SEROPREVALENCE OF EQUINE HERPES VIRUS TYPE-1 IN HORSES AND DONKEYS IN QALUBIAH GOVERNORATE IN EGYPT

Hazem M. El Moghazy[1], Mohamed G. Abdelwahab[2], Faiysal I. Hamouda[2], Elsayed M. Ibrahim[2] and Safaa M.A. Warda[3]

[1] Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Benha University.
[2] Animal Medicine Department, Faculty of Veterinary Medicine, Benha University.
[3] Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

ABSTRACT

Equine hepesirus-1 (EHV-1) is among the infectious diseases that threaten equine health. EHV-1 infects horses causing epidemic respiratory disease, abortion in pregnant mares, neonatal foal death, myeloencephalopathy, and chorioretinpathy. The present study was designed to investigate the presence of EHV-1 in blood serum of horses and donkeys in different localities in Qalubiah Governorate. A total of 185 blood serum samples were collected from horses and donkeys. All animals were evaluated based off whether they were of Indigenous breeds, different ages, apparent health, and clinical signs to equine herpes. The collected blood serum samples were investigated against equine herpes virus type-1 by using an indirect ELISA. The results revealed that 39.5% (73/185) of all horses and donkeys' sera samples were EHV-1 seropositive while 42.86 % (33/77) of the horses' sera samples and 37 % (40/108) of the collected donkeys' sera samples were EHV-1 seropositive. Meanwhile, 57.14 % (44/77) in horses and 63 % (68/108) in donkeys' sera samples were found to be seronegative for the EHV-1 infections. Analysis of data revealed that, young animals (<2 years) were represent (50%) (14/28) while adult animals (≥2years) were (37.6%) (59/157) EHV-1 antibodies seropositive. Further analysis showed that, EHV-1 antibodies seropositive young horses (9/17) (52.9%) were higher than their adults (40%) (24/60). While, EHV-1 antibodies seropositive young donkeys (45.5%) (5/11) were higher than their adults (36.1%) (35/97). In the present

study, the total number of (185) examined horses and donkeys' sera were differentiated into (79) males and (106) females. Out of (79) male cases, 15.2% (12/79) of total male horses and donkeys were EHV-1 seropositive while 15.15% (5/33) of male horses' sera and 15.2% (7/46) of male donkeys' sera were EHV-1 seropositive. However, out of (106) female cases, 57.5% (61/106) of total female horses and donkeys were EHV-1 seropositive. While, the percentage of seropositive sera samples of female horses and donkeys were 63.6% (28/44) and 53.2% (33/62) respectively.

Key Words: EHV-1; Seroprevalence; indirect ELISA.

1. INTRODUCTION

Equine herpes virus (EHV) is a highly contagious disease that affects equids probably caused by either of the two closely related herpes viruses, equine herpes virus-1 (EHV-1) or equine herpes virus-4 (EHV- 4) **(AAEP, 2013).**

Equine herpes virus-1 (EHV-1) is an important equine viral pathogen that exerts its major impact by inducing abortion storms or sporadic abortions in pregnant mares, early neonatal death in foals, respiratory disease in young horses and myeloencephalopathy in adult horses **(van Maanen, 2002; Patel and Heldens, 2005).**

Infections caused by EHV-1 are particularly common in young horses, and typically result in establishment of latent infection within the 1st weeks or months of life **(Foote, et al., 2004)** with subsequent viral reactivation causing clinical disease and viral shedding during periods of stress. Therefore, rapid diagnostic methods are useful for managing the disease **(OIE, 2015).**

Due to latency being a general feature of the herpes viruses, the conduct of routine serological tests bears significance for the control of herpes virus infections **(Siedek, et al., 1999).** Thus, ELISA is frequently used in the laboratory diagnosis of EHV-1 and EHV-4 as a serological test **(Yasunaga, et al., 1998 and Gilkerson, et al., 1999).**

The goal of this research was to determine the actual situation of EHV-1 infections in horses and donkeys in Qalubiah Governorate and to make recommendations for testing and dealing with infections in order to prevent economic losses.

2.MATERIAL AND METHODS

2.1. Materials:

2.1.1. Animals:

2.1.1.1. Horses and donkeys:

One hundred and eighty five horses and donkeys of different ages and sexes from different localities in Qalubiah Governorate in addition to the animals conducted to the veterinary Teaching Hospital, Faculty of Veterinary Medicine, Benha University) as shown in T**able (1 & 2).**

Table (1): Number of horses and donkeys used for seroprevalence of EHV-1 antibodies in Qalubiah Governorate, according to the age.

Species	**Animals**		
Age	**Horses**	**Donkeys**	**Total No.**
< 2 years	17	11	28
2-5 years	26	28	54
>5 years	34	69	103
Total	77	108	**185**

Table (2): Number of horses and donkeys used for seroprevalence of EHV-1 antibodies in Qalubiah Governorate, according to the sex.

Sex	Animal Species		Total No.
	Horses	Donkeys	
Male	33	46	79
Female	44	62	106
Total	77	108	**185**

2.1.2. Antigen:

A partially purified antigen of EHV-1 local isolate propagated on Vero cell line was prepared according to the method described by **Dutta et al., (1983) and Azmi and Field (1993)** used in serological test.

2.1.3. Antisera:

Horse anti-EHV-1 hyperimmune sera, prepared in Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, used as positive control in serological test.

2.1.4. Reagent and solution for serological test:

2.1.4.1. Reagent and solutions used in solid phase ELISA;

2.1.4.1.1. Phosphate buffered saline (PBS):

Sodium chloride	8.0 gm
Potassium chloride	0.2 gm
Potassium dihydrogen phosphate	0.2 gm
Di-sodium hydrogen phosphate 2 H2O	3.1 gm
Deionized Distellad water to	1000 ml

2.1.4.1.2. Coating buffer:

Na_2CO3	1.86 gm
NaHCO3	2.93 gm
DDW	1000 ml

2.1.4.1.3. Washing buffer:

PBS + 0.05 % Tween 20

2.1.8.2.4. Blocking buffer:

PBS + 2.3 % bovine serum albumin

2.1.4.1.5. Diluting buffer:

PBS + 0.05 % Tween 20 + 0.1 % bovine serum albumin

2.1.4.1.6. Substrate solution:

Citric acid	7.74 gm	
Sodium dihydrogen phosphate	17.3 gm	
DDW	1000 ml	pH 5.5-6.0

2.1.4.1.7. Substrate working solution:

This was freshly made by addition of ortho-phenelene diamine-2 HC1 (OPD), Sigma in a concentration of 1 gm per 1 ml of substrate stock solution and 0.8 ul of H_2O_2 (30%) per 1 ml buffer added just before use and kept in a dark brown bottle.

2.1.4.1.8. Anti-horse IgG:

Anti-horse IgG whole molecule conjugated with preoxidase produced by binding site Birmingham Research Park, UK.

2.1.4.1.9. Stopping solution: ' •

2 N sulphuric acid solution prepared by mixing 1 ml concentrated sulphuric acid with 6.5 ml water to be used immediately.

2.2. Methods:

2.2.1. Samples collection from horses:

Volumes of 6 to 8 mL blood samples were collected from each of the Egyptian horses and donkeys, using external jugular venipuncture method. Species, age, sex, previous vaccination and last trip history were also recorded.

2.2.2. Preparation of blood serum samples:

The serum samples were prepared according to the standard procedures after blood clotting at ambient environment and overnight incubation at 18 - 26°C controlled room temperature. Serum samples were separated to stock tubes and kept in a freezer (-20 °C) until use.

2.2.3. Enzyme linked immunosorbent assay (Solid phase ELISA):

- Partially purified EHV-1 antigen was diluted 1:100 in coating buffer.
- A Microtiter plate (96-wells flat bottom plate) was coated with 100ul/well of the diluted antigen and left overnight at 4°C.

- The following day, the plate was washed 3 times with a washing buffer and dried.
- 100 ul of blocking solution per well was added and incubated at 37°C for 1 hour.
- The plate was washed 3 times and dried.

• The serum samples as well as the negative and positive control sera were diluted 1/100 in a diluting buffer, then 100 ul of each sample was added to two wells from the coated plate and the plate incubated at 37°C for 1 hour.

• The plate was washed 3 times and dried.

• A peroxidase conjugate (anti-species IgG) was diluted 1/800 in the diluting buffer and 50 ul was added to each well, then the plate was incubated at 37°C for 1 hour.

• The plate was washed 3 times and dried.

• 100 ul of the substrate working solution were added to each well.

• The plate was incubated at room temperature in a dark place till a convenient colored reaction develops.

• The reaction was rapidly stopped by addition of 10 ul/well of stopping solution.

• The plate was read on an ELISA reader using a filter of wavelength 490 nm.

• The cut -off point of optical density was 0.1.

The end titre of the tested sample was calculated according to **William (1987)** as follow:

$$\frac{\text{OD of tested sample - OD of negative control}}{\text{OD of positive control - OD of negative control}} \times \text{titre of positive control}$$

3. RESULTS:

3.1.The seroprevalence of EHV-1 specific antibodies in horses and donkeys sera using ELISA in Qalubiah Governorate:

A total of 185 sera samples (77 from horses and 108 from donkeys) were tested for EHV-1 specific antibodies using ELISA. Overall results revealed that 42.86 % (33/77) of the horses sera samples and 37 % (40/108) of the collected donkeys sera samples were EHV-1 seropositive while 39.5% (73/185) of all horses and donkeys sera samples were EHV-1 seropositive. Meanwhile 57.14 % (44/77) in horses and 63 % (68/108) in donkeys sera samples was found to be seronegative for the EHV-1 infections. These results were shown in T**ables 3, 4, 5 & Fig. 8.**

Table. 3: Seroprevalence of EHV-1 antibodies positive samples in horses and donkeys in Qalubiah Governorate.

Animal	Number of tested sera	Seropositive		Seronegative	
		No.	%	No.	%
Horse	77	33	**42.9%**	44	**57.14%**
Donkey	108	40	**37%**	68	**63%**
Total	**185**	**73**	**39.5%**	**112**	**60.5%**

No. = number of examined animals. **%** = Percent.

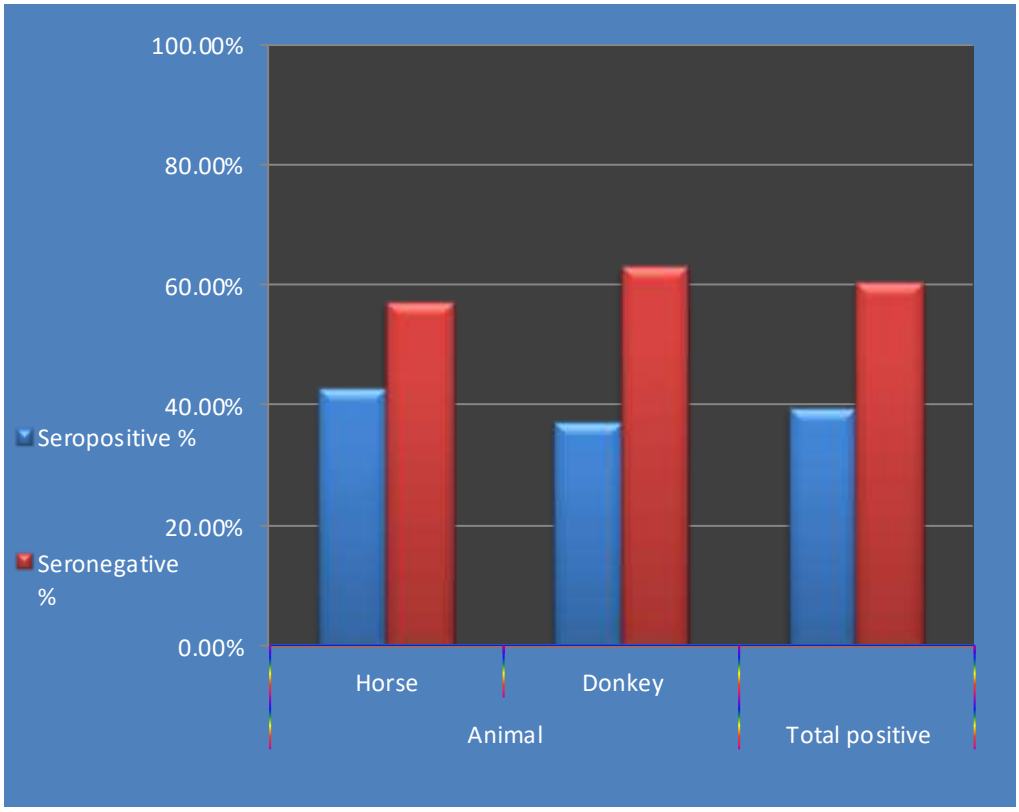

Fig. 8 : Seroprevalence of EHV-1 antibodies positive samples in horses and donkeys in Qalubiah Governorate.

Table. 4 : EHV-1 antibodies titer of collected horse's sera samples using ELISA in Qalubiah Governorate.

No.	ELISA titer	No.	ELISA titer	No.	ELISA titer	No.	ELISA titer
1	**740**	24	**460**	47	**490**	70	**580**
2	**350**	25	**69**	48	**69**	71	**60**
3	**90**	26	**74**	49	**514**	72	**566**
4	**50**	27	**580**	50	**64**	73	**77**
5	**388**	28	**66**	51	**86**	74	**494**
6	**46**	29	**422**	52	**86**	75	**499**
7	**388**	30	**498**	53	**70**	76	**64**
8	**46**	31	**59**	54	**44**	**77**	**592**
9	**80**	32	**633**	55	**611**		
10	**96**	33	**86**	56	**65**		
11	**40**	34	**47**	57	**590**		
12	**66**	35	**611**	58	**74**		
13	**450**	36	**78**	59	**48**		
14	**91**	37	**86**	60	**671**		
15	**29**	38	**490**	61	**55**		
16	**512**	39	**69**	62	**680**		
17	**69**	40	**602**	63	**593**		
18	**574**	41	**515**	64	**69**		
19	**58**	42	**96**	65	**530**		
20	**660**	43	**520**	66	**77**		
21	**78**	44	**58**	67	**82**		
22	**601**	45	**518**	68	**429**		

23	**88**	46	**58**	69	**68**

No. = sample number of examined horse's sera.

Table. 5 : EHV-1 antibodies titer of collected donkey's sera samples using ELISA in Qalubiah Governorate.

No.	**ELISA titer**	**No.**	**ELISA titer**	**No.**	**ELISA titer**	**No.**	**ELISA titer**	**No.**	**ELISA titer**
1	**75**	24	**528**	47	47	70	**580**	93	**520**
2	**90**	25	**37**	48	**610**	71	**35**	94	**94**
3	**515**	26	**40**	49	**31**	72	**598**	95	**89**
4	**85**	27	**522**	50	**613**	73	**65**	96	**579**
5	**45**	28	**25**	51	**87**	74	**76**	97	**105**
6	**590**	29	**35**	52	**599**	75	**603**	98	**88**
7	**86**	30	**710**	53	**66**	76	**87**	99	**518**
8	**28**	31	**33**	54	**535**	77	**79**	100	**96**
9	**602**	32	**45**	55	**69**	78	**480**	101	**66**
10	**33**	33	**634**	56	**710**	79	**98**	102	**599**
11	**40**	34	**96**	57	**87**	80	**83**	103	**87**
12	**509**	35	**88**	58	**93**	81	**595**	104	**92**
13	**35**	36	**598**	59	**528**	82	**71**	105	**520**
14	**27**	37	**84**	60	**65**	83	**87**	106	**97**
15	**701**	38	**87**	61	**720**	84	**564**	107	**498**
16	**44**	39	**589**	62	**28**	85	**98**	**108**	**88**
17	**38**	40	**89**	63	**548**	86	**93**		
18	**528**	41	**99**	64	**85**	87	**604**		
19	**42**	42	**558**	65	**498**	88	**86**		

20	**605**	43	**76**	66	**90**	89	**89**
21	**37**	44	**559**	67	**538**	90	**79**
22	**599**	45	**36**	68	**87**	91	**585**
23	**32**	46	**40**	69	**79**	92	**59**

No. = sample number of examined donkey's sera.

3.2. Age, sex and species susceptibility:

Among (185) horses and donkeys sera screened by ELISA, (73) were found positive for EHV-1 antibodies. Out of (73) positive cases, (33) were found positive for horses sera and (40) were found positive for donkeys sera.

As shown in T**able. 6 and Fig. 9.** Analysis of data revealed that, young animals (<2 years) were represent (50%) (14/28) while adult animals (≥2years) were (37.6%) (59/157) EHV-1 antibodies seropositive. Further analysis showed that, the EHV-1 antibodies seropositive young horses (9/17) (52.9%) were higher than their adults (40%) (24/60). While EHV-1 antibodies seropositive young donkeys (45.5%) (5/11) were higher than their adults (36.1%) (35/97).

Table. 6: Seroprevalence of positive EHV-1 antibodies in sera of young and adult horses and donkeys using ELISA in Qalubiah Governorate.

Species		**Animal**		**Total positive**
Age		**Horse**	**Donkey**	
Young <2 years	**No.**	9	5	**14**
	%	**52.9%** (9/17)	**45.5%** (5/11)	**50%** (14/28)
Adult ≥2years	**No.**	24	35	**59**
	%	**40%** (24/60)	**36.1%** (35/97)	**37.6%** (59/157)
Total		33	40	**73**

No. = number of tested animals.
% = Percent of positive sample from the total number of the same age group.

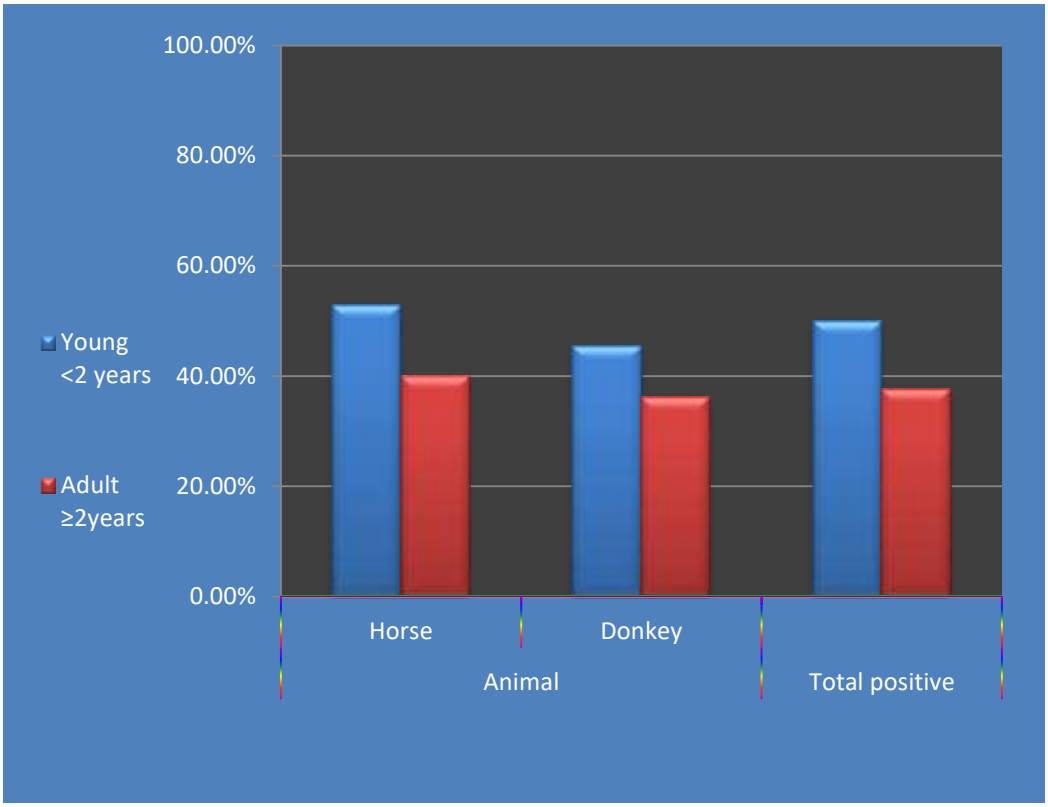

Fig. 9: Seroprevalence of positive EHV-1 antibodies in sera of young and adult horses and donkeys using ELISA in Qalubiah Governorate.

In the present study, the total number of (185) examined horses and donkeys sera were differentiated into (79) males and (106) females. As shown in T**able. 7 and Fig. 10.** Out of (79) male cases, 15.2% (12/79) of total male horses and donkeys were EHV-1 seropositive while 15.15% (5/33) of male horses sera and 15.2% (7/46) of male donkeys sera were EHV-1 seropositive. However, Out of (106) female cases, 57.5% (61/106) of total female horses and donkeys were EHV-1 seropositive while the percentage of seropositive sera samples of female horses and donkeys were 63.6% (28/44) and 53.2% (33/62) respectively.

Table. 7 : Seroprevalence of EHV-1 antibodies positive sera of male and female horses and donkeys using ELISA in Qalubiah Governorate.

Species		**Animals**		**Total positive**
		Horses	**Donkeys**	
Sex				
Male	**No.**	5	7	12
	%	**15.15%** (5/33)	**15.2%** (7/46)	**15.2%** (12/79)
Female	**No.**	28	33	61
	%	**63.6%** (28/44)	**53.2%** (33/62)	**57.5%** (61/106)
Total	**No.**	33	40	**73**
	%	**42.9%** (33/77)	**37%** (40/108)	**39.5%** (73/185)

No. = number of tested animals.

% = Percent of positive sample from the total number of the same sex group.

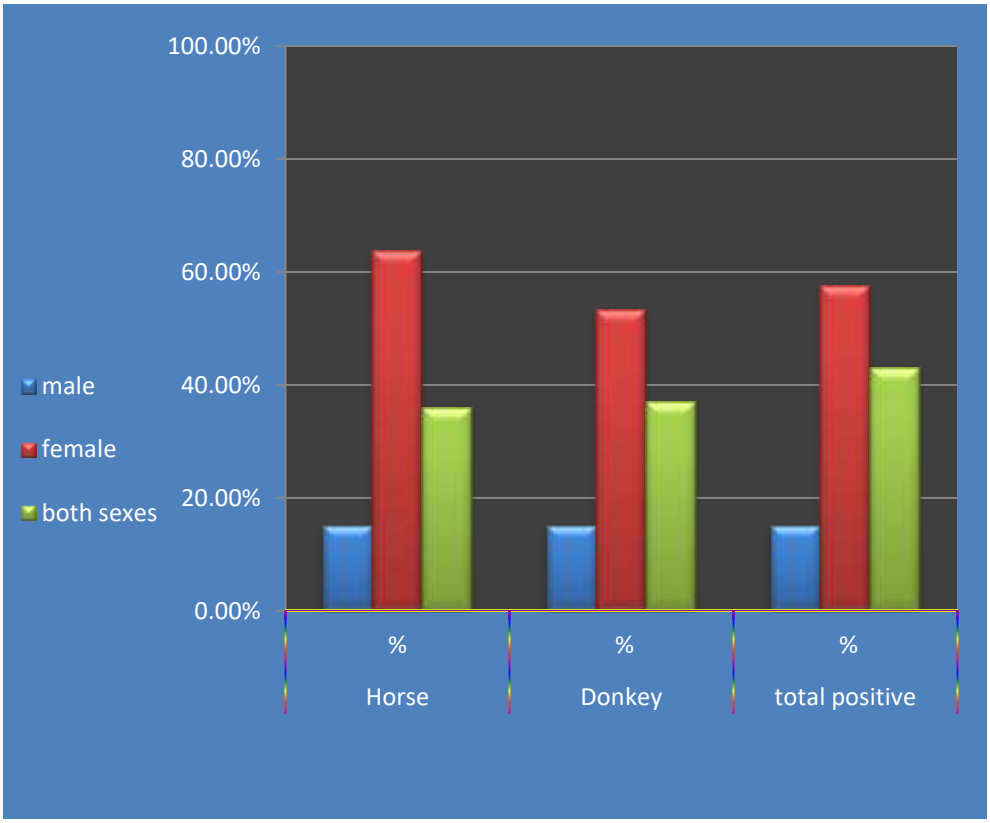

Fig. 10 : Seroprevalence of positive EHV-1 antibodies in sera of male and female horses and donkeys using ELISA in Qalubiah Governorate.

4. DISCUSSION:

In Egypt, the first recorded result for the presence of EHV-1 specific antibodies in horse's serum through serological evidence was done by **(Matumoto et al., 1965)**, while EHV-1 was first isolated by **(Hassanein et al., 2002)** in Cairo from aborted mares at the last trimester of pregnancy.

The serological investigation of EHV-1 that conducted in this study aimed to determine the prevalence of the disease, as well as the age and sex susceptibility.

Regarding the seroprevalence of EHV-1, out of 185 sera sample (77 from horses and 108 from donkeys) examined for EHV-1 specific antibodies using ELISA. Overall results revealed that 42.86 % (33/77) of the horses sera samples and 37 % (40/108) of the collected donkeys sera samples were EHV-1 seropositive while 39.5% (73/185) of total horses and donkeys sera samples were EHV-1 seropositive as shown in **Tables (3, 4 ,5).** The same results obtained by (**Avci, et al., 2014)** who recorded that, 34.66% (52/150) were found to be positive for EHV-1 antibodies. Also

this result agrees with (**Abdelgawad, et al., 2015)** who reported, that 33.3% (4/12) EHV-1 seropositive in donkey by using peptide-based ELISA. (**Ataseven *et al*., 2009)** revealed that the seropositivity for EHV-1 was 14.5%. However, other studies, reported higher levels of infection; for example**: (Yildirim et al., 2015)** who reported that 52.48% (222/423) of the horses sampled and 51.85% (126/243) of the donkeys sampled were EHV-1 seropositive. When the horse and donkey samples were evaluated together, 52.25% were seropositive for EHV-1. Also **(Wegdan, et al., 2016)** reported that 65.9% of the total horse and donkeys serum samples examined were seropositive for EHV-1.

None of the animals that tested positive for antibodies to EHV-1 exhibited clinical symptoms indicative of herpesvirus infection. This is characteristic of natural viral host species **(Abdelgawad et al., 2015).**

Regarding the Age, sex and species susceptibility, analysis of data revealed that, young animals (<2 years) were represent (50%) (14/28) while adult animals (≥2years) were (37.6%) (59/157) EHV-1 antibodies seropositive. Further analysis showed that, the EHV-1 antibodies seropositive young horses (52.9%) (9/17) were higher than their adults (40%) (24/60). While EHV-1 antibodies seropositive young donkeys (45.5%) (5/11) were higher than their adults (36.1%) (35/97). As shown in **Table (6) & Fig. (9).** The same results were obtained by **(Pusterla, et al., 2016)** who reported that EHV-1 generally affects horses less than three years of age with the majority of affected horses being less than one year of age. Although study cases represented a wide range of ages, 65% of them were ≤10 years of age. This reflects the higher susceptibility of young animals to infectious respiratory pathogens **(Mumford and others 2003). Also (Foot et al., 2004)** reported that, **i**nfections caused by EHV-1 are particularly common in young performance horses. (**Allen, 2008)** estimated that 80 to 90% of horses have been exposed to EHV1 infections by two years of age and respiratory disease associated with EHV1 is most commonly seen in young animals at the time of weaning. While experimental infection proved that older horses are more predisposed to the development of neurological disease as compared to young to young/middle aged horses. Adult horses may develop viremia 100 times higher than young horses and they are 8 times more likely to develop the disease.

In the present study, the total number of (185) examined horses and donkeys sera were differentiated into (79) males and (106) females. Out of (79) male cases, 15.2% (12/79) of total male horses and donkeys were EHV-1 seropositive while 15.15% (5/33) of male horses sera and

15.2% (7/46) of male donkeys sera were EHV-1 seropositive. However, Out of (106) female cases, 57.5% (61/106) of total female horses and donkeys were EHV-1 seropositive while 63.6% (28/44) of female horses sera and 53.2% (33/62) of female donkeys sera were EHV-1 seropositive. As shown in **Table (7) & Fig. (10).** This result came in similarity with results of (**Goehring, et al., 2006)** who reported higher infection rate among mares in Netherlands. This higher detection among females differ with the findings of **(Hafshejani et al., 2015)** in Isfahan who suggest that the stallions EHV-1: 18.18% were infected more than the mares EHV-1: 9.67%, and therefore had more active infection by the viruses. But the result of Shahrekord (EHV-1: stallion (8.96%), mare (7.14); suggested otherwise. This finding suggests that sex may not have played a role in EHV infection. (**Momtaz and Hematzadeh 2003)** reported that sex did not significantly affect infection of horses in Chaharmahal and Bakhtiari province, Iran. Other authors elsewhere had reported that sex is a factor in the epidemiology of infection by EHV-1 **(Goehring, et al., 2006 and Lunn, et al., 2009).** The differences in infection rate among sexes in these studies may be due to differences in rate of exposure to infection, health status (such as pregnancy and suckling in mares), age, previous vaccinations, or immune status of the animals sampled in the study areas.

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