Serological detection of H3N8 equine influenza A virus in some Egyptian provinces

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Abstract:

Equine influenza virus (EIA) type A virus (H3N8 and H7N7) is a leading cause of serious respiratory disease in horses causing worldwide morbidity and economic losses. In this study, serological evidence of EIV subtype H3N8 was investigated in horses and donkeys in 4 Egyptian provinces (Cairo, Giza, Sharkia and Gharbia) in between (December 2017 and January 2019). Serum samples of 164 horses and 98 donkeys were examined by ELISA and HI tests giving 63 positive horses serum samples (38.41%) for EIV antibodies, while 32 donkey serum samples were positive (32.65%). Sharkia province showed the highest EI prevalence in horse serum samples with 47.87 % prevalence rate followed by Gharbia, Cairo, and Giza provinces with 40%, 11.11% and 11.76% respectively by ELISA. Furthermore, Sharkia province also showed the highest EI prevalence rate (44.44 %) in donkey sera followed by Gharbia and Cairo provinces with (23.07%), and (16.67 %) respectively by ELISA. The results of this study showed higher predominance of the virus antibodies in horses in winter season with 43.18% and lower rate in summer with 27.94% by ELISA. Considering the age, EIV antibodies varied considerably in different age groups by ELISA, animals of 1-3 years old showed the highest EIV prevalence (43.96) %. while, the lowest rate (24.14 %) was observed in animals younger than one year. in contrast HI test results showed variations in different age groups; animals of less than one-year-old showed the highest viral prevalence, with an average of (8.62 %). While the lowest prevalence rate (1.13 %) was observed in animals older than three years.

Keywords: EIV H3N8, ELISA, HI test and Equine influenza.

Introduction

Equine influenza (EI) is a globally distributed extremely contagious viral disease of horses caused by Influenza A virus subtypes H3N8 and H7N7 ***(Sovinova et al., 1958)***

causing high morbidity and low to moderate mortality rates. EIV is known to infect both human and dogs ***(Crispe et al., 2011)*** EIV belongs to family Orthomyxoviridae genus Influenza A virus which is an enveloped virus with helical RNA genome ***(Webster and Thomas, 1993).*** It possesses eight single segmented negative sense RNA strands that sub-typed based on hemagglutinin (HA) and neuraminidase (NA) encoding at least 10 viral proteins which are HA, NA, NP, M1, M2, PB1, PB2, PA, NEP and NS1 ***(Cullinane and Newton, 2013).*** EI is mainly caused by H7N7, first isolated in Eastern Europe in 1956 ***(Sovinova et al., 1958)*** H7N7 EIV has not been isolated since1980s ***(Karamendin et al., 2016)*** and subtype 2 named H3N8, first isolated in USA in 1963 ***(Waddell, 1963).*** Egypt is a hub center for raising and marketing of the pure breed Arabian horses ***(Kalad et al., 2013).*** The number of equines exceeded 3.4 million heads: 64000 horses, 3.4 million asses and 1160 mules ***(FAO, 2011).*** Therefore, the contribution of equine influenza in the expansion of the gene pool of Influenza A viruses in Egypt should not be neglected ***(Kalad et al., 2008).*** Egypt is considered a hotspot for the evolution of a pandemic potential Influenza A virus (IAV), either via antigenic drift of the H5N1 to increase its adaptation to humans ***(Watanabe et al., 2011)*** or through assortment with other IAV subtypes, especially H3N2 virus ***(Fuller et al., 2013)*** or H9N2 ***(Arafa et al., 2012).*** Egypt experienced 3 outbreaks of equine influenza H3N8 in horses, mules and donkeys. The first outbreak was in 1989 ***(Ismail et al., 1990).*** The second outbreak was in 2000 ***(Hamoda et al., 2001)*** and the most severe third outbreak in 2008 ***(Aly et al., 2009)***. The overall aim of the present work was designed to evaluate the current situation of equine influenza virus in Egypt through Serological detection of EIV H3N8 antibodies in serum of randomly collected samples from horses and donkeys in 4 provinces in Egypt by enzyme linked immunosorbent assay (ELISA) and hemagglutination inhibition (HI) test.

Materials and methods

Samples: Two hundred and sixty-two blood samples (164 from horses & 98 from donkey) were collected from apparently healthy horses and donkeys in four provinces of Egypt. All examined animals are household animals, non-vaccinated and age ranged from results display age less than 3 years old and more than 1-year-old. Distribution of samples in each province was shown in table (1). 5 ml was collected from the jugular vein then allowed to clot without anticoagulant and then serum was collected and kept frozen at -20˚c till screening of EIV antibodies.

Studied area: Four provinces in Egypt were studied for presence of EIV in different equines (horses and donkeys) during the period from December 2017 to January 2019 based on high population of equine species.

Screening of influenza-A antibodies by competitive ELISA: ID vet. FLUACA ver 0917 EN, France (www.IDvet.com) is ID Screen® Influenza A Antibody Competition Multi-species Competitive ELISA a commercial diagnostic ELISA test kits designed to detect antibodies against the internal nucleoprotein of influenza A virus. The procedures were done according to manufacturer's instructions. The Kit contains microplates coated with nucleoprotein of influenza A, anti NP peroxidase conjugate, dilution buffer, washing buffer, substrate, stopping solution, positive control and negative control.

Haemagglutination Inhibition Assay: The haemagglutination inhibition (HI) test was performed in microtiter plates according to standard procedures ***(OIE, 2018).*** The concentrations of antibody/virus are expressed as the reciprocal of the minimum dilution required to completely inhibit haemagglutination. Subtyping of equine influenza viruses was determined by haemagglutination inhibition using H3N8 specific antisera. Samples were firstly treated with Tween 80/ether, which destroys viral infectivity, reduces the risk of cross contamination, and enhances the HA activity ***(John and Fulginiti, 1966).***

Results

Serological evidence of EIV in horses: A total of 164 horse's serum samples were examined for serological evidence of EIV antibodies by competitive ELISA and HI test. 63 horses '(38.41 %) had positive antibodies to EIV by ELISA (Table 2). The highest prevalence was observed in Sharkia province with 47.87 % followed by Gharbia province with a percentage of 40%. The seroprevalence for EIV in Cairo and Giza were 11.11% and 11.76% respectively. HI test results revealed 50 positive samples (30.48 %) had antibodies to EIV. The highest prevalence was observed in Sharkia province with 41.48 % followed by Gharbia province with a percentage of 25.71 %. The highest antibody titer 256 was evidenced in Sharkia and the lowest titer 16 was observed in Giza province.

Serological evidence of EIV in donkeys: ninety-eight donkeys were examined for EIV antibodies, 32 produced positive results (32.65%) by ELISA (Table 3). The highest EIV prevalence was observed in Sharkia province (44.44%) followed by Gharbia province (23. 07%).The seroprevalence for EIV in Cairo was (16.67%). The highest ELISA titer (0.131) was evidenced in

Sharkia, and the lowest titer (0.036) was observed in Cairo province

While using HI (H3 antigen) test reveal 24 positive samples (24.48 %) had antibodies to EIV. The highest prevalence was observed in Sharkia province with 30.55 % followed by Gharbia province with a percentage of 23.21 %.

Distribution of seropositive samples of horses and donkeys according to the age: EIV seropositive samples in horse and donkeys were distributed in three different age groups for the 4 studied provinces (Table 4). In general, the group of 1–3-year-old age showed the highest incidence in all four-provinces studied with an average percentage of 36.26 %. The lowest incidence rate 24.14% was observed in animals less than one-year-old and 34.09 % in animals more than 3 years old. Meanwhile when examined by HI-H3 antigen, the results revealed that average percentage of the highest antibody titer in all four-provinces studied was 38.79% for animals aging 1-3years old.

Table (1): *Distribution of blood samples in 4 provinces of Egypt*

Province	Serum Samples	
	Horses	Donkey
Sharkia	113	36
Gharbia	35	56
Cairo	18	6
Giza	17	0
Total	164	98

(N.B): all samples collected from apparently healthy non vaccinated animals

Table (2): *Seroprevalence of equine influenza virus in horses at 4 provinces study sites*

Province	Total No	Elisa (M protein)		HI test (H3 protien antigen)	
		+ve	Mean OD	+ve	Mean HI log 2
Cairo	18	2	0.061	1	7
Giza	17	2	0.059	1	5
Sharkia	94	45	0.103	39	5.41
Gharbia	35	14	0.127	9	5.22
Total	164	63		50	

Table (3): *Seroprevalence of equine influenza in donkey at 4 provinces study sites*

Province	Total No	Elisa (M protein)		HI test (H3 protien)	
		+ve	Mean OD	+ve	Mean HI log 2
Cairo	6	1	0.036	0	0
Giza	0	0	0.000	0	0
Sharkia	36	16	0.131	11	5.81
Gharbia	56	15	0.128	13	5.31
Total	98	32		24	

Table (4): *Distribution of seropositive EIV in 4 studied sites related to age*

Age	Total No	Elisa (M protein)		HI (H3 protien)	
		+ve	Mean OD	+ve	Mean HI log 2
< 1 y	58	14	0.196	5	4.4
1-3 y	116	51	0,050	45	5.42
> 3 y	88	30	0,112	24	5.54
Total	262	95		74	

Discussion:

Equine influenza is widespread infective disease of equine throughout the world. Consider as one of the most important Equidae species diseases that affect equine and their owners ***(Waghmare et al., 2010)***. Due to inconvenience in horse stables, explosive outbreak and high economic losses in horse industry, it consider one of the most important equine diseases that have capacity to close the racing championship in a country for a period of months due to restriction of horse movement and quarantine periods ***(Powell et al., 1995).*** since the last H3N8 outbreak in 2008 in Egypt, EIV current situations in Egyptian equine populations are unclear and not fully understood ***(Soliman et al., 2008)***, hence the overall aim of the present work was designed to evaluate the current situation of equine influenza virus in Egypt and designing a control strategy for control and prevention of equine influenza by investigating the presence of antibodies to the infectious agents in serum of animals through serological detection of EIV antibodies in serum of apparently healthy horses and donkeys. Serological investigation was found the most effective method of detecting EIV infections in equines during USA outbreaks ***(Morley et al., 2000).*** According to the owners, none of the animals in the current study had a history of travel and none had been vaccinated against EIV. All the animals appeared healthy at the time of sampling. Serum samples were tested for antibodies to influenza A by competitive ELISA. The test utilizes a well conserved influenza A virus nucleoprotein ***(Gorman et al., 1991)***, and can detect antibodies to all influenza A virus subtypes including H3N8 and H7N7. A total of 262 horses and donkeys at 4 study sites were examined for serological evidence of influenza-A antibodies in the period between December 2017 and January 2019. Overall, 63 positive samples (38.41%) horses and 32 (32.65%) donkeys had antibodies to influenza A by ELISA (Table 2 and 3). The highest prevalence was observed in Sharkia province followed by Gharbia province in both horse and donkeys. These results are correlated with the large number of collected samples and with the fact that Sharkia and Gharbia provinces had a high

intensity of Arabian horse industry when compared to Giza and Cairo provinces. In the present study, the prevalence rates of influenza-A antibodies in equine sera were 38.41% in horses and 32.65% in donkeys with an overall prevalence rate of 24.04 %. These results are parallel to that obtained in Turkey by ***(Ataseven and DALY, 2007)*** who recorded the prevalence of influenza A antibodies in horses, mules and donkeys, 41.8%, 12.8% and 9.4% respectively. On the other hand, ***(Rose et al., 1970)*** has believed that donkeys are more susceptible to the disease than horses. The diagnosis of EIV could be based on virus isolation in embryonated eggs from equine with acute infection. However, the demonstration of an antibody response is evidence of previous infection ***(Adeyefa and McCauley, 1994).*** We noticed that total positive percent of serum samples by ELISA test was higher than HI test as we use only H3 subtype in HI test while in ELISA it detects any influenza A virus with any subtype. The high antibody titers observed in this study (Table 2 and 3) cannot be accounted for vaccination. Age group of 1-3 year showed the highest incidence in all four-provinces studied with an average percentage of 43.96 %. The lowest incidence rate (24.14%) was observed in animals less than one-year-old and 34.09 % in animals more than 3 years. The same results were obtained by ***(Morley et al., 2000)*** whose noted that young horses have a much greater risk of contracting the disease than older horses. Moreover, naïve horses may be infected with avian H3N8 or canine H3N8 influenza virus which may cause high mortality ***(Harder and Vahlenkamp, 2010)***. Internationally, the movement of horses has been identified as key for spreading disease between countries ***(Sluyter, 2001).*** In conclusion, the present study clearly shows presence of influenza-A antibodies in horses and donkeys in 4 provinces in Egypt, which is possibly resulted from natural infection since the tested animals were not vaccinated. Further epizootiology investigation of EIV at the country level is important to monitor and determine the magnitude of EIV infection and its economic impact on Egyptian equine breeding.

Conclusion

Based on the obtained results we can conclude that EIV antibodies can be detected successfully through serological investigation in horses and donkey's serum that confirm the spread of EIV in Sharkia, Gharbia, Cairo and Giza provinces of Egypt during the period of 2017-2019 by HI and ELISA tests. The key management and environmental factors that determine the risk of equines to contracting EIV and control strategies in Egypt were identified

also horses is the most susceptible species to EIV H3N8 than donkeys. With higher Incidence of EIV in 1-3 years' age and the low incidence was observed in animals less than one-year-old. Also higher rate of EIV were observed in Sharkia and Gharbia provinces when compared to Cairo and Giza provinces.

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الملخص العربى
الكشف المصلي عن فيروس أنفلونزا الخيول H3N8 A في بعض المحافظات المصرية

ان فيروس انفلونزا الخيول نوع أ (ه3 ن8) لهو سبب رئيسى خطير لامراض الخيول التنفسية مسببا انتشارا عالميا و خسائر اقتصادية كبيرة. فى هذه الدراسة تم ايجاد دليلا سيرولوجيا لتواجد فيروس انفلونزا الخيول فى عينات سيروم الدم فى كلا من الخيول والحمير فى 4 محافظات مصرية وهى القاهرة والجيزة والغربية والشرقية فى الفترة ما بين يناير 2017 ويناير 2019 ولذلك تم تجميع عدد 164 عينة سيروم من الخيول و عدد 98 عينة سيروم من الحمير حيث تم فحصها باستخدام اختبار الاليزا واختبار منع التجلط الدموى السيولوجى معطية عدد 63 عينه موجبة من سيروم دم الخيول بنسبة 38.41% لمضادات الاجسام الخاصة بفيروس انفاونزا الخيول (ه3 ن8)باستخدام اختبار الاليزا وعدد 32 عينة موجبة فى سيروم دم الحمير بنسبة 32.65% لمضادات الاجسام الخاصة بفيروس انفاونزا الخيول (ه3 ن8)باستخدام اختبار الاليزا. كما وجدنا اعلى نسبة تواجد للفيروس فى سيروم دم الحيوانات بمحافظة الشرقية بنسبة 47.87% يليها محافظات الغربية والقاهرة والجيزة على التوالز على صعيد اخر اثبتت محافظة الشرقية اعلى نسبة تواجد (44.44%) فى سيروم دم الحمير باستخدام اختبار الاليزا يليها محافظات الغربية والقاهرة بنسبة 23.07% و نسبة 16.67% على التوالى . اما فيما يخص موسم انتشار الفيروس وجدت اعلى نسبة تواجد للفيروس فى موسم الشتاء بنسبة 43.18% مقارنة بموسم الصيف والذى اعطى نسبة تواجد 27.94% باستخدام اختبار الاليزا كما وجدت اعلى نسبة لانتشار وتواجد الفيروس كانت فى الاعمار مابين 1-3 سنوات من العمر بنسبة 43.96% واقل نسبة تواجد فى العمر اصغر من سنه بنسبة 24.14%. لذلك وجب تتبع الفيروس بشكل اكبر لمعرفة وتحديد مدى تواجد الفيروس فى بقية المحافظات المصرية.