



## Prevalence of equine infectious anemia virus in horses and donkeys determined by comparison of ELISA and AGID in Mexico

[Prevalência do vírus da anemia infecciosa equina em cavalos e burros, determinada por comparação de ELISA e AGID, no México]

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### ABSTRACT

From February to December 2021, 960 serum samples from different climate regions in two Mexican states were analyzed for antibodies against EIAV using competitive enzyme-linked immunosorbent assay (cELISA) and agar gel immunodiffusion (AGID) tests. The overall seroprevalence of EIAV infection in equids determined by ELISA and AGID tests was 19.69% and 17.60%, respectively. The highest rate of seropositivity was observed in the state of Veracruz (23.67%, using AGID test), and the lowest rate was found in the state of Puebla (13.12%, using cELISA test). In total, 17.45% (85/487, using AGID test) and 15.40% (75/487, using cELISA test) of horses were positive for EIAV infection. Donkeys showed a prevalence of 19.87% (94/473) in cELISA and 21.99% (104/476) in AGID. The cELISA was compared with AGID as the gold standard test. The diagnostic sensitivity for horses and donkeys was 90.4% and 92.2%, and the specificity was 97.5% and 97.3%, respectively, with an almost perfect degree of agreement (kappa values of 0.871 and 0.888). Therefore, the overall seroprevalence rate of EIAV infection in equines in the sampled states of Puebla and Veracruz was 19.3%, with 91.4% sensitivity, 97.4% specificity, and an agreement of 96.25%, indicating almost perfect agreement (kappa = 0.880).

Keywords: AGID test, ELISA, epidemiology, equine infectious anemia virus, prevalence

### RESUMO

De fevereiro a dezembro de 2021, 960 amostras de soro de diferentes regiões climáticas em dois estados mexicanos foram analisadas quanto à presença de anticorpos contra o EIAV, por meio de testes de imunoabsorção enzimática competitiva (cELISA) e imunodifusão em gel de ágar (AGID). A soroprevalência geral da infecção por EIAV em equídeos, determinada pelos testes ELISA e AGID, foi de 19,69% (189 de 960 amostras de soro) e 17,60% (169 de 960 amostras de soro), respectivamente. A maior taxa de soropositividade foi observada no estado de Veracruz (23,67%, usando-se o teste AGID), e a menor taxa foi encontrada no estado de Puebla (13,12%, usando-se o teste cELISA). No total, 17,45% (85/487, usando-se o teste AGID) e 15,40% (75/487, usando-se o teste cELISA) dos cavalos foram positivos para a infecção por EIAV. Os jumentos apresentaram uma prevalência de 19,87% (94/473) no cELISA e 21,99% (104/476) no AGID. O cELISA foi comparado com o AGID como o teste padrão-ouro. A sensibilidade diagnóstica para cavalos e jumentos foi de 90,4% e 92,2%, e a especificidade foi de 97,5% e 97,3%, respectivamente, com um grau de concordância quase perfeito (valores kappa de 0,871 e 0,888). Portanto, a taxa de soroprevalência geral da infecção por EIAV em equinos nos estados de Puebla e Veracruz amostrados foi de 19,3%, com sensibilidade de 91,4%, especificidade de 97,4% e concordância de 96,25%, indicando concordância quase perfeita (kappa = 0,880).

Palavras-chave: teste AGID, ELISA, epidemiologia, vírus da anemia infecciosa equina, prevalência

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## INTRODUCTION

Equine infectious anemia (EIA) is recognized as one of the most important viruses infecting the *Equidae* family. The vast majority of studies have been carried out on horses, with comparatively few publications for other equid species such as donkeys and mules (Barrandeguy and Carossino 2018; Issel *et al.*, 2013; Jara *et al.*, 2020). Equine infectious anemia virus (EIAV) has a worldwide distribution, causing important economic losses in the equine industry. The disease is mainly characterized by fever, thrombocytopenia, anemia, rapid weight loss, and dependent oedema (Barrandeguy and Carossino 2018; Issel *et al.*, 2014). EIAV infection is predominantly transmitted by blood-feeding horseflies, but iatrogenic transfer of blood can also occur through contaminated needles (Barrandeguy and Carossino 2018).

At present, no treatment exists for the disease; animal movement restrictions and culling of infected animals are used to control outbreaks within the equine population (Cook *et al.*, 2001). However, no commercial vaccine is currently available, so accurate tests are important to rapidly diagnose infections when studying the epidemiology, surveillance and control of the disease (Issel *et al.*, 2013). Currently, the diagnosis of EIAV is limited to indirect detection of virus-specific antibodies, including enzyme-linked immunosorbent assay (ELISA) and agar gel immunodiffusion (AGID), often considered the gold standard test (Issel *et al.*, 2013; Scicluna *et al.*, 2013). These serological detection methods provide sensitive and specific diagnosis of EIAV infection, except in the early stages of disease or nursing foals of infected mares (Barrandeguy and Carossino, 2018; McConnico *et al.*, 2000). The World Organization for Animal Health (OIE) currently recommends both diagnostic methods (ELISA and AGID tests) to evaluate EIAV infection.

In Mexico, several recent studies using ELISA in equids have indicated that the EIA prevalence in the state of Veracruz ranged between 33.54% and 37.7% (Estrada-Coates *et al.*, 2018; Sánchez-Contreras *et al.*, 2018). Therefore, the objectives of this study were (i) to investigate the seroprevalence of EIAV infection in horses and donkeys by using ELISA and AGID tests and (ii)

to evaluate the performance of diagnostic tests in two Mexican states.

## MATERIAL AND METHODS

The samples of this study were collected in the states of Puebla (33,919 km<sup>2</sup>) and Veracruz (71,826km<sup>2</sup>), located in the east-central and eastern regions of Mexico, respectively. The states belong to different climate groups, predominantly tropical wet (Puebla, Veracruz), tropical subhumid (Puebla, Veracruz), tropical rainforest, temperate with dry winters and semi-arid (Puebla). The climate of Puebla and Veracruz states is dominated by the spring rainy season from April to May and summer rainy season from June to September, with a mean annual temperature of 14.7°C and 23°C, as well as annual precipitation of 900mm and 1500 mm, respectively (INEGI, 2017).

Farms were selected based on convenience, depending on the willingness of the owners or managers to take part in the study. A total of 960 blood samples from different equid species were collected from the blood of EIAV naturally infected and uninfected equines between February 2021 and December 2021, centrifuged at 2000 × *g* for 10 min at 4 °C and then stored at -80°C until use. A total of 487 horses and 473 donkeys were at least six months old and included both sexes. Different categories of equids management were included in the sampling, such as draft power, pack animals, breeding, and some animals used to take part in local races and shows.

The commercially available VMRD EIAV immunodiffusion test detects precipitating antibodies using p26 antigen following the manufacturers' instructions and the OIE guidelines. The results were read with a minimum of 24 h of incubation at room temperature. The AGID tests were considered positive if the precipitation line curvature was observed and negative by the absence of the line. Each test was visually interpreted by at least two agar gel immunodiffusion analysts. In case of disagreement between analysts, the sample was retested.

Detection of antibodies against EIAV in equid sera was performed by a commercial cELISA test (IDEXX Laboratories, USA), designed to

detect antibodies to p26, which has been approved for EIA diagnosis in several countries. The test was performed according to the manufacturer's instructions. Absorbance was measured at 650 nm using the ELISA reader (BioTek ELx800).

Data were analyzed using the software IBM SPSS version 25 for Windows (SPSS Inc., Chicago, IL, USA). The diagnostic performance of the EIAV ELISA assay was compared with the AGID test as a standard test for the determination of sensitivity and specificity. The kappa value was calculated to determine the agreement between diagnostic tests. Four agreement categories were described by (Viera and Garrett, 2005) to classify the calculated kappa values: (1) fair: 0.21–0.40; (2) moderate: 0.41–0.60; (3) substantial: 0.61–0.80; and (4) almost perfect: 0.81–0.99. The association between the two assay methods was verified by McNemar's test, and  $P < 0.05$  was considered significant.

## RESULTS

The overall EIA prevalence based on the AGID test in horses and donkeys in two Mexican states was 19.69% (189 out of 960 serum samples), and 17.60% (169 out of 960 serum samples) using the cELISA test. The seroprevalence of EIAV infection in horses and donkeys using AGID test in the states of Puebla and Veracruz were 18.26% (107 out of 586 serum samples) and 21.93% (82 out of 374 serum samples), respectively. A summary of the serological detection using diagnostic tests in horses and donkeys is presented in Table 1. The percentage of equids with antibodies detected by ELISA against EIAV (20.05%) in Veracruz was higher than for Puebla state (16.04%). The prevalence in the state of Puebla using AGID (21.05%) and ELISA (18.75%) test were higher for donkeys than those for horses (15.25% and 13.12%), respectively. Our results show that positive donkeys in Veracruz state is higher using both AGID (23.67%) and ELISA (21.89%) tests compared with horses (20.49% and 18.54%).

Table 1. Prevalence of equine infectious anemia virus (EIAV) infection in horses and donkeys analyzed by serum enzyme-linked immunosorbent (ELISA) test based on p26 protein and agar gel immunodiffusion (AGID) assay

Species	AGID				ELISA			
	Puebla		Veracruz		Puebla		Veracruz	
	Infected animals	Prevalence (%)						
Horse (n=487)	43/282	15.25	42/205	20.49	37/282	13.12	38/205	18.54
Donkey (n =473)	64/304	21.05	40/169	23.67	57/304	18.75	37/169	21.89
Total	107/586	18.26	82/374	21.93	94/586	16.04	75/374	20.05

The cELISA was carried out initially on 960 equid samples to compare with the AGID test. The overall positive samples were 185 in comparison with the AGID assay. As shown in Table 2, there were 41 positive and 241 samples in horses from Puebla, 62 positive and 242 samples in donkeys from Puebla, 42 positive and 163 samples in horses from Veracruz, and 40 positive and 129 negative samples in donkeys from Veracruz. The highest prevalence of EIAV infection (23.7%, 40 of the 169 donkey serum samples) was obtained in Veracruz state, followed by horses from Veracruz (20.5%,

42/205), donkeys from Puebla (20.4%, 62/304) and horses from Puebla (14.3%, 41/282; Table 2).

The cELISA and AGID tests showed the highest sensitivity ranged from 90.2% to 92.5% and specificity ranged from 97.1% to 97.7%, respectively (Table 3). Higher sensitivity (92.2%) and lower specificity (97.3%) was observed in donkeys while horses had even lower sensitivity (90.4%) but higher specificity (97.5%). Serum samples from donkeys in the state of Veracruz had the highest sensitivity

*Prevalence of equine...*

(92.5%) and specificity (97.7%) and the lowest false positive and negative rates. No significant difference existed in the proportion of positive test results when comparing the AGID assay with the commercial cELISA in every establishment and overall samples (McNemar's

chi-squared test;  $P > 0.05$ ; Table 3). Based on the statistical analysis, the agreement between cELISA and AGID tests was almost perfect for every establishment and overall samples, with kappa values ranging from 0.871 to 0.902.

Table 2. Detection of equine infectious anemia virus (EIAV)-specific antibodies in the serum of horses and donkeys, based on enzyme-linked immunosorbent (ELISA) test with agar gel immunodiffusion (AGID) assay as confirmatory test

Species	AGID		Total
	Positive	Negative	
<b>Puebla</b>			
<b>Horse</b>			
Positive	37	6	43
Negative	4	235	239
<b>Total</b>	41	241	282
<b>Donkey</b>			
Positive	57	7	64
Negative	5	235	240
<b>Total</b>	62	242	304
<b>Veracruz</b>			
<b>Horse</b>			
Positive	38	4	42
Negative	4	159	163
<b>Total</b>	42	163	205
<b>Donkey</b>			
Positive	37	3	40
Negative	3	126	129
<b>Total</b>	40	129	169

Table 3. Comparison of detection of equine infectious anemia virus (EIAV)-specific antibodies in the serum of equines, evaluated by commercial enzyme-linked immunosorbent test (ELISA) based on p26 protein, with agar gel immunodiffusion (AGID) assay as confirmatory test

		Prevalence (%)	Agreement (%)	Kappa value	McNemar chi-square	Sensitivity (%)	Specificity (%)	False positive rate (%)	False negative rate (%)
<b>State</b>	<b>Puebla</b>								
	Horse	14.6	96.45	0.860	$P > 0.05$	90.2	97.5	2.49	9.76
	Donkey	20.4	96.05	0.880	$P > 0.05$	91.9	97.1	2.89	8.06
	<b>Total</b>	17.6	96.25	0.872	$P > 0.05$	91.3	97.3	2.69	8.74
	<b>Veracruz</b>								
	Horse	20.5	96.10	0.880	$P > 0.05$	90.5	97.5	2.45	9.52
	Donkey	23.7	96.45	0.902	$P > 0.05$	92.5	97.7	2.33	7.50
	<b>Total</b>	21.9	96.26	0.891	$P > 0.05$	91.5	97.1	2.40	8.54
<b>Species</b>	Horse	17.0	96.30	0.871	$P > 0.05$	90.4	97.5	2.48	9.64
	Donkey	21.6	96.19	0.888	$P > 0.05$	92.2	97.3	2.70	7.84
<b>Total</b>		19.3	96.25	0.880	$P > 0.05$	91.4	97.4	2.58	8.65

**DISCUSSION**

To our knowledge, this is the first study to conduct a serological survey to determine the seroprevalence of EIAV infection in horses and donkeys using ELISA and AGID tests, as well as to evaluate its performance for serological

diagnosis in two states of Mexico. The overall EIA prevalence among horses and donkeys in the states of Puebla and Veracruz was 19.69% using the Coggins or AGID test, higher than that observed in Colombia (9.7%) and Brazil (11.8%) and lower than that reported in the northern

region of Brazil (24.5%) (Borges *et al.*, 2013; Cruz *et al.*, 2020; Tique *et al.*, 2015).

The seroprevalence in horses and donkeys using cELISA assay in the states of Puebla and Veracruz was 17.60%. Previous serological surveys for EIA conducted in this country and published for Veracruz state have relied exclusively on the ELISA test, reporting a higher seroprevalence between 33.54% and 37.7% (Estrada-Coates *et al.*, 2018; Sánchez-Contreras *et al.*, 2018). However, previous research in Italy (ranging from 0.18% to 0.27%) and Ethiopia (0.1%) might have overestimated prevalence by using only the AGID test in equids; it may have been underestimated in Sudan (5.58%) by ELISA assay in horses and donkeys (Gebremeskel *et al.*, 2018; Maresca *et al.*, 2012; Wegdan *et al.*, 2017).

The lowest percentages of prevalence during the study period were found in Puebla state (18.26% and 16.04%), with a five-month summer rainy season and annual precipitation of 900 mm. The highest estimated prevalence of EIAV was observed in Veracruz state (21.93% and 20.05%), which has a four-month spring rainy season and annual precipitation of 1500 mm. Studies in Italy, Sudan and Brazil have reported correlations between prevalence and regions or seasonality, predicting relatively low hematophagous insect vector burdens and showing less seropositivity when compared to other regions within each country (Maresca *et al.*, 2012; Oliveira *et al.*, 2017; Wegdan *et al.*, 2017).

In our study, the seroprevalence of EIAV in donkeys was 21.99% (104 out of 473 serum samples) by AGID. The lowest estimated prevalence of EIAV has been observed in northeast Brazil (1.6%) and the northeast region of Brazil (0.81%) (Costa *et al.*, 2022; Oliveira *et al.*, 2017). Donkeys showed a prevalence of EIAV (19.87%, 94 out of 473 serum samples) using cELISA that was relatively higher than that reported in another study conducted on feral donkeys in the northeast region of Brazil, which reported a prevalence of 14.4% (Oliveira *et al.*, 2017). Additionally, a lower serum prevalence (10.5%) of EIA-positive donkeys was observed in the Brazilian northeast region (Costa *et al.*, 2022). However, discordant results in donkeys between AGID, two ELISAs (rgp90 protein and p26 protein) and immunoblot in detecting the

seroprevalence of EIAV infections showed false-positive results in ELISA tests and AGID associated with false-negative results. Explanations for the discordance might be experimental conditions, laboratory personnel or differences in antigen preparation (Oliveira *et al.*, 2017). In addition, previous studies have suggested that donkeys take longer to produce antibody levels detectable by the AGID test than horses and ponies after infection with EIAV (Cook *et al.*, 2001).

The seroprevalence results found in horses (17.45%, 85 out of 487 serum samples) using AGID agree with those of Márquez-Alvarado *et al.* (2015) who reported low prevalence values (4%) in creole horses from Venezuela. Antibodies against EIAV antigens diagnosed by cELISA in horses (15.40%, 75 out of 487 serum samples) were higher than those in Venezuela (10.2%, creole horses) and the northeast region of Brazil during subsequent dry periods of 2017 (1.22%) and 2018 (1.32%) (Bezerra *et al.*, 2021; Márquez-Alvarado *et al.*, 2015). The AGID test has high specificity and has become established as the gold standard for serological diagnosis of EIA. However, its key disadvantages are the long turnaround time for the diagnostic results, high antibody concentrations and difficult visual reading of the precipitation line (Cullinane *et al.*, 2007; Issel and Cook 1993; Scicluna *et al.*, 2013). Serological diagnosis by ELISA is the most rapid and potentially sensitive technique for EIA, detecting infections earlier than the AGID test (Espasandin *et al.*, 2021; Nardini *et al.*, 2017).

For the detection of anti-EIAV antibodies in equids, we used the AGID as a confirmatory test to validate the sensitivity and specificity of the commercial cELISA. For the overall samples tested, the diagnostic sensitivity was 91.4% and the specificity was 97.4%, with an almost perfect degree of agreement (kappa value = 0.880). Using a commercial IDEXX kit in samples of Venezuelan horses showed a moderate agreement value of 0.53, with 100% sensitivity and 97% specificity compared with AGID test. Paré and Simard (2004) reported a sensitivity of 100%, a specificity of 93.3% and a kappa value of 0.93 for the detection of EIA antibodies using a cELISA in comparison with a reference AGID kit in Canadian horses. In a recent study, Espasandin *et al.* (2021) tested equine samples

for EIA using AGID and cELISA tests. They showed 92% sensitivity and 90% specificity, with a very good agreement (0.75 and 0.9). Scicluna *et al.* (2013) observed an increase in the efficiency of EIA diagnosis when they used an ELISA screening test and confirmed positive results with an AGID test, concluding conflicting results among ELISA and AGID tests with the immunoblot as a supplemental assay.

### CONCLUSIONS

Geographic and climatic regions had different seroprevalences of EIAV infection in horses and donkeys. This highlights the importance of continuous monitoring programs on wide-scale testing to provide useful information about the status of infection for establishing an efficient movement control of equids. These data showed that the combination of ELISA and AGID tests had high-performing characteristics for the diagnosis of EIAV infection and can improve the efficacy of surveillance programs.

### REFERENCES

- BARRANDEGUY, M.E.; CAROSSINO, M. Infectious diseases in donkeys and mules: an overview and update. *J. Equine Vet. Sci.*, v.65, p.98-105, 2018
- BEZERRA, C.D.S.; ANJOS, D.M.D.; FALCÃO, B.M.R. *et al.* True prevalence and spatial distribution of Equine Infectious Anemia Virus (EIAV) in horses from northeast Region of Brazil. *Acta Sci. Vet.*, v.49, p.1-6, 2021.
- BORGES, A.M.; SILVA, L.G.; NOGUEIRA, M.F. *et al.* Prevalence and risk factors for equine infectious anemia in pocone municipality, northern Brazilian Pantanal. *Res. Vet. Sci.*, v.95, p.76-81, 2013.
- COOK, S.; COOK, R.; MONTELARO, R. *et al.* Differential responses of equus caballus and Equus asinus to infection with two pathogenic strains of equine infectious anemia virus. *Vet. Microbiol.*, v.79, p.93-109, 2001.
- COSTA, V.M.D.; CURSINO, A.E.; FRANCO LUIZ, A.P.M. *et al.* Equine Infectious Anemia Virus (EIAV): Evidence of circulation in donkeys from the Brazilian Northeast Region. *J. Equine Vet. Sci.*, v.108, p.103795, 2022.
- CRUZ, A.P.M.; AQUINO, M.H.C.; HELAYAEL, M.J.S.A. *et al.* Seroprevalence for equine infectious anaemia in equidae seized in the municipality of Petrópolis, State of Rio de Janeiro, Brazil, 2015/2018. *Ciênc. Rural*, v.50, p.e20190073, 2020.
- CULLINANE, A.; QUINLIVAN, M.; NELLY, M. *et al.* Diagnosis of equine infectious anaemia during the 2006 outbreak in Ireland. *Vet. Rec.*, v.161, p.647-652, 2007.
- ESPASANDIN, A.G.; CIPOLINI, M.F.; FORLETTI, A. *et al.* Comparison of serological techniques for the diagnosis of equine infectious Anemia in an endemic area of Argentina. *J. Virol. Methods*, v.291, p.114101, 2021.
- ESTRADA-COATES, A.T.; ALVA-TRUJILLO, M.; MUÑOZ-MELGAREJO, S. *et al.* Seroprevalencia de anemia infecciosa equina en équidos del estado de Veracruz, México. *Agrociencia*, v.52, p.33-38, 2018.
- GEBREMESKEL, A.K.; GETACHEW, A.; ADAMU, D. A cross sectional study on prevalence of cattle fasciolosis and associated economical losses in cattle slaughtered at Gondar Elfora Abattoir, northwest Ethiopia. *J. Vet. Med. Anim. Health*, v.10, p.101-105, 2018.
- INEGI. Anuario estadístico y geográfico de los Estados Unidos Mexicanos, 2017.
- ISSEL, C.; SCICLUNA, M.; COOK, S. *et al.* Challenges and proposed solutions for more accurate serological diagnosis of equine infectious anaemia. *Vet. Rec.*, v.172, p.210-210, 2013.
- ISSEL, C.J., COOK, R.F. A review of techniques for the serologic diagnosis of equine infectious anemia. *J. Vet. Diag. Invest.*, v.5, p.137-141, 1993
- ISSEL, C.J.; COOK, R.F.; MEALEY, R.H. *et al.* Equine infectious anemia in 2014. live with it or eradicate it? *Vet. Clin. North Am. Equine Pract.*, v.30, p.561-577, 2014.
- JARA, M.; FRIAS-DE-DIEGO, A.; MACHADO, G. Phylogeography of equine infectious anemia virus. *Front. Ecol. Evol.*, v.8, 2020.
- MARESCA, C.; SCOCCIA, E.; FACCENDA, L. *et al.* Equine infectious anemia: active Surveillance in Central Italy 2007-2009. *J. Equine Vet. Sci.*, v.32, p.596-598, 2012.

- MÁRQUEZ-ALVARADO, Y.; MÁRQUEZ-ALVARADO, A.; MELÉNDEZ-PEREIRA, C. *et al.* Comparación de las técnicas de IDGA y cELISA para el diagnóstico de anemia infecciosa equina en caballos criollos venezolanos. *Rev. Cient.*, v.25, p.381-385, 2015.
- MCCONNICO, R.; ISSEL, C.; COOK, S. *et al.* Predictive methods to define infection with equine infectious anemia virus in foals out of reactor mares. *J. Equine Vet. Sci.*, v.20, p.387-392, 2000.
- NARDINI, R.; AUTORINO, G.L.; ISSEL, C.J. *et al.* Evaluation of six serological ELISA kits available in Italy as screening tests for equine infectious anaemia surveillance. *BMC Vet. Res.*, v.13, p.105, 2017.
- OLIVEIRA, F.G.; COOK, R.F.; NAVES, J.H.F. *et al.* Equine infectious anemia prevalence in feral donkeys from Northeast Brazil. *Prev. Vet. Med.*, v.140, p.30-37, 2017.
- PARÉ, J., SIMARD, C. Comparison of commercial enzyme-linked immunosorbent assays and agar gel immunodiffusion tests for the serodiagnosis of equine infectious anemia. *Can. J. Vet. Res.*, v.68, p.254-258, 2004.
- SÁNCHEZ-CONTRERAS, A.A.; ESTRADA-COATES, A.T.; ALVA-TRUJILLO, M. *et al.* Diagnóstico serológico de anemia infecciosa equina y piroplasmosis en équidos de trabajo del municipio de Veracruz, Veracruz, México. *Agrociencia*, v.52, p.39-46, 2018.
- SCICLUNA, M.T.; ISSEL, C.J.; COOK, F.R. *et al.* Is a diagnostic system based exclusively on agar gel immunodiffusion adequate for controlling the spread of equine infectious anaemia? *Vet. Microbiol.*, v.165, p.123-134, 2013.
- TIQUE, V.; POLO, F.; BENAVIDES, J. *et al.* Seroprevalencia de anemia infecciosa equina en los departamentos de Córdoba y Bolívar, Colombia. *Rev. Facul. Cienc. Vet.*, v.56, p.96-104, 2015.
- VIERA, A.J.; GARRETT, J.M. Understanding interobserver agreement: the kappa statistic. *Fam. Med.*, v.37, p.360-363, 2005.
- WEGDAN, H.; SAHAR, M.; BALLAL, A. *et al.* Sero prevalence of Equine Infectious Anemia (EIA) virus in selected regions in Sudan. *Microbiol. Res. J. Int.*, v.18, p.1-6, 2017.