELISA results) in different governorates.

Also, ELISA testing results of sera samples allocates of the country upper, lower and central regions showed that, the highest percentage of positivity was noticed upper Egypt region in (Beni Suef - Fayoum - Qena - Luxor) at a percentage of 28% and the delta region was (17%)

in (Alexandria - Kafr El-Sheikh - El Beheira-Gharbia) governorates, while the lowest positive percentage was found in the central region was 13% in (Cairo - Giza) governorates as shown in Fig. (2). The results of high prevalence of Equine influenza in the equidae in Upper Egypt may be due to the very bad hygienic measures as well as neglected vaccination protocols of the reared equidae also intensive collection of those animals in a restricted condensed area that facilitates the easy transmission and spreading of the infection among those animals.

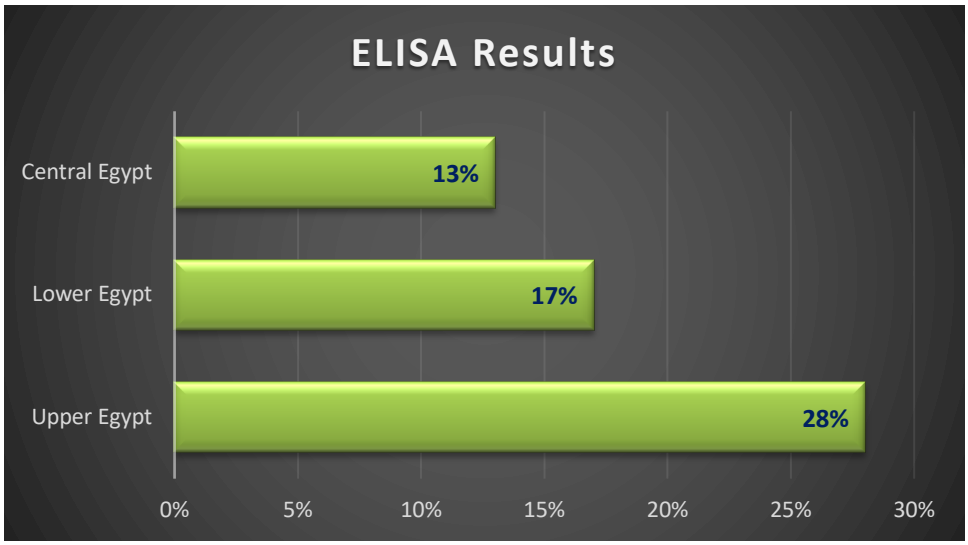

Fig. (2): ELISA results among sera samples of equidea in the three localities.

High prevalence of EI in the region of Upper Egypt were may confirm that, the region that has low biosecurity measures show the high positivity % of the infection of EIV. This result is agree with, **Annon, 2006; Kickingbird, 2008 and Clement *et al.*, 2016.** Concerning the epidemiological data of EIV infection, the prevalence of equine influenza infection depending upon the results of indirect ELISA showed that the highest percentage of positivity for the age group more than 3 years old (32%) and the lowest percentages was for the age group of (1-3) years old (17%), while the age group of up to one year was (9%) as shown in Fig. (3). The high prevalence of infection among the age group more than 3 years (32%) may be due to heavy working and stress as well as parasitic infections and neglected anthelmintic treatment that predisposed the immune suppression that made the viral infection easier beside gathering and grouping of the adult age category that made the infection rate high.

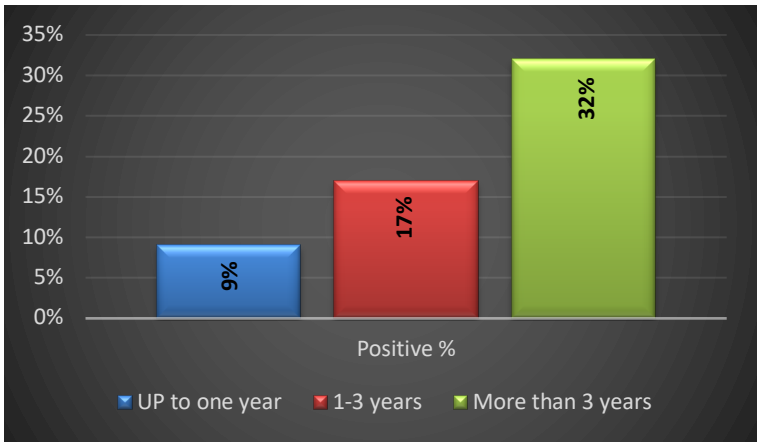

Fig. (3): Prevalence of equine influenza infection in relation to the age.

Also, the results of indirect ELISA showed the highest positivity in Donkeys as 34.8% (72 out of 207) and lowest was for the mules as 5.8% (12 out of 207), while in Arabian horses the positive percentage was 33.3% (69 out of 207) and in native horse's positivity percentage was 26% (54 out of 207) as shown in figure (4). High prevalence of EIV in donkeys may be due to very weak immune status and high prevalence of parasitic infections among donkeys as well as very bad nutrition status may play a role in the infection rate among donkeys. The uncontrolled mixing of un-examined donkeys and horses from unknown backgrounds at these events is the perfect medium for disease transmission. Endemic diseases such as respiratory viruses and bacteria, and fungal skin infections can spread with ease from infected horses, ponies or donkeys in close contact with other equidae or where equipment is shared. Other endemic transmissible equine diseases have similar clinical presentations in donkeys and horses although there is a perception as evidenced that donkeys (as compared to horses) show fewer signs of disease and might thus act as reservoirs of same. Also, the Arabian and native horses showed high prevalence that may be due to immunosuppression as well as bad handling and management practices.

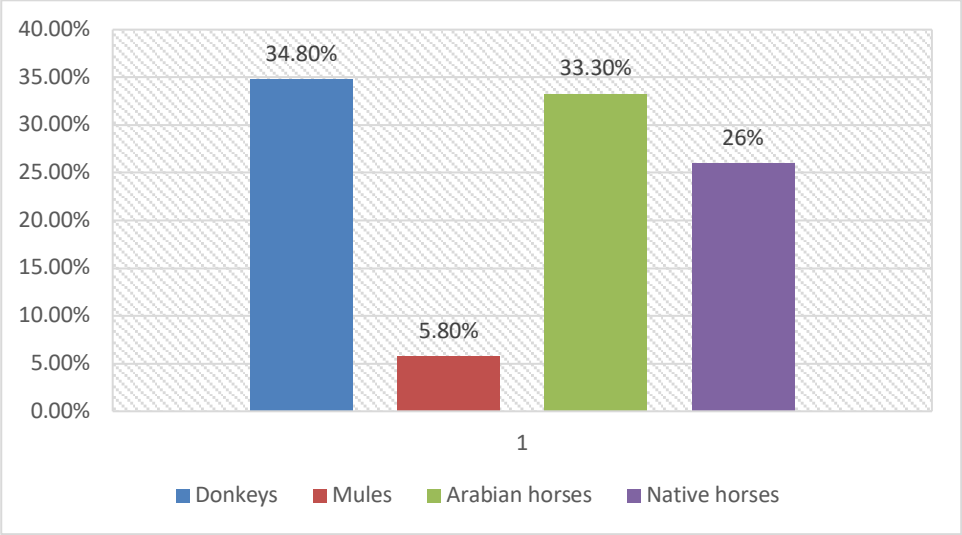

Fig. (4): Prevalence of equine influenza infection in relation to the equine species.

Also, the results of indirect ELISA showed the highest positivity was for unvaccinated equidea 96.2% (199 out of 207) and the lowest was for vaccinated 3.8% (8 out of 207) as shown in Fig. (5).

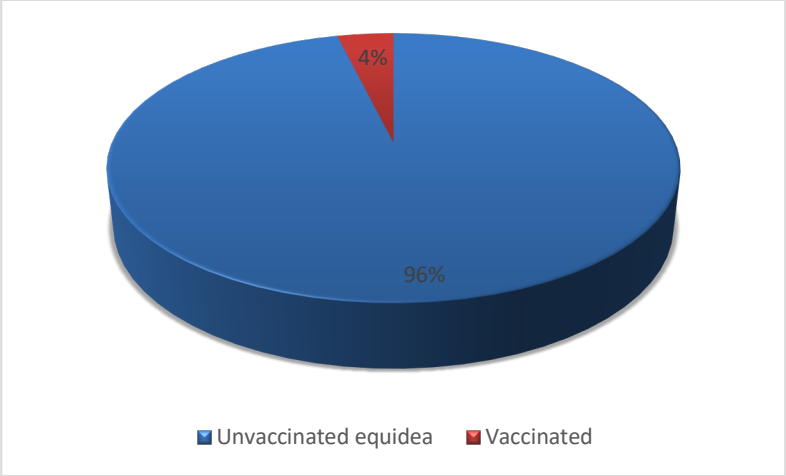

Fig. (5): Prevalence of equine influenza infection among vaccinated and unvaccinated equidea.

Haemagglutination Inhibition test was also used as a direct and quick diagnostic tool of Equine influenza virus, where 67% (240 out of 357) were HI positive and those animals were concentrated in Upper Egypt region and the rest 33% were react HI negative as shown in Fig. (6). Biosecurity measures includes the three principle steps for biosecurity: Physical segregation, cleaning-for removing contamination and Disinfection- for killing any remaining virus. These biosecurity measures were applied on farm from the central region against

another farm with no biosecurity measures application for a period from 2014 till 2016 and the results showed reduction in the percentage of the clinically suspected cases (from 33% till 10%) in the farm which apply the biosecurity measures and the other farm still showing increasing rate in the new clinically cases (from 46% to 50%).

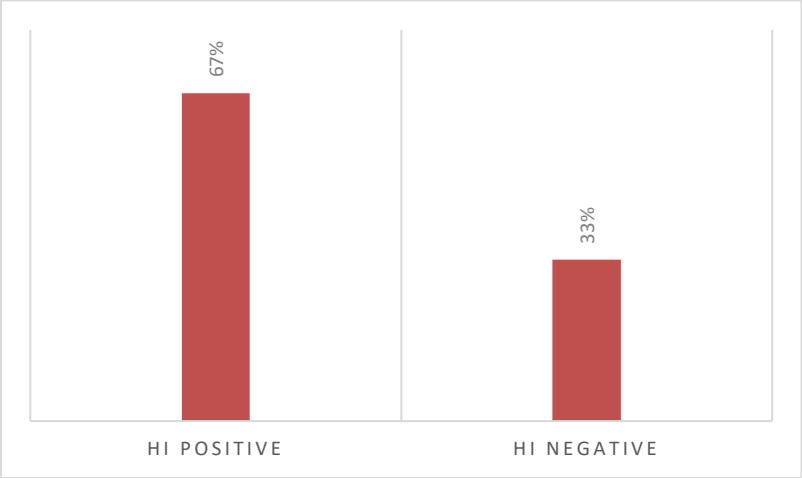

Fig. (6): Prevalence of equine influenza infection among equidea using HI test.

CONCLUSION

In conclusion, a rapid accurate sensitive diagnostic technique should be developed for detection of EI infection. The RT-PCR can be reliably used for the quantification of equine influenza virus in vaccine efficacy studies. Given the propensity of influenza A virus to cross the species **(Webster and Guo, 1991 and Daly *et al.*, 2004)** and their zoonotic potential **(Dowdle and Hattwick, 1977; Koopmans *et al.*, 2003 and de Jong *et al.*, 2005)** this assay can be used with relative ease and speed to aid virus surveillance that is advantageous not only to the equine industry but also to public health.

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