Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 1, 158-160

Seroprevalence of strangles in horses and donkeys in Mosul city, Iraq

Israa A. Al-Robaiee, Wisam S. Hassan, Maab I. Al-Farwachi*

Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

ARTICLE INFO

Recieved: 13 October 2023

Accepted: 25 December 2023

***Correspondence:**

Corresponding author: Maab I. Al-Farwachi
E-mail address: maabalfrwche@yahoo.com

Keywords:

Strangles
Horses
Indirect ELISA
Prevalence
Streptococcus equi

ABSTRACT

Strangles is considered an important equine contagious bacterial caused by *Streptococcus equi*. This study planned to evaluate the seroprevalence of anti-*S. equi* antibodies in horses located in Mosul city, Iraq for the first time. The sera were collected from 184 animals (156 horses and 28 donkeys), and then they were screened by using indirect ELISA (iELISA) to effectively check the seroconverted animals. The results of this study showed that the total prevalence rate of strangles in horses was 12%, (0.0% in donkeys) and the seroprevalence rate in animals less than 3 years old was 20%, while in animals of ages 3 years and more, it was 7% ($P < 0.05$). The seroprevalence rate in racing and horses with respiratory signs was significantly higher than ones with Draught and apparently normal conditions ($P < 0.05$). Any significant relationship was not found between seropositive animals and sex, and among racing horses ($P < 0.05$). To close, the presence of anti-*S. equi* antibodies in the examined horses might require more attention to reduce the incidence of the disease in horse breeding centers found in the study zone.

Introduction

Strangles is a contagious equine disease which is principally characterized by respiratory signs and superficial suppurative lymphadenitis. The causative agent is the bacteria of *Streptococcus equi*, which are Gram positive, pus-forming microorganisms, and produce hemolysis on blood agar (Paillot *et al*., 2010; Chhabra *et al*., 2023). The disease is primarily spread via the direct and/or indirect routes (Lindahl *et al*., 2011; Tartor *et al*., 2019). Clinically, strangles is mainly marked by inflammation of the upper respiratory tract (Seromucoid nasal discharge which progressively become mucopurulent), abscessation in the adjacent lymph nodes (Boyle *et al*., 2018) and severe inflammation of the mucosa in head and throat regions with extensive swelling, and often ruptures of superficial lymph nodes (Paillot *et al*., 2010; Richmond, 2015).

The Equidae could serve as carriers or reservoirs; therefore, they play a significant role in the circulation of the causative agent within the epidemics, which makes the management and avoidance of the disease more challenging (Duran and Goehring, 2021). The morbidity rate of the disease among horses may reach 100%, whereas the case rate for fatalities cannot be more than 10% (Chanter *et al*., 2000). In Iraq, the disease was reported in horses by molecular tools and bacterial isolation of the causative agent (Jawad, 2000; Jabbar, 2014), also in the neighboring countries, the disease was reported in Saudi Arabia with an infection rate of 44.8% and a fatality rate of 17.24% and the total prevalence rate was 44.2% (Osman *et al*., 2021), and in Iran the prevalence rate was 37.5% (Mohammadi *et al*., 2016).

The disease has major drawbacks in the Equine industry around the world because its long-term illness, prolonged convalescence time, cost of prevention and treatment, and finally the related sever consequences (Cohen *et al*. 2020).

To our knowledge, there is limited documentation about the status of strangles in Iraq. Therefore, the objective of this study was to evaluate the presence of anti-*Streptococcus equi* antibodies in horses and donkeys in the different regions of Mosul City, Iraq.

Materials and methods

Ethical Statement

This study plan was approved for animal handling by the Institutional Animal Care and Use Committee at the College of Veterinary Medicine, University of Mosul, Iraq.

Animal sampling and Laboratory tests

Blood samples from 184 horses were randomly collected, and the animals (draught and racing horses) were populated in different areas of Mosul city in Iraq, as mentioned in Table 1. However, the majority of animals were apparently normal; some of those animals had respiratory signs. Their ages were between 2-6 years old, and they were included in both sexes (56 males, and 128 females). In addition, blood samples were collected from 28 donkeys (18 males, and 10 females). The period of study was between September 2022 and September 2023. The samples of blood were collected directly from the jugular vein using plain vacutainer tubes to subsequently acquire the sera for indirect ELISA (iELISA) test. After that, the collected blood samples were transported im-

ISSN: 2090-6277/2090-6269/

mediately in cooled sterile conditions to the laboratory of the College of Veterinary Medicine/ University of Mosul/ Iraq. The sera were stored at -20°C until the application of iELISA. The sera were screened for the presence of anti- *S. equi* antibodies by the Horse anti-streptococcus antibody (anti-streptococcus) ELISA Kit (SunLong Biotech Co., LTD, Zhejiang, China). The procedure was done following the instructions of the applied commercial kit provided by the manufacturer. The iELISA plate was read at 450 nm using ELISA plate reader (Bio Tek, USA). Finally, the data of the current investigation was retrieved by calculating the O.D. values of the sera and the provided controls of the testing kit.

Table 1. Number of examined horses and their regions.

Region	Number of horses
Al-Shalalat	81
Bab-Sinjar	52
Rabia'a	24
City center	9
Badoosh	7
Al-Shamsiat	6
Al-Namrud	5
Total	184

Statistical analysis

The SAS statistical analysis system (Ver. 9.2, 2010, SAS Inc., USA) was used to analyze the data from the current study. The calculated proportions were compared using the Chi-square test, with P <0.05 was deemed significant.

Results

The outcomes of this study revealed that the seroprevalence of strangles among the screened horses in Mosul City, Iraq. The results of iELISA showed the presence of anti-*Streptococcus equi* antibodies which were reflected in the prevalence of strangles in the tested animals. Generally, the total percentage for the prevalence of the disease in horses was 12%. The seroprevalence of anti-*Streptococcus equi* antibodies was nil in donkeys in both males and females. Any significant relationship was not found between seropositive animals and sex (p>0.05).

Further, the results of screening the included horses by iELISA in animals, that were less than 3 years old, showed the presence of 14 positive cases out of 70 samples (20%). They were significantly higher at P< 0.05 compared to the older animals. On the other hand, the animals of age 3 years and older were reported to have 8 positive cases, for strangles, out of 114 samples (7%) as mentioned in Table 2.

Moreover, the results of testing the animals that were physically classified according to their respiratory condition; animals with respiratory signs showed 13 seropositive out of 48 samples (27.1%), whereas animals with normal respiratory status were 6.6%. The seroprevalence rate in horses with respiratory signs was significantly higher than the rate in the apparently healthy animals as expressed in Table 3.

Additionally, the seroprevalence of anti-*Streptococcus equi* antibodies in racing horses was 14%, while it was 9.1% in the draught horses (Table 4).

Discussion

Strangles is one of the main contagious illnesses that mostly affect horses, and it has major drawbacks in the equine industry in general. Limited information is available about the epidemiology of disease in Mosul City, Iraq; therefore, this study was designed to provide a preliminary insight into the seroprevalence of the disease in Mosul city at the northern part of Iraq.

In this study, the screening of horse sera was done by iELISA to investigate the presence of anti-*S. equi* antibodies. The iELISA has a high sensitivity detection ability compared to the traditional bacterial isolation techniques (Chen *et al*., 2021); therefore, it was used in this scientific investigation. The total percentage for the disease prevalence was showed 12% in horses in Mosul city, Iraq. This result was different from the reported prevalence rate in the study of Al-Gharban (2017) who was showed a rate of 22.29% in horses and donkeys in Baghdad, Iraq. This variation in the results might be attributed to the differences in the geographical regions, climate conditions, laboratory test type and timing of the study. Furthermore, the regions included in that study (Al-Gharban, 2017) are considered as centers for the racing horses in Iraq, and therefore this might increase the risks of infection because of the presence of carrier animals from either local or imported sources.

A higher prevalence of strangles was recorded by Osman *et al* (2021) who reported a prevalence rate of about 44.2% in Saudia Arabia, 37.5% in Iran (Mohammadi *et al*., 2016), and 45.2% in Pakistan (Ijaz *et al*., 2012) may be due to the presence of carrier horses that lead to spread and persistence of infection.

In the current study, the seroprevalence rate of strangles was 20%

Table 2. Seroprevalence of anti-*Streptococcus equi* antibodies by iELISA in different sex and age groups.

Sex	Number of samples	Number of seropositive horses (%)	P-value	Age	Number of samples	Number of seropositive horses (%)	P-value
Male	56	6 (10.7)	0.73	< 3 years	70	14 (20*)	0.01
Female	128	16 (12.5)		≥ 3 years	114	8 (7)	
Total	184			22 (12)			

* Significant difference at P< 0.05

Table 3. Seroprevalence of anti-*Streptococcus equi* antibodies by iELISA in horses based on their respiratory condition.

Samples	Number of samples	Number of seropositive horses	Percentage (%)	P-value
Animals with respiratory signs	48	13	27.1 *	0.00
Animals without respiratory signs	136	9	6.60	
Total	184	22	12	

* Significant difference at P< 0.05

Table 4. Seroprevalence of anti-*Streptococcus equi* antibodies by iELISA in the racing and draught horses.

Samples	Number of samples	Number of seropositive horses	Percentage (%)	P-value
Racing horses	95	17	18*	0.01
Draught horses	89	5	5.60	

* Significant difference at P< 0.05

in the animals under 3 years of old, and it was significantly higher than the recorded 7% in the animals of age 3 years and more. Regarding the infected age groups, however, the disease is more frequently observed in younger animals, but it could also infect horses of any age (Ijaz *et al*., 2010; Manzoor *et al*., 2008). In that scope, the study of Neamat-Allah and Damaty (2016) recorded a prevalence rate of 20% in animals with ages between 1-2 years old. This result is in harmony with our results for that age group of horses. Furthermore, in this study, it was showed that the seroprevalence rate in animals with respiratory signs (27.1%) was significantly higher than the rate observed in the animals without those signs (6.6%). This finding is relevant to the published literature about the disease, as it is generally characterized by upper respiratory signs as mentioned by Sweeney *et al*. (2005) and Boyle (2017). In addition, the results showed that the seroprevalence rate was significantly high in the racing horses group (18%) when compared with the reported rate in the draught horses (5.6%). This difference might be explained by the majority of the race horses were young under 3 years old, whereas the other group was included older animals. Since the young animals are more susceptible to strangles than adults; therefore the incidence of the disease in these age group is generally high (Ijaz *et al*., 2010; Manzoor *et al*., 2008). Finally, no seropositive cases were noticed in donkeys as screened by iELISA in Mosul City. This result could be occured due to these animals live in a small groups with less exposure to the pathogen from carrier and /or active cases, also they are relatively considered more resistant to infection than horses and mules (Thiemann, 2012).

Conclusion

This investigation has revealed the seroprevalence of anti-*S. equi* antibodies in horses and donkeys, and this is the first report for evaluation of the status of strangles in Mosul City, Iraq.

Acknowledgments

The researchers are grateful for the financial support provided by the College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

Conflict of interest

There was no conflict of interest as it was approved by the authors.

References

Al-Gharban, H.A.A.J., 2017. Seroepidemiological detection and culture utilization for diagnosis of carrier horses and donkeys with strangles. Journal of Education College Wasit University 1, 649-660. DOI: 10.31185/eduj.Vol1.Iss28.30.

Jabbar, A.A., 2014. Used of Real-Time PCR technique for detection of *Streptococcus equi* from horses infected by strangles in AL-Diwanyia Provence, Iraq. International Journal of Advanced Research 2, 208-213.

Boyle, A., 2017. Strangles and its complications. Equine Veterinary Education 29, 149-157. DOI:org/10.1111/eve.12568.

Boyle, A.G., Timoney, J.F., Newton, J.R., Hines, M.T., Waller, A.S., Buchanan, B.R., 2018. *Streptococcus equi* Infections in Horses: Guidelines for Treatment, Control, and Prevention of Strangles-Revised Consensus Statement. Journal of Veterinary Internal Medicine 32, 633-647. DOI: 10.1111/jvim.15043.

Chanter, N., Nicola, C., Talbot, J., Newton, R., Hewson, D., Verheyen, K., 2000. *Streptococcus equi* with truncated M-proteins isolated from outwardly healthy horses. Microbiology 146, 1361–1369. DOI: 10.1099/00221287-146-6- 1361.

Chen, R., Shang, H., Niu, X., Huang, J., Miao, Y., Sha, Z., Qin, L., Huang H., Peng, D., Zhu, R., 2021. Establishment and evaluation of an indirect ELISA for detection of antibodies to goat Klebsiella pneumonia. BMC Veterinary Research 17, 107. DOI: 10.1186/s12917-021-02820-1.

Chhabra, D., Bhatia, T., Goutam, U., Manuja, A., Kumar, B., 2023. Strangles in equines: An overview. Microbial pathogenesis 178: 106070. DOI: 10.1016/j.micpath.2023.106070

Cohen, N.D., Cywes-Bentley, C., Kahn, S.M., Bordin, A.I., Bray, J.M., Wehmeyer, S.G., Pier, G.B., 2020. Vaccination of yearling horses against poly-N-acetyl glucosamine fails to protect against infection with *Streptococcus equi* subspecies equi. PLoS One 15, e0240479. DOI: 10.1371/journal.pone.0240479

Duran, M.C., Goehring, L.S., 2021. Equine strangles: An update on disease control and prevention. Austral journal of veterinary sciences 53, 23-31. DOI: https://doi.org/10.4067/S0719-81322021000100023.

Ijaz, M., Khan, M., Khan, M. ,Avais, A., Maqbool, M., Ali, M., Shahzad W., 2010. Prevalence and serum protein values of strangles (*Streptococcus equi*) affected mules at Remount Depot, Sargodha (Pakistan). Equine Veterinary Education 22, 196-198. DOI.org/10.1111/j.2042-3292.2010.00052.x

Ijaz, M., Khan, M., Dourani, A., Saleem, M., Chaudhry, A., Ali, M., Mehmood, K., Shahzad, W., 2012. Prevalence and haemato-biochemical studies of strangles (*Streptococcus equi*) affected horses in Pakistan. The Journal of Animal and Plant Sciences 22, 295-299.

Jawad, N., 2000. Clinical Study on strangles in Iraqi horses. The Iraqi Journal of Veterinary Medicine 24, 1-4.

Lindahl, S., Söderlund, R., Frosth, S., Pringle, J., Båverud, V., Aspán, A., 2011. Tracing outbreaks of *Streptococcus equi* infection (strangles) in horses using sequence variation in the seM gene and pulsed-field gel electrophoresis. Veterinary Microbiology 153, 144-149. DOI: 10.1016/j.vetmic.2011.03.027

Manzoor, S., Siddique, M., Ashraf, M., 2008. Occurrence of lancefield group C streptococcal species in strangles cases of foals in Punjab, Pakistan. Pakistan Veterinary Journal 28, 17-20.

Mohammadi, A., Borujeni, M.P., Gharibi, D., Mashhadi, A.G., 2016. A serological survey on strangles disease in horses of some areas in Khuzestan province by ELISA. Journal of Veterinary Research 71, 373-379.

Neamat-Allah, A.N., Damaty, H.M., 2016. Strangles in Arabian horses in Egypt: Clinical, epidemiological, hematological, and biochemical aspects. Veterinary World 9, 820-826. DOI:10.14202/vetworld.2016.820-826

Osman, S.A., Tharwat, M., Saeed, E.M., 2021. An outbreak of strangles in arabian horses in saudi arabia: Epidemiology, clinical signs and treatment outcomes. International Journal of Veterinary Science 10, 323-328.

Paillot, R., Robinson, C., Steward, K., Wright, N., Jourdan, T., Butcher, N., Heather, Z., Waller, A.S., 2010. Contribution of each of four Superantigens to *Streptococcus equi*-induced mitogenicity, gamma interferon synthesis, and immunity. Infection and Immunity 782, 1728-1739. DOI: 10.1128/IAI.01079-09

Richmond, R., 2015. Practical management of strangles outbreaks in horses. Australian Veterinary Journal 93, N20.

Štritof, Z., Mitchell C., Turk N., Habuš J., Hađina, S., Perharić, M., Waller, A.S., 2021. Seroprevalence of *Streptococcus equi* subspecies equi in Croatia - Short communication. Acta Veterinaria Hungarica 68, 361-363. DOI: 10.1556/004.2020.00061

Sweeney, C.R., Timoney, J.F. , Newton , J.R. , Hines, M.T., 2005. *Streptococcus equi* infections in horses: guidelines for treatment, control, and prevention of strangles. Journal of Veterinary Internal Medicine 19, 123-134.

Tartor, Y.H., Gharieb, N., Ali, W., EL-Naenaeey, E.S., Ammar, A., 2019. Rapid and Precise Diagnostic Tests for *S. equi*: An Etiologic Agent of Equine Strangles. Zagazig Veterinary Journal 47, 146-159. DOI:10.21608/zvjz.2019.9896.1024

Thiemann, A.K., 2012. Respiratory disease in the donkey. Equine Veterinary Education 24, 469-478. DOI.org/10.1111/j.2042-3292.2011.00292.x