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Preliminary Detection of Rickettsiae Using PCR Targeting *OmpA* Gene Among Dogs and Horses in Cairo, Egypt

Hend H. Abdullah[1], Amal El-Molla[2], Fayez A. Salib[2], Alaa A. Ghazy[1], Nesreen A. Allam[1], and Sobhy Abdel-Shafy[1]

[1] *Department of Parasitology and Animal Diseases, Veterinary Research Division, National Research Centre, Dokki, Cairo, Egypt.*

[2] *Department of Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt.*

BACKGROUND: Rickettsioses are intracellular bacterial infectious diseases causing high morbidity and sometime high mortality with public health importance.

Aim: The aim of present study was to detect rickettsiae in dogs and horses in Cairo, Egypt by using Polymerase Chain Reaction (PCR) targeting *OmpA* gene.

Methods: A total number of 200 blood samples (100 from dogs and 100 from horses) were collected from Cairo province, Egypt. All blood samples were investigated by PCR using *OmpA* gene to detect the rickettsial infection. Furthermore, hematological examination for all blood samples was performed.

Results: PCR detection of rickettsiae using *OmpA* gene amplification revealed that the infection rate was 18 % and 72 % in dogs and horses, respectively. Regarding hematological changes, hypochromic anemia, significant increase in neutrophils, lymphopenia and monocytopenia were recorded in rickettsiae positive dogs. Meanwhile, thrombocytopenia, leucopenia and monocytosis were recorded in rickettsiae positive horses.

Conclusion: The detection of rickettsiae in dogs and horses indicated that dogs and horses play a critical role as sentinels in an epidemiological approach of rickettsiae in Cairo, Egypt.

Keywords: Rickettsiae, Dogs, Horses, PCR, *OmpA* gene.

Introduction

Dogs and horses are important in spotted fever group (SFG) rickettsioses epidemiology. These animals act as sentinels and amplifier hosts because of their contacts to nature, vegetation and ticks [1].

Dogs play an important role in maintaining the infection of rickettsiae in nature. Dogs can act as reservoir for rickettsiae and have acquired infection of *Rickettsia conorii* from infected *Rhipicephalus sanguineus* ticks and transmitting rickettsiae to other uninfected ticks [2,3]. In USA, some cases of *Rickettsia rickettsii* (*R. rickettsii*) were reported in dogs and their owners [4-6], as well as in Brazil [7,8]. Also, dogs were considered to be the sentinels of *R. conorii* infection [9,10].

Horses are playing important role in spreading of infected ticks as they move from place to another in the country [11-13]. However, Sangioni et al. [12] and Riveros-Pinilla et al. [14] reported that the identification of antibodies in horses is important diagnostic method for detection the presence of *R. rickettsii* in certain areas. In a study in North America, *R. rickettsii*-experimentally infected horses showed fever and had rickettsemia for only one day. *R. rickettsii*-infected horses did not present other clinical signs of the disease, but they reported high titers of IgG antibodies with long-lasting persistence [15].

Corresponding auther: Sobhy Abdelshafy, E-mail: aasobhy@yahoo.com, P.O.: 12622. Tel.&Fax. +2023337093,
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Generally, rickettsioses have low mortality but with high morbidity except some *Rickettsia* spp. which had high mortality in dogs and people as *R. rickettsii*. After occurrence of tick bite to the animal, the main clinical symptoms of rickettsioses begin to appear after 4-10 days. The main clinical manifestations were in the form of high temperature, headache, muscular pain, skin rash, local lymphadenopathy and a characteristic eschar (tache noire) at the site of bite. Rickettsioses-related common non-specific laboratory abnormalities include mild leukopenia, anemia, and thrombocytopenia [9,16,17].

In Egypt, a few studies were conducted on the diagnoses of Spotted Fever Group rickettsiae (SFG) in ixodid ticks. Two SFG species, *Rickettsia africae* and *Rickettsia aeschlimanni* were recorded in *Hyalomma* spp collected from camels, and *R. africae* was only detected in the camel host [18-20]. Knowledge about rickettsiae in dogs and horses are still lack. Therefore, this study was designed to detect the rickettsial infections in dogs and horses by using PCR targeting *OmpA* gene. Moreover, hematological changes associated with rickettsioses in dogs and horses were also investigated.

Materials and Methods

Animals and sampling

In this study, clinical signs in all animals were observed and recorded. Blood samples were collected from 200 animals (100 dogs and 100 horses) from Cairo province, Egypt. A total of 10 ml blood sample was taken from each animal from cephalic or saphenous vein in dogs and jugular vein in horses. The blood sample for each animal was poured in EDTA sterile tube for hematological examination, and the remaining was stored at -20°C forPCR investigations.

DNA extraction

Genomic DNA was extracted from blood samples using GF-1 Tissue Blood Combi DNA Extraction Kit (SNF Medical Company, Vivantis, Malaysia) according to the manufacturer's instructions.

Screening of rickettsiae by PCR using OmpA gene

PCR technique was carried out on 200 blood samples (100 from dogs and 100 from horses). Standard PCR was performed to detect rickettsiae using *OmpA* gene. A pair of primers was designed according to Fournier et al [21] targeting 590-634 bp of *OmpA* gene (Table1). The PCR reactions were performed in a PTC-100™ thermal cycler (MJ Research Inc., USA) under complete aseptic condition. The protocol of the reactions was applied according to Abdullah et al. [19]. PCR products were visualized by electrophoreses in 1.5% agarose stained with ethidium bromide. A 100 bp ladder (Alliance Bio, USA) was used with each gel. Finally, Lab Image software (Bio-Rad) was used for gel photos analyzed.

Hematological profiles

A total of 200 animal species (100 dogs and 100 horses) were used for hematological study. Hematological parameters were determined according to Merck Veterinary manuals [22-24]. The hematological parameters included RBCs count, Hb concentration, PCV, calculation of erythrocyte indices (MCV, MCH and MCHC), platelets count, WBCs count and differential leucocytic count.

Statistical analysis

The hematological parameters were performed according to Student's t test (SPSS 14.0 for Windows Evaluation Version). Probability values (P-value) < 0.05 were considered of statistically significant and < 0.001 were considered of high statistically significant.

Results

Clinical signs

Most of the examined animals were apparently healthy (90 dogs and 83 horses). Meanwhile, the main observed clinical signs in the rest of animals were in the form of fever (n = 5&13), anorexia (n = 4&9), lethargy and depression (n = 2&10), anemia (n = 2&7), enlargement of lymph nodes (n = 3&2), ocular signs (n = 2& 0), and emaciation (n = 2 &3) in dogs and horses, respectively.

TABLE 1. Primers utilized in amplification of *OmpA* gene.

DNA Marker	5'- Primers Sequences-3'	Amplified Fragments	Reference
OmpA gene			
190.70-F	5'-ATGGCGAATATTTCTCCAAAA-3'	590-634 bp	Fournier et al[21]
190.701-R	5'-GTTCCGTTAATGGCAGCATCT-3'		

Detection of SFG rickettsiae in dogs and horses

The obtained fragment products of *OmpA* were 500bp in horses and 600 bp in dogs (Fig. 1). The results revealed that the prevalence of rickettsiae in dogs and horses was 18% (18/100) and72% (72/100), respectively (Table 2).

Hematological parameters in dogs and horses with rickettsioses

The results revealed hypochromic anemia, significant increase in neutrophils, lymphopenia and monocytopenia in rickettsiae positive dogs. While, thrombocytopenia, leucopenia and monocytosis were recorded in rickettsiae positive horses (Tables 3&4).

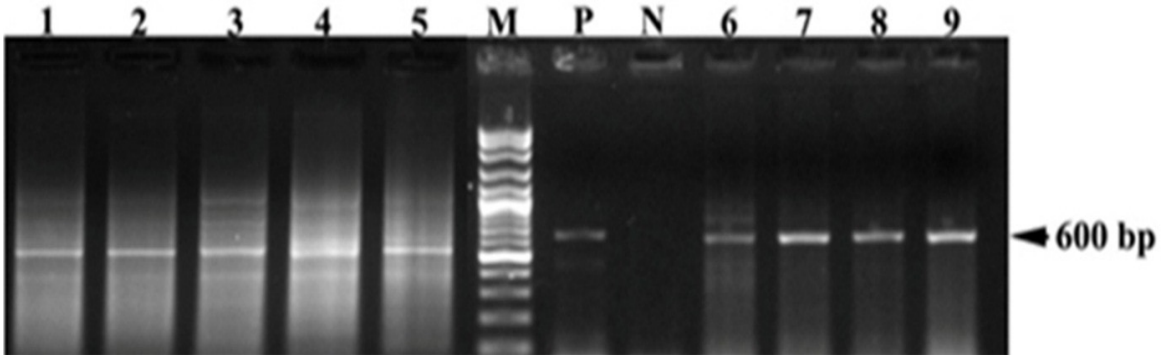

Fig. 1. Molecular identification of rickettsiae by PCR products of the *OmpA* gene detected in animal species using 1.5 % agarose gels stained with ethidium bromide. In figure, lane M: 100 bp DNA ladder, lane N: Control negative, lane P: Control positive. Lanes 1 to 5 present 500 bp amplicon of *OmpA* rickettsiae positive samples of horses, while lanes 6 to 9 present 600 bp amplicon of *OmpA* rickettsiae positive samples of dogs.

TABLE 2. The prevalence of rickettsiae among dogs and horses as screened by PCR.

Tick Infestation	Total No. of Tested Animals by PCR		No. of Positive Animals by PCR		The prevalence rate (%)	
	Dogs	Horses	Dogs	Horses	Dogs	Horses
Tick infested animals	41	0	9	0	22.0	0
Tick free animals	59	100	9	72	15.3	72.0
Total	100	100	18	72	18.0	72.0

TABLE 3. Hematological parameters of rickettsiae positive dogs compared with rickettsiae negative dogs as detected by PCR (Mean ± Standard Deviation; SD).

Hematological Parameters	Animal Groups	
	Rickettsiae negative Dogs	Rickettsiae positive Dogs
RBCs ($\times 10^6$)	6.06±0.18	6.28±0.36
Hb (g/dl)	15.97±0.35	15.40±0.98
PCV (%)	45.40±0.91	44.00±1.90
MCV (fl)	71.67±1.07	71.00±2.39
MCH (pg)	26.71±0.50	24.56±0.80*
MCVC (g/dl)	35.50±0.60	34.79±1.11
Platelets ($\times 10^3$)	161.90±13.92	162.30±21.19
WBCs ($\times 10^3$)	17.16±0.73	14.82±1.55
Neutrophils (%)	35.40±4.29	58.30±8.30**
Lymphocytes (%)	54.83±4.00	33.00±7.48**
Monocytes (%)	8.16±0.44	7.00±0.85*
Eosinophils (%)	1.59±0.11	1.69±0.17

* = significant at P< 0.05 ** = high significant at P< 0.01

TABLE 4. Hematological parameters of rickettsiae positive horses compared with rickettsiae negative horses as detected by PCR (Mean ± Standard Deviation; SD).

Hematological Parameters	Animal Groups	
	Rickettsiae negative Horses	Rickettsiae Positive Horses
RBCs ($\times 10^6$)	5.11±0.23	5.69±0.17
Hb (g/dl)	11.02±0.45	11.27±0.33
PCV (%)	32.77±1.45	32.64±1.13
MCV (fl)	64.38±1.42	57.93±1.71
MCH (pg)	21.63±0.29	20.00±0.45
MCVC (g/dl)	33.72±0.43	34.86±0.49
Platelets ($\times 10^3$)	174.07±12.57	123.81±11.99**
WBCs ($\times 10^3$)	7.02±0.32	5.77±0.26*
Neutrophils (%)	29.00±1.39	39.71±3.85
Lymphocytes (%)	57.64±1.75	58.96±1.50
Monocytes (%)	3.85±0.64	10.74±0.49**
Eosinophils (%)	2.21±0.28	2.02±0.09

* = significant at P< 0.05 ** = high significant at P< 0.01

Discussion

The current study is one of the pioneer studies on rickettsiae infecting dogs and horses in Egypt. The great importance has been given to police dogs, as they are used to enforce public order by chasing, holding and locating suspects or finding missing persons or objects, detecting illicit substances such as drugs or explosives and detecting the odour of decomposing bodies. Moreover, horses have been used in security purposes in desert and border areas, also in racing as a traditional sport. The present study was directed to perform hematological and molecular diagnostic investigations on rickettsiae in dogs and horses.

The main clinical signs observed in a few number of animals from the 200 studied dogs and horses were in the form of fever, anorexia, lethargy and depression, anemia, enlargement of lymph nodes, ocular signs and emaciation. Meanwhile, the most of animals were apparently healthy. These recorded results are similar to those mentioned by Gasser et al. [25], Solano-Gallego et al. [26], Piranda *et al*[2,8] and Levin et al. [27], who reported the same symptoms of nonspecific febrile illness associated with rickettsiae. Davidson et al. [28] added that the ocular findings included bilateral conjunctival vascular injection, multifocal retinal hemorrhages, anterior uveitis and petechial hemorrhages in the iris stroma were also recorded. Whereas, ocular hemorrhages were the most common ophthalmic sign of spotted fever rickettsiae positive animals [29]. Meanwhile, these results disagree with Weiser and Greene [30], who reported cutaneous lesions in rickettsioses in the form of oedema as well as petechial and ecchymotic hemorrhages with severe dermal necrosis. Oedema and hemorrhages were severe in scrotum pinnae and limbs. Moreover, the same clinical signs in dogs were observed in horses except ocular signs. Lemos et al. [11] and Medeiros et al. [31] reported that the clinical manifestations of the disease in horses are rare. Concerning the rest of animals in the current study, they were apparently healthy (90 dogs and 83 horses). These results disagree with Kelly et al. [32], Solano-Gallego et al. [33] and Ortuno et al. [10], who reported that no statistically significant differences were found between clinically healthy and sick dogs. Moreover, Riveros-Pinilla et al. [14] detected antibodies against *Rickettsia* spp. in apparently healthy horses.

There were no previous studies that applied to detect rickettsiae in dogs and horses by PCR in Egypt. The diagnosis of rickettsiae was characterized as a challenge, because of non-specific clinical signs and laboratory abnormalities or subclinical infection [9,17,25]. Molecular techniques (including PCR and sequencing) were applied to allow more accurate and rapid detection and identification of rickettsiae with improved sensitivity and specificity of the diagnosis [17,34].

Primer amplifying sequence of rickettsiae was *OmpA* which was less conserved gene in SFG rickettsiae, so it had a high discrimination power in rickettsiae [21].

PCR technique was carried out on 200 blood samples collected from dogs and horses. The blood samples were screened by PCR targeting *OmpA* gene (Spotted fever group specific primer). The obtained fragment products of *OmpA* were ranged from 500 to 600 bp, as shown in figure (1). The results revealed that the prevalence of rickettsiae in dogs and horses was 18 and 72 %, respectively. Hence, there were no previous studies detected rickettsiae in animal hosts by PCR in Egypt. The prevalence of canine SFG rickettsiae in dogs in the present study (18.0 %) was similar with Solano-Gallego et al. [26] in Italy, who reported that the rate of *Rickettsia* spp. DNA in the blood of sick dogs was 14 %. Kamani et al. [35] detected DNA of *Rickettsia* spp. in dog blood samples (8.8 %) in Nigeria. Meanwhile in horses, the prevalence of SFG rickettsioses in the present study (72.0 %) agreed with Lemos et al. [11], Horta et al. [36], Medeiros et al. [31], Alves et al. [37] and Riveros-Pinilla et al. [14], who had detected rickettsiae serologically in horses using immunofluorescence. In addition, Vianna et al. [38] and Pacheco et al. [39] found that the prevalence rate of rickettsiae in horses ranged from 68 to 81 %. However, the detection of SFG rickettsiae in the present study indicated the possibility for these pathogens to be present in dogs and horses in Egypt, and the importance of these domestic animals as potential infection amplifiers which play a more dominant role in the persistence of rickettsiae in the nature than previously thought [2,7]. Hence, the detection of tick-borne rickettsiae in dogs and their ticks in the previous studies indicated that both the animal and human populations in Egypt are at risk for these pathogens [19]. In addition to, *Rh. sanguineus* ticks was known to be aggressive to bite humans [40]. These characteristics can facilitate the transmission of rickettsiae to human.

The incidence of rickettsiae among infested and non-infested animals by ticks was detected by PCR; the prevalence of rickettsiae was 22.0 % in the tick infested dogs but 15.3% in the non-infested dogs as shown in Table 2. Similar results were obtained by Ortuno et al. [10], who stated that highly exposed dogs to *Rh. sanguineus* ticks reported higher seroprevalence level with rickettsiae than dogs living as pets or Kennels and subjected to tick control programs. Our results indicated that animals infested by ticks were at high risk to infection withrickettsia spp. because *Rh. sanguineus* ticks was detected as the principle vector of rickettsiae in Egypt [41,42]. Some ticks-infested animals were negative for rickettsiae in the present study. This may be attributed to the fact that attached ticks were free from rickettsiae spp., or infected with rickettsiae but they recently attached to these animals, thus the ticks need some time to transmit rickettsiae to animals. Moreover, the ticks-free dogs and horses (at time of examination) which were proved positive for rickettsiae in the present study might be infested previously by ticks, yet received manual tick removal and/or various acaricide treatments.

Hematological profiles in the studied animals were recorded as shown in Tables 3&4. The presented results revealed that hypochromic anemia, significant increase in neutrophils, lymphopenia and monocytopenia were recorded in rickettsiae positive dogs. Meanwhile, thrombocytopenia, leucopenia and monocytosis were recorded in rickettsiae positive horses. These results disagree with Levin et al. [27], who reported that marked monocytosis and leukocytosis have been found in dogs, while, these authors' reports agree with our results in horses; marked monocytosis. The reported thrombocytopenia and leucopenia in the investigated horses are in agreement with Gasser et al [25], Elchos and Goddard[5] and Parola et al [9,17], who recorded early leukopenia during the course of the disease followed by progressive leukocytosis and severe thrombocytopenia.

Conclusion

This study is the first detection of *Rickettsia* spp. in dogs and horses in Cairo, Egypt using PCR. Hypochromic anemia, marked increase inneutrophils, lymphopenia and monocytopenia were reported in rickettsiae positive dogs, while, thrombocytopenia, leucopenia and monocytosis were recorded in rickettsiae positive horses.

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Conflict of interest

The authors declared that they have no conflict of interest.

Ethical standard

The study was conducted according to the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals. Informed consent was obtained from the owner of the animals included in the study.

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تحديد اولى للركتسيا باستخدام البسي ار لجين او ام بي ايه في الكلاب و الأحصنه بالقاهرة ـ مصر

هنـد حسن عبدالله [١] ، آمال الملا [٢] ، فايــز صليب [٢] ، عــلاء عبدالمنعم غازي [١] ، نسـرين علام [١] وصبحى عبدالشافى حسن [١]

[١] قسم الطفيليات و أمراض الحيوان – شعبة البحوث اليطرية ـ المركز القومي للبحوث ـ القاهرة ـ مصر

[٢] قسم الأمراض المعدية – كلية الطب البيطري – جامعة القاهرة ـ القاهرة ـ مصر.

تم إجراء دراسات هيماتولوجية وتشخيصية لمرض الركيتسيا فى الكلاب والخيول وذلك لتحديد مدى اصابة هذه الحيوانات بمرض الركيتسيا. ولتحقيق هدف هذه الدراسات تم تجميع عدد 200 عينة دم من الحيوانات المختبرة من بينهم 100 من الكلاب, و 100 من الخيول من محافظة القاهرة.

تم تسجيل الأعراض المرضية الظاهرة على الحيوانات فكانت كالآتى: إرتفاع فى درجة الحرارة (٥ كلاب, و١٣ خيل), وفقدان الشهية (٤ كلاب, و٩ خيل) وفتور و اجهاد (٢ كلاب, و١٠ خيل), وأنيميا (٢ كلاب, و٧ خيل) , وتورم فى الغدد الليفاوية (٣ كلاب, و٢ خيل), وأعراض فى العين (٢ كلاب, و٠ خيل), ونحول و هزال (٢ كلاب, و٣ خيل). بينما باقى الحيوانات كانت ظاهريا طبيعية (٩٠ كلاب, و٨٣ خيل).

تم إجراء المسح الجزيئى للركيتسيا فى عينات الدم بأستخدام مناطق مشفرة من الجينوم(*OmpA*)) فى اختبار تفاعل البلمرة المتسلسلPCR)) على ٢٠٠ عينة دم (١٠٠ كلب, و١٠٠ خيل). وأظهرت النتائج أن نسبة الأصابة بالركيتسيا كانت١٨,٠ ٪ فى الكلاب, و٧٢,٠ ٪ فى الخيول.

تم عمل دراسات هيماتولوجية على جميع الحيوانات, وأظهرت النتائج وجود أنيميا, وزيادة معنوية فى خلايا النيتروفيل والمونوسيت مع وجود انخفاض معنوى فى خلايا الليمفوسيت فى الكلاب المصابة بالركيتسيا. كما تم تسجيل انخفاض معنوى فى كلا من صفائح الدم وكرات الدم البيضاء وزيادة فى المونوسيت فى الخيول المصابة بالركيتسيا.