Seroepidemiological detection and Culture Utilization for Diagnosis of Carrier Horses and Donkeys with Strangles

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Abstract

This study was aimed for detection of carrier equines with strangles disease through identification of anti-*Streptococcus equi* antibodies in blood sera of apparently healthy horses and donkeys that inhabitant in different regions belonging to three Iraqi provinces. For this purpose, an overall of 462 equine animals (308 horses and 154 donkeys) were submitted for examining by using of indirect-ELISA that revealed on 22.29% total positive infections, comprised from 29.87% seropositive horses and 7.14% seropositive donkeys. In addition, the culture results of nasopharyngeal swabs samples that obtained, randomly, from 30 horses, seropositively, were revealed on 86.76% positive samples.

In horses, the seropositive results in Baghdad, Al-Qadisiyah and Wasit provinces were 28.13%, 30.27% and 30.37%, respectively; while in donkeys; they were 9.41% and 4.35% in Al-Qadisiyah and Wasit provinces, respectively. The seroprevalence in worker and races horses were 30.96% and 18.52% of, respectively, while in age groups, they were 47.54% and 25.51% in of ≤ 3 years and > 3 years groups, respectively. Also, the seropositive results were 28.68% in males and 30.81% in females; whereas, they were 33.49% of seropositive horses with a history of respiratory signs and 21.88% without it.

Keywords: Strangles, Carrier, Seroepidemiological, Culture, Diagnosis, Horses, Donkeys

الكشف المصلي الوبائي واستخدام الزرع لتشخيص الخيول والحمير الحاملة لداء الخناق

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الخلاصة

هدفت الدراسة الحالية الى تحديد الحيوانات الخيلية الحاملة لداء الخناق من خلال كشف الاجسام المضادة للمكورات السبحية الخيلية في مصول دم الخيول والحمير السليمة ظاهريا التي تستوطن في مناطق مختلفة تعود لثلاث محافظات عراقية. لهذا الغرض، اجماليا خضع ٤٦٢ حيوان خيلي (٣٠٨ خيل و ١٥٤ حمير) الى الفحص المصلي باستعمال تقنية الاليزا غير المباشر والذي كشف عن ٢٢.٢٩% انتشار مصلي كلي موجب ، تضمنت ٢٩.٨٧% خيول و ٧.١٤% من الحمير. اضافة الى ذلك ، اظهرت نتائج زرع عينات المسحات الانفية البلعومية التي اخذت ، عشوائيا ، من ٣٠ خيل موجبة مصليا ، عن ٨٦.٧٦% عينة موجبة.

في الخيول، بلغت النتائج الموجبة مصليا في محافظات بغداد والقادسية وواسط ٢٨.١٣ و ٣٠.٢٧% و ٣٠.٣٧% ، على التوالي ؛ اما في الحمير ، فقد كانت ٩.٤١% و ٣٠.٣٧% في محافظتي القادسية وواسط ، على التوالي . بلغ الانتشار المصلي في خيول العمل والسباق ٣٠.٩٦% و ١٨.٥٢% ، على التوالي ، اما في مجاميع العمر، فقد كان ٤٧.٥٤% و ٢٥.٥١% في مجموعتي ≤ ٣ سنوات و > ٣ سنوات ، على التوالي . ايضا ، بلغت النتائج الموجبة مصليا ٢٨.٦٨% في الذكور و ٣٠.٨١% في الاناث ؛ في حين كانت ٣٣.٤٩% من الخيول تمتلك تاريخ للعلامات التنفسية و ٢١.٨٨% بدونها .

الكلمات المفتاحية : داء الخناق ، حاملة ، مصلي وبائي ، زرع ، تشخيص ، خيول ، حمير

Introduction

Strangles is a contagious and infectious disease of upper respiratory tract in horses, donkeys and mules of all ages, caused by *Streptococcus equi* subsp. *equi* (*S. equi*) (1).Worldwide, it is considered as one of the most frequently diagnosed disease in most countries, which occurs with high morbidity and low mortality rates, causing an economic impact due to veterinarian expenses and the need to restrain animals from their activities (2). Although, the transmission can be occur by direct or indirect contact with clinically diseased animals, the recovering and subclinical cases is represented the main important source for infection to susceptible equines (3). Mainly, the acute infections are characterized by high fever, an abscessation of lymph nodes in head and neck, particularly submandibular and retropharyngeal lymph nodes, dysphagia and mucopurulent nasal discharge (4). Nonetheless, the lack of clinical signs exhibited by persistently infected carriers emphasizes the need to implement effective quarantine or testing procedures for their identification and treatment before they come into contact with an existing herd (5). Although, the isolation of organisms by culture still remains the gold standard for diagnosis of acute infections, the absence of bacterial sheds during the persistence phase suggesting the possibility of quantification of specific antibody response (6). Recently, ELISA can be used to detect asymptomatic carriers especially in non-vaccinated animals, as well diagnosing of bastard strangles and immune mediated complications such as purpura haemorrhagica and streptococcal myositis (7, 8). The goals of present study were to detect the seroprevalence of specific antibodies against strangles in horses and donkeys of some regions in Baghdad, Al-Qadisiyah and Wasit provinces by using of an indirect ELISA, and estimation the association of positive results in horses with some epidemiological risk factors that included the region, type of work, age, sex as well as a history of respiratory signs.

Materials and methods

Data and samples collection

From different areas related to Baghdad, Al-Qadisisyah and Wasit provinces, and during the period of February/2015 to October /2016, a totally of 462 non-vaccinated equines (308 horses and 154 donkeys) were selected randomly for this study. From each one, about 6 ml of blood samples was drained from jugular vein

by using of a disposable syringe, evacuated in EDTA anticoagulant tubes and centrifuged at 3000 rpm/15 minutes for serum obtaining. The serum samples were saved in numbered 1ml eppendorf safe-lock tubes that kept at -20°C until is used (9). Also, the required data related to the region, type (Races or workers), age (≤ 1 year and > 1 year), sex (males and females) and a history of respiratory signs, were reported as epidemiological risk factors.

Serological assay

The indirect-ELISA (IDvet-USA), based on detection of *SeM* surface protein, was used in this study for screening of specific IgG-antibodies against *S. equi*. According to manufacturer instructions, all samples and controls sera were diluted, incubated and, finally, measured at OD_{450nm} by using of a microplate photometer ELISA-reader (BioTek-USA). The test validation and results interpretation of samples and controls (positive and negative) have been performed in dependence on manufacturer guidelines.

Nasopharyngeal swabs

In order to confirm the carrier infection by culture, a totally of 30 seropositive horses were selected randomly and submitted for naopharyngeal swabs. The samples were transported in cooled inoculated containers with ice, transported to the lab, and submitted for culturing onto blood agar media as described by (10). The beta hemolytic colonies were considered as positive samples for guttural pouch carriers.

Statistical analysis

All data were arranged, classified and tabled by using of a computerized Microsoft Office Excel (2007), and analyzed by using of the descriptive statistic and Chi-square test (X^2) in IBM/SPSS program (*v.23*). The significant differences ($P \leq 0.05$) were used to assess the prevalence of seropositive equines and to compare between them. Also, the association of positive results with some epidemiological risk factors was estimated (11).

Results

As shown in (Table 1), a totally of 462 equine animals (308 horses and 154 donkeys) were examined by using of indirect-ELISA that revealed on 103/462 (22.29%) overall seropositive equines, comprised of 92/308 (29.87%) and 11/154 (7.14%) horses and donkeys, respectively. A high significant difference (P≤0.05) was reported in seropositive prevalence between horses and donkeys.

Table (1): Seropositive results of 462 equine animals by indirect-ELISA

Species		Total No.	Seropositives	Seronegatives
1	Horse	308	92 (29.87 %) [a]	216
2	Donkey	154	11 (7.14 %) [b]	143
Total		462	103 (22.29%)	359

Variation in small letters, vertically, referred to a significant difference at P≤0.05

The results of culturing nasopharyngeal swabs of 30 seropositive horses were revealed on 26/30 (86.67%) positive swab samples and 4/30 (13.33%) negative samples (Fig. 1).

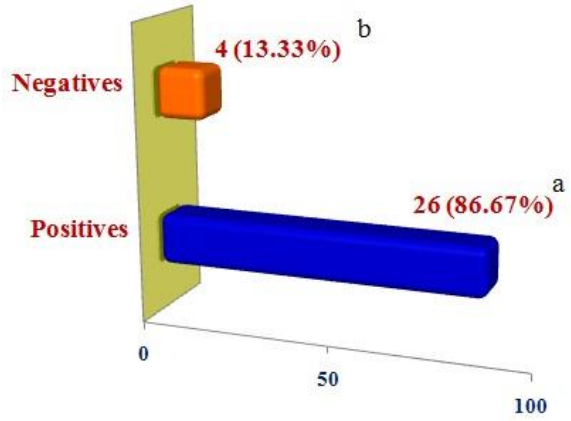

Figure (1): Results of nasopharyngeal swabculture

In (Table 2): According to horses residence factor, the study's results of horses and donkeys were reported that in Baghdad, Al-Qadisiyah and Wasit provinces, the seropositive prevalence was 18/64 (28.13%), 41/135 (30.37%) and 3/69 (4.35%), and 33/109 (30.27%) and 8/85 (9.41%), respectively.

Table (2): Seropositive results in according to residence factor

Residence		Horses		Donkeys	
		No.	Seropositivity	No.	Seropositivity
1	Baghdad	64	18 (28.13%) [b]	-	-
2	Al-Qadisiyah	109	33 (30.27%) [b]	85	8 (9.41%) [a]
3	Wasit	135	41 (30.37%) [b]	69	3 (4.35%) [b]
Total		308	92	154	11

Variation in small letters, vertically, referred to a significant difference at P≤0.05

In regarding to work's type factor, the study were exhibited on 5/27 (18.52%) and 87/281 (30.96%) seropositive races and worker horses, respectively, (Fig. 2). Whereas in age factor, the study horses were involved 29/61 (47.54%) and 63/247 (25.51%) seropositive horses in ≤ 3 years and > 3 years group, respectively, (Fig. 3).

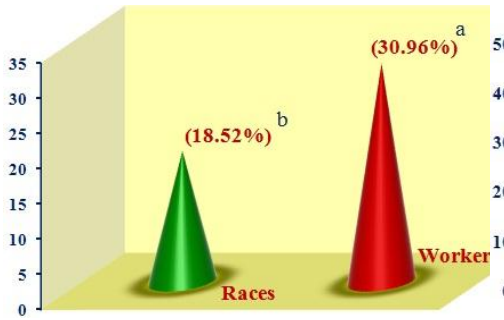

Figure (2): Seropositive horses in according to

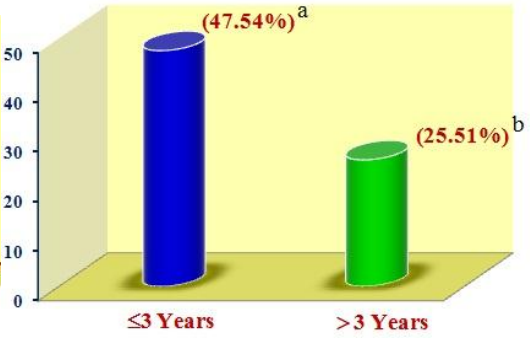

Figure (3): Seropositive horses in according to age factor

In according to horse's sex factor, the positive seroprevalence were 39/136 (28.68%) males and 53/172 (30.81%) females (Fig.4). Also, the results showed that 71/212 (33.49%) and 21/96 (12.88%) of seropositive horses were with a history of respiratory signs and without it, respectively, (Fig.5).

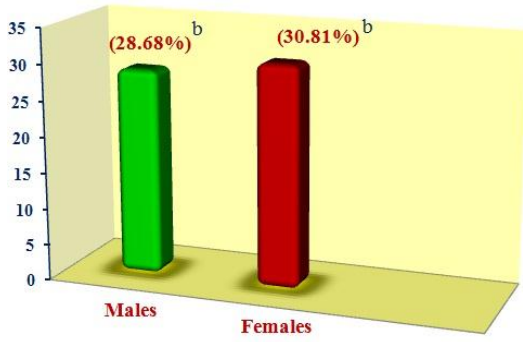

Figure (4): Seropositive horses in according to sex factor

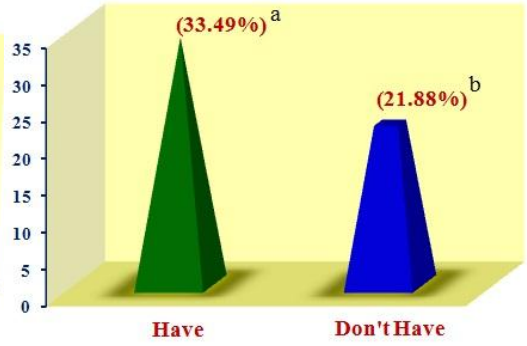

Figure (5): Seropositive horses in according to a history of respiratory signs factor

Discussion

Carrier horses with strangles as defined by the Animal Health Trust (AHT) are meaning "they infected with *S. equi*, not show any clinical signs and act as a source of infection for other susceptible individuals, which be determined, only, if they have an evidence of an antibody response" (12). In this study, though the total seropositive prevalence of strangles was 22.29%, it was indicated that the horses (29.87%) were more susceptible for *S. equi* infection than donkeys (7.14%) (Table1). Although, the true causes were fully unknown and required for further investigation, the low prevalence in donkeys could be attributed to the low number of obtained samples, less-exposure for infection sources, no imported donkeys and natural resistance. However, the positive results of horses were lower than those reported, locally, by (13, 14).

Globally, the studies that pertained to the prevalence or incidence rates of strangles and the role of some risk factors in development of strangles are very rare. Nevertheless, the prevalence of disease in horses, as reported by previous disease,

was 37.5% in Iran (15), 28% in Saudi Arabia (16), 45.2% in Pakistan (17), 18.4% in Canada (18), and 13% in British (19). In Iraq, strangles is considered as one of most important endemic diseases that affected equine animals at all seasons of year as a result of continual importation of races horses from different worldwide origins, persistence of carrier animals that seemed to be healthy (12), lack of actual bio-security and an absence of active vaccinations schemes, especially, for worker horses.

The current codes of practice on equine diseases published by the Horserace Betting Levy Board (HBLB) suggested that" No convalescent horse or in contact can be considered free from infection until three negative nasopharyngeal swabs and the horse has been tested by guttural pouch endoscopic examination and lavage" (5). Though, the detection of asymptomatic carriers by using of classical screening methods as bacteriological culture or endoscopic assessment of guttural pouches is difficult because of these methods are very cost, impractical for routine screening of large numbers of horses at low risk of being infected with strangles, low sensitive and non-specific, as well as, the intermittent shedding of bacteria from carrier equines (20, 21).

As shown in (Fig.1), the results of indirect-ELISA were confirmed through culturing of the nasopharyngeal swabs that obtained from seropositive horses and revealed on 86.67% positive samples. Hence, the current available serological ELISA that developed by the AHT has been allowed to detect the specific IgG-antibodies for two novel *S. equi* specific antigens (19) Also, this assay has the ability for revealing of recent exposure to *S. equi* with a high sensitivity (92%) and specificity (91%), and detecting of asymptomatic carriers with 90.9% 82.6% of sensitivity and specificity, respectively (22).

In regarding to residency factor, although the prevalence of seropositive horses didn't show any significant differences ($P \leq 0.05$) between the studied provinces, it's more prevalent in donkeys of Al-Qadisiyah than Wasit (Table 2). As well as, some factors were discussed in, only, horses due to rarity of data that related to donkeys. In a factor of work's type (Fig.2), the worker horses reported a high seroprevalence of *S. equi* when compared to races horses, and this might be attributed to genetic variation (٢٣), high exercise, decrease attention to therapy and absence of vaccination program, poor hygienic conditions (٢٤), waning immunity and frequent exposure to different pathogens (3).

In regarding to age factor (Fig.3), the study reported that the younger horses with ≤ 3 years were appeared to be having a high seropositive prevalence than older > 3 years horses, and these results were in agreement with those detected by (11; 13). Typically, strangles can be affected horses at all age categories but the yearlings are most severely affected with longer duration of clinical signs (4, 17). Whereas, the older equines with residual immunity have limited susceptibility or develop a mild form of strangles and shedding the pathogen with production of a sever disease in more susceptible or younger animals (٢٥).

Among horses of both sex groups, the study reported that females (30.81%) have more seropositive prevalence than males (28.68%), but without significant differences (Fig.4), which could be attributed to that both sexes were at the same risk for infection.

The prevalence of strangles in a history of presence respiratory signs group was showed to be more than a group of absence it (Fig.5). Despite a severity of clinical signs during acute phase of disease, the vast majority of equines could be recovered from strangles over a period of weeks, and the exposure of horse to strangles might be occurred without knowledge of its owner (٢٦). In addition, many worldwide outbreaks were thought to be happened but with atypical clinical signs that attributed to the difference in *S. equi* strains (٢١, ٢٧). However, respiratory signs in horses could be attributed to several pathogenic and non-pathogenic (allergic) that occurred, mostly, due to the poor management, and the ability of *S. equi* to establish persistent infection is critical to inter-epizootic transmission, recurrence of strangles and a high incidence of infection around the world (٢٨, ٢٩).

In conclusion, this was the first study concerned with identification of carrier equines with strangles by using of indirect-ELISA in apparently healthy horses and donkeys, and confirmation of seropositive horses result by culture. Also, the study evaluated the role of seropositive carriers among the different regions of three Iraqi provinces, ages and sexes. Hence, the efficient identification and treatment of carriers is very important for prevention and/or eradication of this disease.

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