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Original article

SEROPREVALENCE OF EQUINE HERPES VIRUS-1 IN ENDEMIC AREA OF EGYPT WITH RISK FACTORS ASSESSMENT

E. BESHIR ATA[1], A. SALAMA[2], A. ZAGHAWA[2], A. A. GHAZY[1], A. ELSIFY[2], M. NAYEL[2], Y. HEGAZY[3], E. H. ABDEL-RAHMAN[1] & S. WARDA[4]

[1]Department of Parasitology and Animal Diseases, Veterinary Research Division, National Research Centre, Dokki, Giza, Egypt; [2]Department of Animal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, University of Sadat City, Menoufia, Egypt; [3]Department of Animal Medicine, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt; [4]Department of Equine Diseases, Veterinary Serum and Vaccine Research Institute, Egypt

Summary

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Although equine herpesvirus-1 (EHV-1) infection occurs throughout the world; causing various health problems within horse population such as respiratory disease, abortion and myeloencephalopathy, there is information shortage concerning the epidemiological situation of EHVs in Egypt. This paper is the first study of EHV-1 prevalence rate in Monufia province (as a model for other provinces). During 2015, two hundred and seventy serum samples from EHV non-vaccinated horses were randomly collected from 9 centres of Monufia province. The indirect ELISA was used to detect the prevalence rate of the disease while assessment of the associated risk factors was conducted using univariate and multivariate logistic regression models. The results showed that EHV-1 infection was widespread among horses at Monufia province (apparent prevalence rate 64% and true prevalence rate 28%) and posed risk for the health of other equines in the region. Results of risk factors identification showed that horses > 5 years of age were at significant risk of getting EHV-1 infection than < 1-year-old horses (OR: 5; $P<0.02$), while males were twice more prone than females of getting the EHV-1 infection (OR: 2 and $P<0.03$). There was a significant effect of different localities on the prevalence of EHV-1 infection. The obtained results could be extrapolated to the different districts and governorates of Egypt because of the similarity of the husbandry system of equines all over Egypt.

Key words: EHV-1; ELISA; risk factors; seroprevalence, Egypt

INTRODUCTION

Equine herpesvirus-1 (EHV-1) is a large DNA enveloped virus that belongs to *Alphaherpesviridae* in the order Herpesviruses and is closely related to EHV-4 (Dunowska, 2014). Infection with EHV-1 causes various health problems within horse population with extensive economic losses with three main clinical consequences of infection: respiratory disease, storm of abortion occurring during the last third of pregnancy, equine herpes myeloencehalopathy (EHM) (Lunn *et al.,* 2009; Ata *et al.*, 2018).

Although multiple studies were carried out to characterise the EHV-1 (Kasem *et al.,* 2010; Badr *et al.,* 2018), the prevalence of EHV-1 infection and risk factors affecting the infection end result have been investigated in few studies (Hartley *et al.,* 2005; Goehring *et al.,* 2006). Different methods could be used for detection of prevalence including enzyme linked immune assay (ELISA) and virus neutralisation test (VNT) which are highly recommended by OIE (2017), virus isolation, and PCR (Dunowska, 2014).

The clinical presentation of EHV-1 outbreaks influenced by multiple risk factors including known factors such as the presence of an infected, shedding horse, introduction of new horses to a herd, season, age, previous exposure to the virus, pregnancy status, breed and sex; and suspected factors such as geographical location, stressors and immunological status (Lunn *et al.,* 2009).

In Egypt, there are few publications about the EHV infection, EHV-2 antibodies were detected early in horse sera during a serological survey at 1965 (Matumoto *et al.,* 1965). The virus was isolated from aborted foetus on the chorioallantoic membrane as a first record (Hassanain *et al.,* 2002). This trial was followed by others in which the virus was identified and isolated from aborted Arabian mares and internal organ of their foeti (Nehal *et al.,* 2007; Safaa *et al.,* 2013).

The information shortage concerning the epidemiological situation of EHVs in Egypt necessitates conducting many comprehensive studies. No previous results had been published on the prevalence of EHV infection in Monufia province in Egypt. Therefore, the aim of the current study was to detect the apparent and true prevalence of the EHV-1 infection among horses in this governerorate as a model for other provinces using indirect ELISA with special reference to the associated risk factors including age, sex, state of pregnancy and different localities.

MATERIALS AND METHODS

Study area

This study was carried out in the nine centres of the Monufia province (Shibin El Kom, Menouf, Ashmoun, El Bagour, Birket El Sab, El Shohada, Tala, Quesna and Sers El Lyan) during 2015. The Monufia province lies in the middle of Nile Delta between 30◦44'32.75"N, 31◦15'11.94"E and 30◦10'54.50"S, 30◦32'49.51"W, in the south-east of Beheira, west of Qalyubia, and north to Giza provinces (Fig. 1).

Study animals

Ethical approval for this study was obtained from the Ethics Committee of National Research Centre, Giza, Egypt. Two hundred and seventy EHV non-vaccinated horses of various ages were randomly selected and used in the present study. The data of the animals were collected through a questionnaire containing mainly

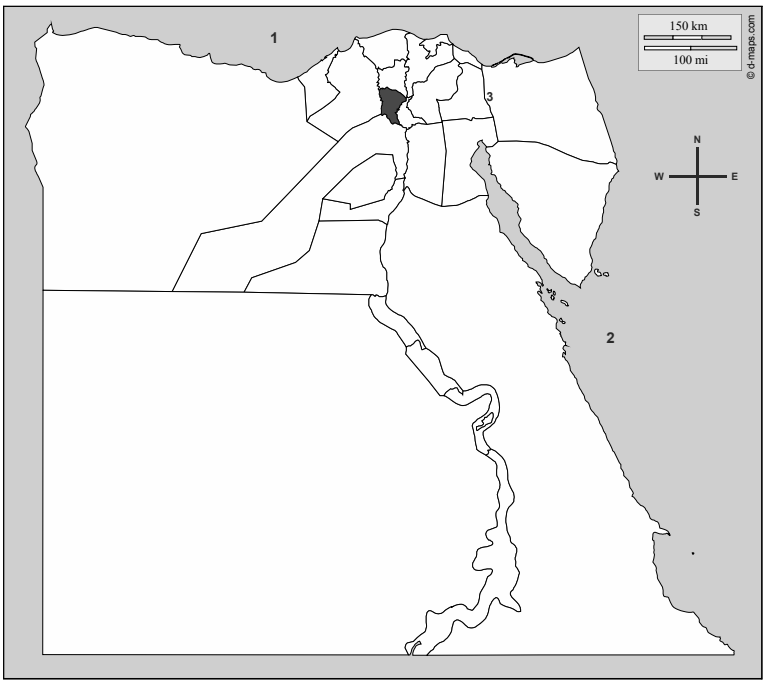

Fig. 1. The study area: Egypt map with location of Monufia province map; 1 – The Mediterranean Sea, 2 - The Red Sea, 3 - The Suez Canal.

closed-ended questions about the sex, age, location and pregnancy status. Regarding the age, the animals had been subdivided into 4 groups, the first one representing animals under 1 year of age, the second – with animals aged between 1 & 2 years, the third included animals aged between 2 & 5 years and the fourth one: animals over 5 years of age. Animals were divided into 2 groups according to sex and pregnancy status.

Serum samples

Blood samples were collected from the examined animals by jugular venipuncture followed by serum separation. The sera samples were kept at –20 °C until used (OIE, 2017). EHV-1 positive and negative sera were kindly supplied by the Veterinary Serum and Vaccine Research Institute (VSVRI), Egypt.

Indirect ELISA

Indirect ELISA was used for determination of the prevalence of EHV-1 according to Nehal (2006). Whole antigen of local EHV-1 isolate was used for plate coating; it was kindly supplied from the VSVRI, Egypt. Anti-horse IgG (H+L) peroxidase antibody conjugate and orthophenylenediamine dihydrochloride (OPD) as peroxidase substrate were supplied from Sigma-Aldrich, respectively Cat. No SAB3700159 and P9187-5. In brief; optimal working concentration of antigen, primary and secondary conjugated antibodies were determined by checkerboard titration (Lang *et al.,* 2013); were

10 µg/mL, 1:100 and 1:1000 respectively. ELISA plates were coated overnight at 4 °C with the whole antigen in 50 mM carbonate/bicarbonate buffer pH 9.6. Plates were washed 3 times with 150 mM PBS PH 7.2 containing 0.1% Tween-20. The unbound binding sites were blocked with 3% bovine serum albumin (BSA) for 1 h at 37 °C. The diluted tested sera were added and incubated for 1 h at 37 °C. Anti-Horse IgG secondary antibody was added and incubated at 37 °C for 1 h. The colour was developed within 15 min at room temperature by adding 0.4 g/L ortho-phenylenediamine. The reaction was stopped with 2M sulfuric acid. The optical density was measured spectrophotometrically at 450 nm (ELX800 Universal Microplate Reader, Bio.TEK instrument). The sensitivity and specificity of the ELISA test has been determined according to Gonzalez-Sapienza *et al.* (2000); through comparing the ELISA with the VNT results. The sensitivity and specificity were 100% and 50% specificity respectively with no significant statistical difference between these 2 tests.

Apparent and true prevalence calculation

The apparent prevalence (AP) of EHV1 infection was estimated according to Thrusfield (2007) as percentage ratio of the number of ELISA-positive animals to the total number of examined animals. The true prevalence (TP) was calculated as followed:

$$TP = \frac{(AP + SP - 1)}{(SP + SE - 1)} \times 100$$

where AP = apparent prevalence, SP = specificity, and SE = sensitivity

The 95% confidence interval (CI) for the prevalence (P) was estimated according to Thrusfield (2007):

$$CI = p \pm z \times \sqrt{\frac{P(1-p)}{n}}$$

where z=1.96; n: the number of samples.

Evaluation of risk factors effect

The association between the potential risk factors; age, sex, and state of pregnancy and EHV-1 positive status was examined using a univariate logistic regression model, where variables for which P>0.4 were excluded from further analysis. In the next step, collinearity between pairs of variables was assessed by calculating the Phi correlation coefficient. The significance of this collinear association was assessed using chi-square test. In the case of a pair of variables with a significant association (P<0.05), the variable judged as most biologically plausible was used as a candidate in multivariate analysis. A manual stepwise approach was used in the multivariate logistic regression model constructed for the selection of variables; all variables with P<0.05 were kept in the final model. All two-way interactions between variables retained in the model were assessed. The analyses were carried out using SAS 9.2 (SAS Institute Inc 2008).

RESULTS

Seroprevalence of EHV-1 infection

From all collected 270 serum samples, the average apparent prevalence (AP) was 64% (173/270). The highest apparent prevalence rate was established in El Shohada – 73.3% (22/30), Tala – 73.3% (22/30), Ashmoun, Sers El Layan and El Bagour – each with 70% (21/30). Lower prevalence was determined in Menouf and Quesna: 66.6% (20/30) and 63.3% (19/30) respectively while the lowest rates have been recorded in Birket El Sab

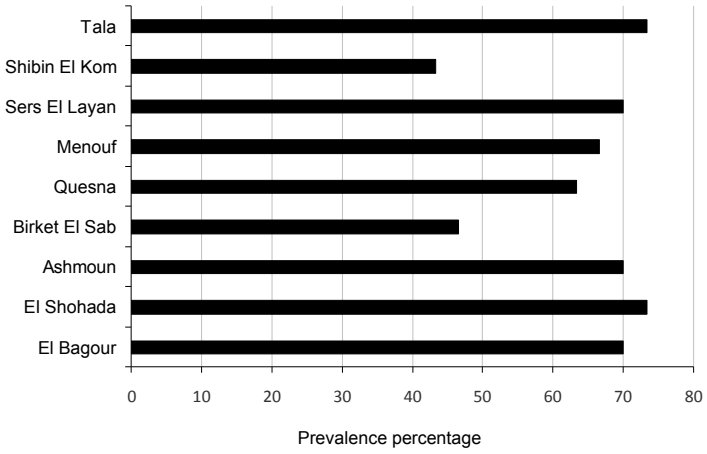

Fig. 2. Seroprevalence of EHV detected by ELISA in horses in the different centres of Monufia province.

Table 1. Apparent and true prevalence of EHV-1 seropositive cases in Monufia province

Risk factors		Total number	Number of positive	Apparent prevalence (%)	True prevalence (%)
Age	<1 y	27	9	33.3	0
	1–2 y	32	21	65.6	31.2
	2–5 y	91	57	62.2	24.4
	>5 y	120	86	71.7	43.4
Sex	Male	79	52	65.8	31.6
	Female	191	121	63.3	26.6
Pregnancy	Pregnant	61	39	63.9	27.8
	Not pregnant	130	82	63.0	26.1

(14/30; 46.6%) and Shibin El Kom (13/30; 43.3%) (Fig. 2).

According to the age, the percent of seropositive animals (AP) in the first group was 33.3% (9/27) which was significantly lower compared to other groups. On the other side, the AP in the second group was 65.6% (21/32), in the third group: 62.2% (57/91) and in the fourth group: 71.7% (86/120) without significant differences between them (Table 1).

Regarding the sex, the percent of the seropositive males (AP) was 65.8% (52/79) vs 63.3% (121/191) in females without statistically significant difference. The percentage of the seropositive (AP) pregnant mares was 63.9% (39/61), while in non-pregnant it was 63.07% (82/130) (Table 1).

Risk factors analysis

Univariate relationships between independent variables and EHV-1 infection status of examined animals are shown in Table 2. Pregnancy status was removed from the multivariate analysis (P>0.54).

Table 2. Results of the univariate logistic regression model for the association between selected potential risk factors and individual horse EHV-1 infection status.

Factors	OR	95% CI	P
Age	1.5	1.20–2.10	0.001
Sex	0.68	0.40–1.16	0.18
Location	1.05	0.95–1.15	0.36
Pregnancy	1.2	0.63–2.82	0.58

OR: odds ratio; CI: confidence interval; P: P value.

The final model was built using location, sex, and age as test variables (Table 3). Male horses were at twice higher risk of getting EHV-1 infection than females. Horses younger than 1 year of age and <2 years of age were nearly 5 and 3 times, respectively less likely to be affected with EHV-1 compared to the reference group > 5 years of age. There was a difference in EHV1 infection rate among different locations but the most significantly low prevalence was in Shibin El Kom.

DISCUSSION

Equine herpesvirus-1 is one of the most discriminated disease causing respiratory manifestations in horses which may be accompanied by abortion and nervous manifestations (Allen *et al.,* 2004; Brosnahan *et al.,* 2009; OIE, 2017).

Many serological methods were used to detect antibodies produced against EHV-4 and EHV-1 (Studdert *et al.,* 2003). Using complement fixation and virus neutralisation tests had been substituted with the highly sensitive ELISA and has found common use in veterinary medicine (Hartley *et al.,* 2005; Ataseven *et al.,* 2009).

The present study revealed an overall apparent prevalence rate of 64% (173/270). On the other side, the true prevalence rate was in average 28%. Previous EHV infection rate was 36.5% (34/93) samples recorded by Amer *et al*. (2011). Higher seroprevalence was recorded in different countries as high as 82% for

Table 3. Results of the multivariate logistic regression model for the association between selected potential risk factors and individual horse EHV-1 positive status

	Categories	OR	95% CI	P
Location	Ashmoun	1.08	0.37–1.18	0.49
	Birket El Sab	0.42	0.14–1.14	0.06
	El Shohada	1.45	0.48–4.38	0.14
	Shibin El Kom	0.28	0.10–0.38	0.003
	Tala	1.18	0.40–3.47	0.35
	Quesna	0.89	0.31–2.61	0.85
	Sers El Lyan	1.06	0.36–3.13	0.51
	El Bagour	0.94	0.32–2.74	0.74
	Menouf	–	–	–
Sex	Male	2.00	1.10–3.72	0.03
	Female	–	–	–
Age	< one year	0.23	0.09–0.58	0.02
	1-2 year	0.40	0.17–0.95	0.44
	>2 –5 years	0.72	0.39–1.31	0.12
	> 5 years	–	–	–

OR: odds ratio; CI: confidence interval; P: P value

EHV-1 and 95% for EHV-4 using specific ELISAs (Lang *et al.,* 2013). It is worth to note that serological surveys of EHV-1 and EHV-4 have always been complicated due to extensive antigenic cross-reactivity; the two viruses cannot be distinguished antigenically using polyclonal antisera (Dunowska, 2014). The difference between the current findings and the other studies may be attributed to many factors including the difference in the epidemiology of EHV-1 among different horse populations, the difference in the testing methods, and the antibody titre could be under the detection limit especially in latently infected animals (Dunowska *et al.,* 2015).

Vaccination against EHVs in the study region was not practiced and the present findings indicated the presence of natural infection with the virus and/or latent cases reactivation. This widespread of the virus and its latency accompanied with massive workload on these animals could result in the continuous shedding of the virus into the environment (Lunn *et al.,* 2009). This could explain the several outbreaks of cases with respiratory manifestation among these animals in the last few years which may be misdiagnosed with other respiratory diseases. On the other hand, the developed antibodies could protect these animals against the neurological signs and also could decrease the amount of virus shedding (Damiani *et al.,* 2014).

Regarding to the age as a risk factor, the present study showed that EHV-1 seroprevalence was significantly increased with age which agrees with Goehring *et al.* (2006); Henninger *et al.* (2007); Allen (2008). The finding that old horses >5 years of age were at significantly higher risk of getting EHV-1 infection than young animals was supported by Donald (1998) who reported that the EHV-1 seroprevalence among New Zealand thoroughbreds increased with age from 29% among 6–12 months old, 48% among 1–2 years old, to approximately 70% among adult (>2 years old) horses. Similarly, 56% of 21 yearlings and 67% of 45 horses from outbreaks of respiratory symptoms tested positive for EHV-1 antibody (Dunowska *et al.,* 2002). On the other hand, serological evidence of EHV-1 infection during the first month after birth in foals born to vaccinated mares was obtained by Dunowska *et al.* (2002). Also, the prevalence of EHV-1 infection among young foals was higher than that in mares or aged animals (Dunowska *et al.,* 2015). The difference between these findings and the current one may be attributed to horses in foreign countries being bred together in studs or in collections with close contact between mares and their foals and also close contact between foals and each other not like individual animals in Egypt.

Regarding sex as a risk factor, the number of seropositive females was significantly lower than males *(*$P<0.04$), and males were twice more likely to be infected than females. Sex (females) and season (winter-spring) were identified as risk factors associated with increased risks for EHM based on the analysis of six outbreaks of EHM in the Netherlands (Goehring *et al.,* 2006). Also, the obtained results were in contrast with Pronost *et al.* (2012) and Traub-Dargatz *et al.* (2013) who recorded that mares were 2.8 times more likely than male horses to be EHM cases.

From the present study, the percent of the seropositive pregnant mares was insignificantly lower than non-pregnant, this result is different with previous data cleared that the pregnancy has been shown to induce physiological immunosuppression in the horse which provides an expla-

nation for a silent circulation of EHV-1 among pregnant mares (Noronha & Antczak 2012), the later finding induced pregnant mares segregation from all other horses on the premises as a rationale for prevention of abortion or neurologic disease in pregnant mares (Allen *et al.,* 2004).

Regarding the location, there was variation in the seroprevalence of EHV-1 among different localities, besides Shibin El Kom was significantly lower than other places ($P<0.003$).

The results of the present study have shown that EHV-1 infections are widespread among horses at Monufia province, and thus, constitute a health risk for the other equine species in the region. This is the first study to investigate the seroprevalence of EHV-1 infection in the study region. The obtained results could be extrapolated to the different districts and governorates of Egypt because of the similarity of the husbandry system of equines all over Egypt.

CONCLUSION

EHV infection is widespread among horses in Monufia province and thus constitute a health risk for other equines in the region. Further structured surveys are required to study the epidemiological aspects such as the role of mules and donkeys and to examine the efficacy of control strategies. Also, these surveys could look for different risk factors for spreading of EHV infection among different populations.

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Correspondence:

Prof. Dr. Ahmed Zaghawa
Department of Animal Medicine and Infectious Diseases,
Faculty of Veterinary Medicine,
University of Sadat City,
Menoufia 32897, Egypt
tel: +2 01016062619
email: zagahmed@hotmail.com