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Article

Molecular detection of *Borrelia anserina* in *Argas persicus* (Acari: Argasidae) ticks collected from Lorestan province, west of Iran

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ABSTRACT
Borrelia anserina is the agent of avian borreliosis, an acute septicemic disease of a variety of avian species in tropical and warm temperate regions of the world. Avian borreliosis is one of the most widespread poultry diseases in Iran, and is of great economic importance. The present study was designed to detect *B. anserina* in *Argas persicus* ticks. Specimens were collected from the cracks of aviary in Lorestan province of Iran. Then the salivary glands, ovaries and uterus of ticks were dissected to detect *B. anserina* within the specific organ using molecular methods. DNA was extracted by Phenol-chloroform method and then a fragment of flagellin gene (*fla*B) of *B. anserina* was amplified by polymerase chain reaction. According to our results, the *fla*B target fragment was detected in *Argas persicus* ticks collected from Lorestan province. It seems that *B. anserina* is widely distributed in *A. persicus* vector ticks. Based on the result, *B. anserina* strain of Iran is similar to *B. anserina fla*B sequences reported from other parts the world.

KEY WORDS: *Argas persicus*; Avian borreliosis; *Borrelia anserina*; flagellin gene; Iran.

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INTRODUCTION

Borrelia anserina (Spirochaetales: Spirochaetaceae) is the agent of avian borreliosis (fowl spirochaetosis), an acute septicemic disease of a variety of avian species e.g. chickens, turkeys, pheasants, geese, and ducks (Abdul-Aziz and Barnes 2013). The disease causes serious losses of domestic chickens and other fowl in tropical and warm temperate regions of the world (Hoogstraal 1985; Ataliba *et al.* 2007; Lisbôa *et al.* 2009; Abdul-Aziz and Barnes 2013). The outbreak and incidence of fowl spirochaetosis were reported from poultries in America, Africa and Asia (Samberg *et al*. 1972; Cooper and Bickford 1993; Mohammed 2008). Affected birds become weak and anemic, many of them show greenish diarrhea and splenomegaly (Cooper and Bickford 1993). The disease is responsible for sporadic losses in subsistence flocks and small scale commercial units (Shane 1997). *B. anserina* is usually transmitted by the fowl tick, *Argas persicus* (Oken) (Acari: Argasidae) and

other *Argas* spp. as well as, by *Culex* mosquitoes (Dickie and Barrera 1964; Lisbôa *et al.* 2009); however, Hungerford and Hart (1937) reported the transmission of *B. anserina* by the red mite, *Dermanyssus gallinae* (De Geer). It has been revealed that other *Argas* ticks can transmit the pathogen agent and retain the infection through the different organs (Diab and Soliman 1977; Zaher *et al.* 1977; Lisbôa *et al.* 2009). Adult ticks may remain alive without feeding and they may carry the spirochete for as long as 3 years (Abdul-Aziz and Barnes 2013). McNeil *et al.* (1949) believed that the vector ticks may harbor *Borrelia* infection for many days, but it is uncertain whether they can transmit virulent *B. anserina* agent to fowl host after many years or not. The spirochete infects all tissues of *Argas* ticks (McNeil *et al.* 1949). The genus *Borrelia* evolved as symbionts of ticks, especially argasid ticks, but act as parasites in mammals and birds (Hoogstraal 1985). Avian borreliosis is one of the most widespread poultry diseases with remarkable economic impact on Iran which was diagnosed in Iran for the first time by RAZI-Institute (Sohrab *et al.* 1957). The clinical field observation in Lorestan province showed typical symptoms of avian borreliosis in the poultries. Moreover, *A. persicus* vector tick is widespread in the country as well as in Lorestan province (Hosseini-Chegeni and Tavakoli 2013; Kayedi *et al.* 2016). In this regard, the present study was designed to detect *B. anserina* agent in *Argas* vector ticks, following the characterization of flagellin gene (*fla*B) as a gene for taxonomic and phylogenetic studies on the bacterial agent.

MATERIALS AND METHODS

Tick specimens

Argas persicus ticks were collected from the cracks of aviary (Figure 3), in many rural areas located in Lorestan province includes; Aleshtar county: Qalayi rural district, Cheshmeh Tala village (33° 46' 40.61" N, 47° 55' 11.29" E), Borujerd county, Darreh Seydi rural district, Kalleh village (33° 57' 9.12" N, 48° 57' 41.56" E), Khorramabad county: 1. Papi district, Sarkaneh village (33° 18' 33.60" N, 48° 42' 54.29" E), 2. Veysian district, Chamdivan village (33° 29' 14.44" N, 48° 0' 13.24" E) and Pol-e Dokhtar county, Mamulan district, Kur Shurab-e Alishah village (33° 27' 49.51" N, 47° 51' 26.86" E). The live tick specimens were transported to the laboratory. Argas tick species were identified according to taxonomical key by Hosseini-Chegeni and Tavakoli (2013).

Figure 1. The aggregated *Argas persicus* ticks at different stages around a thatched birdhouse in Lorestan province.

Dissection of ticks

The un-engorged/semi-engorged tick specimens in different reproductive conditions containing semi-gravid and gravid were dissected in normal saline under a light stereomicroscope (SZX12-Olympus®, Japan). The salivary glands, ovaries and uterus (Figure 2) of male and female ticks were dissected using incision tools e.g. pin, scalpel and tiny forceps. As well as, the gut and diverticula of ticks completely were removed. To avoid these organs with hemolymph and contents of tick's gut, the dissected organs were washed two or three times with clean normal saline. Then, each dissected organ was placed in a distinct microtube for each individual tick. In some cases salivary glands of four different male or female specimens were pooled together.

Examination of avian blood

To confirm the spirochete *B. anserina* in peripheral blood stream of domestic avian, blood smears from seven avian hosts were prepared individually according to Quinn *et al.* (1999). So the blood smears were stained using Giemsa dye and observed under a light microscope.

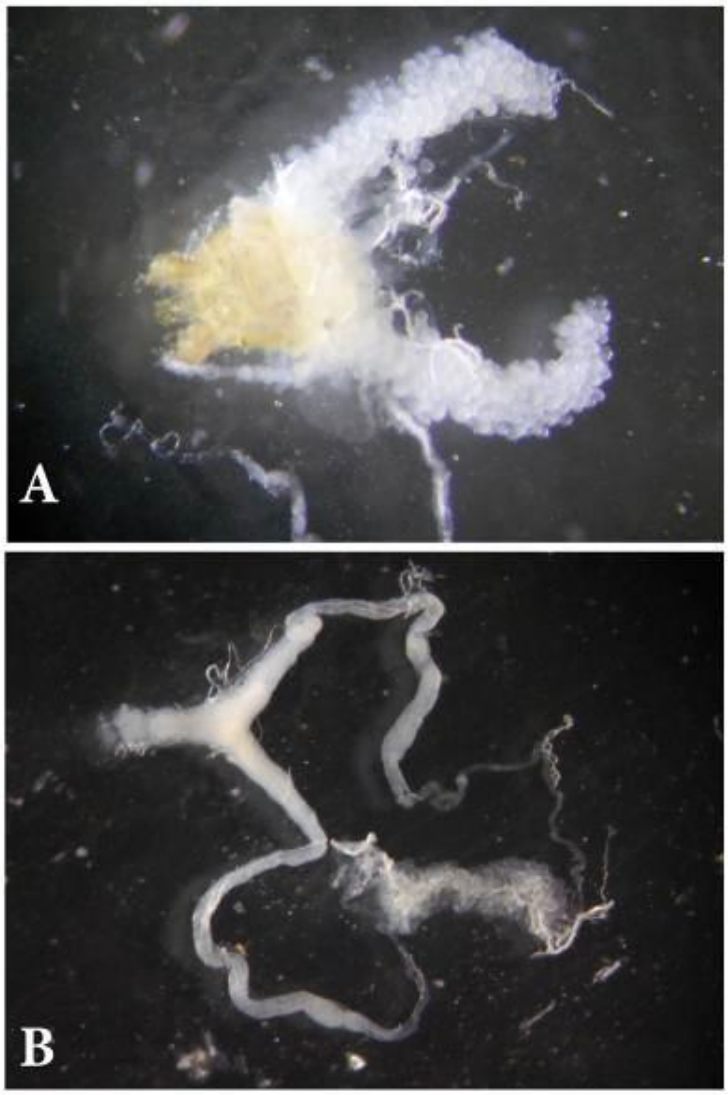

Figure 2. The stereomicroscopic view of dissected salivary glands (A); ovary and uterus (B) of female *Argas* tick in normal saline.

Molecular identification of B. anserina

DNA of dissected organs was extracted using Phenol-chloroform according to our previous lab

protocol set up by Hosseini-Chegeni (2015). A fragment of flagellin gene (*fla*B) of *B. anserina* was amplified by polymerase chain reaction (PCR) using our forward (Fans: 5′- CTC AAA TTA GAG GAT TAT CTC AAG C -3′) and reverse primers (Rans: 5′- TGC TAC AAT TTC ATC TGT CAT TG -3′). PCR reactions for *fla*B gene were carried out in thermocycler (Corbett®, Australia) by initial denaturation (5 min. at 95°C), 30 cycles of denaturation (60 sec. at 95°C), annealing (60 sec. at 55°C), extension (50 sec. at 72°C), and a final extension step (5 min. at 72°C). PCR for each 25 µl final volume reaction was done using 12.5 µl RedMaster PCR® 2X, 1 µl from each forward and reverse primers (10 pM), 4 µl gDNA template (100 ng/µl), 6.5 µl deionized water. The 725 bp PCR product related to *B. anserina* were visualized by 1% agarose gel electrophoresis and the desired bands were purified using GeneJET Gel Extraction Kit® (Thermo Fisher Scientific, USA), then purified PCR products were submitted for sequencing to Faza Biotech® Company (Iran).

Sequencing and phylogenetic analysis

Only 629 bp out of entire size of *fla*B amplified fragment were sequenced, successfully. Phylogenetic analysis was based on just 503 bp after alignment of all *fla*B sequences (including a single sequence of this study and the sequences of GenBank. The sequences were aligned using ClustalW available in MEGA7 package (Kumar *et al.* 2016), then final DNA sequences (503 bp in size) were translated to corresponding amino acid sequences (167 AA). The evolutionary genetic distances were computed using Poisson correction model (amino acid sequences) and Maximum Composite Likelihood model (DNA sequences), then the *fla*B phylogenetic trees for both amino acid and DNA sequences was constructed based on Neighbor-Joining (NJ) method by MEGA7 package. For this purpose, 12 sequences (comprising ingroup and outgroup) were examined for *fla*B phylogenetic analysis. The clades and distinct branches (JF693808, KX171816) of the constructed phylogenetic tree were reorganized based on both criteria bootstrap value greater than 99% support and or a reasonable genetic distance of each species (5–13%). The outgroups were selected from sister groups as well as successively more distant lineages respectively including *B. hermsii* (KX171816) as the nearest lineage, followed by *B. crocidurae* (JX292911), *B. microti* (JF708951), and *B. duttonii* (GU357617), then a more distance lineage *B. recurrentis* (CP000993). Moreover, the ingroups were chosen from GenBank comprising *B. anserina* (CP005830, X75201, CP013704, CP005829, DQ849626, and JF693808).

RESULTS

Molecular detection of B. anserina

Argas persicus tick specimens were collected from different parts of Lorestan province (Table 1). A 752 bp flaB target fragment of Borrelia anserina was detected in A. persicus ticks collected fromAleshtar county, Qalayi rural district, Cheshmeh Tala village, Khorramabad county, Papi district, Sarkaneh village and Pol-e Dokhtar county, Mamulan district, Kur Shurab-e Alishah village.

Table 1. Data of *Argas persicus* specimens collected on poultries examined for *Borrelia anserina* infection in Lorestan province.

County	No. of examined tick pool	Sex	Infected organ	*Borrelia anserina* infection
***Khorramabad**	8	M, F	Ov, Sg, Ut	Negative
****Khorramabad**	10	M, F	Ov, Sg, Ut	Positive
Aleshtar	15	M, F	Ov, Sg, Ut	Positive
Borujerd	10	M, F	Ov, Sg, Ut	Negative
Pol-e Dokhtar	10	M, F	Ov, Sg, Ut	Positive

Ov: ovary, Sg: salivary gland, Ut: uterus, M: male, F: female
* Veysian district, ** Papi district

Amino acid phylogenetic analysis

A fully resolved phylogenetic tree based on *fla*B amino acids sequence data of 12 *Borrelia* taxa including ingroup (clade I and JF693808) and outgroup (clade II and KX171816) was constructed using MEGA7 software (Figure 3). The presented phylogeny indicates that the Iranian *B. anserina* might be grouped with the GenBank *B. anserina* sequences data submitted from Ethiopia, USA, Brazil and Sweden. Except for a Pakistani strain, no intra species variation was found between *fla*B sequences of the clade I in terms of genetic distance. Apparently, all these different members should be assigned as a single species according to *fla*B gene. However, ~5% genetic distance was found between Pakistani strain of *B. anserina* (JF693808) with the members of main clade I (*B. anserina*). This strain may be seen as an isolate *B. anserina* lineage within the *fla*B amino acids phylogenetic tree (Figure 3). An outgroup clade including *B. crocidurae*, *B. microti*, *B. duttonii* and *B. recurrentis*, as well as, *B. hermsii* as a single outgroup lineage were constructed. Strain of Iran (sequence of this study) was clustered with *B. anserina* (DQ849626) originally isolated from *Argas miniatus* ticks from Brazil.

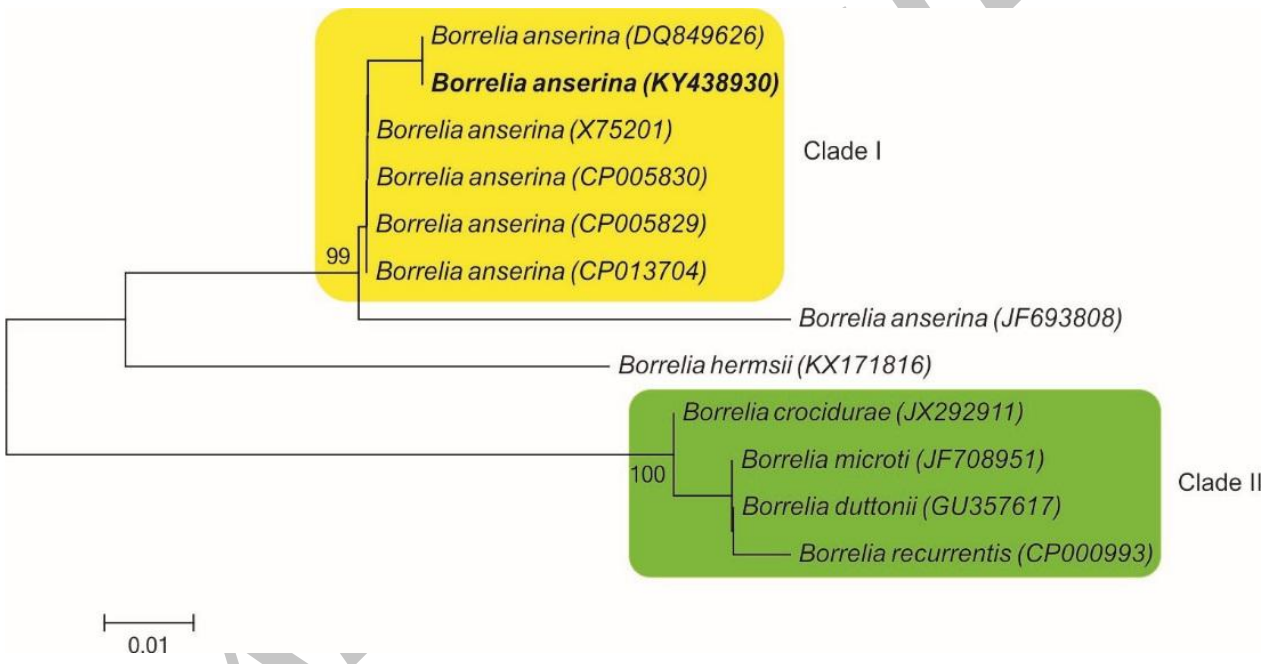

Figure 3. The phylogenetic tree inferred from *fla*B amino acids sequence data of *B. anserina* (clade I) and other *Borrelia* taxa (clade II, KX171816 and JF693808) constructed using Neighbor-Joining (NJ) method with bootstrap test (1,000 replicates). The main clade in right side of tree separated with colored rectangular shape. Taxa are as species name following GenBank accession number, taxon of the present study indicated as bold. Nodes indicated with bootstrap value. Branch lengths are proportional to evolutionary changes, the units of the number of amino acid substitutions per site. The analysis involved 12 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 166 positions in the final dataset. The more diverged Pakistani strain of *B. anserina* (JF693808) located outside the main *B. anserina* (clade I). The members of clade II and *B. hermsii* are as outgroup.

Nucleotide phylogenetic analysis

A fully resolved phylogenetic tree based on *fla*B nucleotide sequence data of 12 *Borrelia* taxa including ingroup (clade I) and outgroup (clade II and KX171816) was constructed using MEGA7 software (Figure 4). The presented phylogeny indicates that the Iranian *B. anserina* might be grouped with the GenBank *B. anserina* sequences data submitted from Ethiopia, USA, Brazil and Sweden. Except for a Pakistani strain, no intra species variation was found between *fla*B sequences of the clade I in terms of genetic distance. Apparently, all these different members should be assigned as a single species according to *fla*B gene. However, ~3% genetic distance was found between Pakistani strain

(JF693808) arising from the *B. anserina* clade I (Figure 4). Therefore, nucleotide phylogenetic tree is poorly resolved according to little resolution in the clade I. An outgroup clade including *B. crocidurae*, *B. microti*, *B. duttonii* and *B. recurrentis*, as well as, *B. hermsii* as a single outgroup lineage were constructed. Strain of Iran (sequence of this study) was clustered with *B. anserina* (DQ849626) originally isolated from *Argas miniatus* ticks from Brazil. A high genetic similarity between 98%–100% was found between *fla*B sequences of GenBank submitted from USA (CP005829, CP005830, CP013704), Brazil (DQ849626), Sweden (X75201) and Ethiopia (JF414732) with a *fla*B of the present study.

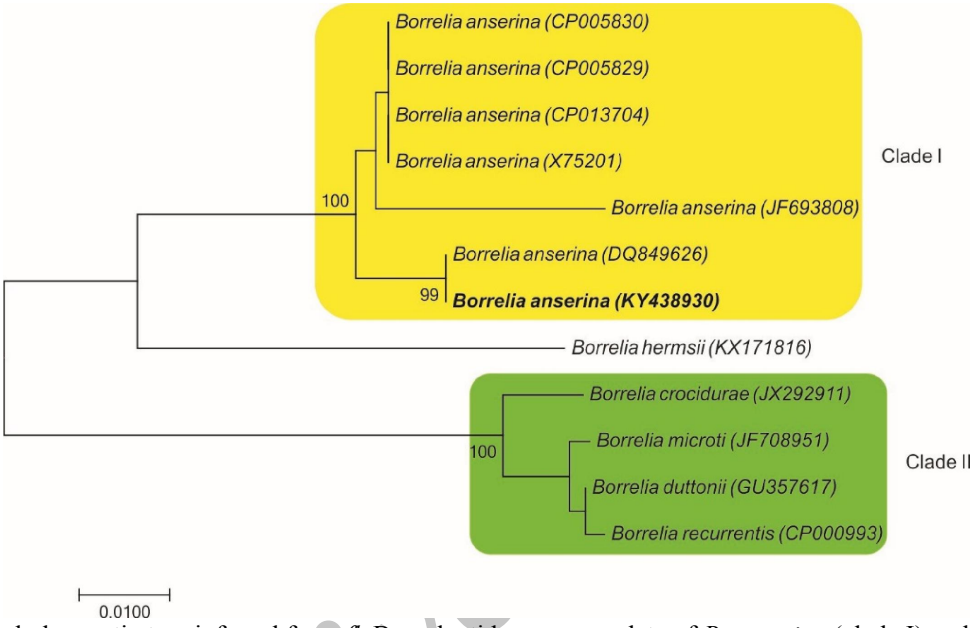

Figure 4. The phylogenetic tree inferred from *fla*B nucleotide sequence data of *B. anserina* (clade I) and other *Borrelia* taxa (clade II and KX171816) constructed using Neighbor-Joining (NJ) method with bootstrap test (1000 replicates). The main clade in right side of tree separated with colored rectangular shape. Taxa are as species name following GenBank accession number, taxon of the present study indicated as bold. Nodes indicated with bootstrap value. Branch lengths are proportional to evolutionary changes, the units of the number of base substitutions per site the units of the number of amino acid substitutions per site. The analysis involved 12 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 500 positions in the final dataset. The more diverged Pakistani strain of *B. anserina* (JF693808) located inside the main *B. anserina* (clade I). The members of clade II and *B. hermsii* are as outgroup.

Examination of avian blood

No spirochete was observed in microscopic examination of avian blood smears prepared from under wing peripheral blood (date not shown).

DISCUSSION

The present study described the first molecular characterization of *B. anserina* strain in Iran. Previously, fowl spirochaetosis has been reported in this country (Sohrab *et al.* 1957; Rak 1976; Rafyi and Rak 1985). But the etiological agent was never characterized by molecular methods. Since the avian hosts such as fowls and domestic birds are considered as the main reservoirs of diseases with more widespread *Argas* vector tick in rural area of Iran thus this study was designed to examine vector status of ticks in terms of infection to *B. anserina*. Occurrence of spirochetosis in tropical or sub-tropical countries corresponds with the distribution of fowl ticks in the genus *Argas*, which serve as both the reservoir and primary vector (Seneviratna 1969; Abdul-Aziz and Barnes 2013). In order to specifically detect *B. anserina* within tick's organs, molecular assay was carried out on salivary

glands, ovaries and uterus of tick specimens, individually or as pooled organs. Although previous studies showed *Argas* ticks are main vectors of *B. anserina* in laboratory conditions (Diab and Soliman 1977; Zaher *et al*. 1977; Lisbôa *et al*. 2009), we tried to detect the disease agent inside these organs which are free of the contents of ingested blood meal inside tick's gut. The morphological, serological and immunological strain variation i.e. mild or virulent isolates, has been reported in various isolates of *B. anserina* (Djankov *et al.* 1972; DaMassa and Adler 1979; Soni and Joshi 1980; Hovind-Hougen 1995; Sambri *et al.* 1999; Abdul-Aziz and Barnes 2013), although, a high genetic similarity between 98%–100% was found between *fla*B sequences of GenBank submitted from USA (CP005829, CP005830, CP013704), Brazil (DQ849626) (Ataliba *et al.* 2007), Sweden (X75201) and Ethiopia (JF414732) (Cutler *et al.* 2012) with a *fla*B of the present study. As well as, a long time ago, five strains of *B. anserina* isolated from different parts of Iran have been identical, antigenetically (Sohrab *et al.* 1957).

Furthermore, all members of clade I with the *B. anserina* sequence of this study clustered in a monophyletic group under 99% bootstrap support. It seems that all these taxa (except for a Pakistani strain) should be considered as a distinct strain in terms of *fla*B gene phylogenetic relationships. However, a high genetic distance difference (~5% in amino acids sequence data and ~3% in nucleotide sequence data) was found between a *fla*B sequence submitted from Pakistan with clade I (including a *fla*B sequence of the present study). Thus, here both amino acids and nucleotide phylogenetic trees are incongruent since it is not clear whether Pakistani strain should be considered inside or outside the clade I. This is a conflict that may be due to insufficient data that needs to investigate in future with more sequences originated from Pakistan and Iran. However, it seems that amino acids phylogenetic tree is more informative than nucleotide phylogenetic tree because regarding to a high genetic distance difference, Pakistani strain positioning as an early-diverging ('basal') lineage relative the clade I, so that this is more reasonable than nucleotide phylogenetic tree. After examining the blood smears prepared from the under wing peripheral blood of fowls (hens and rooster) in a rural house with molecular confirmed *B. anserina* infected *Argas persicus*, no spirochete was observed under the microscopic examination. According to Abdul-Aziz and Barnes (2013), to examine the spirochetes in the chickens exposed to ticks *A. miniatus*, the blood smears should be prepared between day 5 and day 12 post exposure, with the peak number of spirochetemic birds occurring between days 7 and 9 (Abdul-Aziz and Barnes 2013). Whereas, McNeil *et al.* (1949) believed that after primary infection to virulent *B. anserina* agent, *Argas* ticks are unable to transmit bacterial agent to bird host during the later days. Apparently, failure to observing of spirochete in the blood smears may be owing to McNeil hypothesis that is claimed the bacterium may be disappear from the blood stream after febrile period.

Furthermore, the indigenous avian host should be immunologically more resistant than exotic breeds against *B. anserina* infection due to repeated bites of infected *Argas* ticks (Abdul-Aziz and Barnes 2013). An infected tick may remain infective for up to 3 years if the environmental temperature is sufficiently high i.e. 35°C, however, at temperatures below 20°C the spirochetes disappear from the tick body (Seneviratna 1969). So that, the detection of disease agent in the present study can be due to its long survival in vector tick. In the past years, the genus *Borrelia* (including species with human host) was considered in soft tick species collected from Iran (Aghighi *et al.* 2007; Barmaki *et al.* 2010; Rafinejad *et al.* 2011; Naddaf *et al.* 2012; Aghaei *et al.* 2014). Except, *B. anserina*, the bird hosts can be infected by various other species of the genus *Borrelia*, such as *B. burgdorferi s. str.* and *B. garinii*, known as the causative agents of Lyme borreliosis in humans (Kurtenbach and Schäfer 2001). Based on the results of this study, it seems that the spirochete agent is widely distributed in *A. persicus* vector ticks, since more examined *Argas* tick specimens or tick pools in different geographical locations were found to be infected. The soft tick *A. persicus*, which is a cosmopolitan species would be found in different rural situation with high adaptability even higher than *Ornithodoros* ticks in Iran territory (Hosseini-Chegeni and Tavakoli 2013). Since unlike the *Argas* ticks, some *Ornithodoros* species disappear within its distribution area in Lorestan province

during past decay due to reconstruction of animal housing in rural area (personal observation). According to previous studies conducted in Lorestan province, domestic birds may be infect/infest with many parasitic agents including *Argas* tick species (Dezfoulian *et al.* 2011; Dezfoulian *et al.* 2013; Zakian *et al.* 2014; Badparva *et al.* 2015). The disease might be frequently present in rural area with indigenous fowls which are keeping at dirty thatched birdhouse harboring huge number of *Argas* ticks, competent vector of the disease. Since the distribution and prevalence of fowl spirochaetosis caused by *B. anserina* has not being investigated in Iran well, this study provides valuable information about the epidemiology of the disease in Iran and subsequently the control of the disease agent in domestic fowls of rural areas in Lorestan province, west of Iran, where poultries and livestock are abundant. So far, veterinary health system in the area infected by *B. anserina* (including Lorestan province) focused on industrial farms and avicultures rather than native poultries (personal communications). Whereas, domestic fowls potentially may be a risk source of infection for *Argas* ticks in these susceptible areas. Thus control measures need to be implemented on all infected areas and hosts of the disease agent. Basically, control of fowl spirochaetosis should be depend on to prevent the introduction and establishment of *Argas* tick vector in the flock rearing systems. As a further work, it is proposed to study the prevalence of different strains of avian borreliosis in different geographical location of Iran along with vector ticks and a suspected mite, *D. gallinae*.

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REFERENCES

Abdul-Aziz, T. & Barnes, H.J. (2013) Miscellaneous and sporadic bacterial infections. *In*: Swayne, D.E. (Ed.), *Diseases of poultry*. John Wiley & Sons, Oxford UK, pp. 1017–1053.

Aghaei, A., Ghazinezhad, B. & Naddaf, S.R. (2014) Detection of *Borrelia* DNA in *Ornithodoros tholozani* ticks and their eggs. *Journal of Medical Microbiology and Infectious Diseases*, 2(3): 118–120.

Aghighi, Z., Assmar, M., Piazak, N., Javadian, E., Seyedi-Rashti, M.A., Kia, E.B., Rassi, Y. & Vatandoost, H. (2007) Distribution of soft ticks and their natural infection with *Borrelia* in a focus of relapsing fever in Iran. *Journal of Arthropod-Borne Diseases*, 1(2): 14–18.

Ataliba, A.C., Resende, J.S., Yoshinari, N. & Labruna, M.B. (2007) Isolation and molecular characterization of a Brazilian strain of *Borrelia anserina*, the agent of fowl spirochaetosis. *Research in Veterinary Science*, 83(2): 145–149.

Badparva, E., Ezatpour, B., Azami, M. & Badparva, M. (2015) First report of birds infection by intestinal parasites in Khorramabad, west Iran. *Journal of Parasitic Diseases*, 39(4): 720–724.

Barmaki, A., Rafinejad, J., Vatandoost, H., Telmadarraiy, Z., Mohtarami, F., Leghaei, S.H. & Oshaghi, M.A. (2010) Study on presence of *Borrelia persica* in soft ticks in Western Iran. *Journal of Arthropod-Borne Diseases*, 4(2): 19–25.

Cooper, G.L. & Bickford, A.A. (1993) Spirochetosis in California game chickens. *Avian Diseases*, 37(4): 1167–1171.

Cutler, S., Abdissa, A., Adamu, H., Tolosa, T. & Gashaw, A. (2012) *Borrelia* in Ethiopian ticks. *Ticks and Tick-borne Diseases*, 3(1): 14–17.

DaMassa, A.J. & Adler, H.E. (1979) Avian spirochaetosis: enhanced recognition of mild strains of *Borrelia anserina* with bursectomized-and dexamethasone-treated chickens. *Journal of Comparative Pathology*, 89(3): 413–420.

Dezfoulian, O., Zibaei, M., Nayebzadeh, H., Haghgoo, M., Emami-Razavi, A.N. & Kiani, K. (2013) Leucocytozoonosis in domestic birds in southwestern Iran: an ultrastructural study. *Iranian Journal of Parasitology*, 8(1): 171–176.

Dezfoulian, O., Zibaei, M., Nayebzadeh, H., Zakian, N. & Haghgoo, M. (2011) Prevalence and ultrastructural study of *Aegyptianella* spp. in domestic birds from southwestern area, Iran. *Iranian Journal of Veterinary Research*, 12(2): 167–171.

Diab, F.M. & Soliman, Z.R. (1977) An experimental study of *Borrelia anserina* in four species of *Argas* ticks. *Zeitschrift für Parasitenkunde*, 53(2): 201–212.

Dickie, C.W. & Barrera, J. (1964) A study of the carrier state of avian spirochetosis in the chicken. *Avian Diseases*, 8(2): 191–195.

Djankov, I., Soumrov, I. & Lozeva, T. (1972) Use of the immunofluorescence method for the serotype identification of *Borrelia anserina* (Sakharoff, 1891) strains. *Zoonoses and Public Health,* 19(3): 221–225.

Hoogstraal, H. (1985) Argasid and nuttalliellid ticks as parasites and vectors. *Advances in Parasitology*, 24: 135–238.

Hosseini-Chegeni, A. (2015) *Taxonomy and phylogeny of Iranian ticks of the genus Hyalomma (Acari: Ixodidae) with morphologic and molecular methods*. Ph. D. dissertation, Guilan University, Guilan, Iran, 190 pp. (In Persian with English summary).

Hosseini-Chegeni, A. & Tavakoli, M. (2013) *Argas vespertilionis* (Ixodida: Argasidae): A parasite of Pipistrel bat in Western Iran. *Persian Journal of Acarology*, 2(2): 321–330.

Hovind-Hougen, K. (1995) A morphological characterization of *Borrelia anserina*. *Microbiology,* 141(1): 79–83.

Hungerford, T.G. & Hart, L. (1937) Fowl tick fever (spirochaetosis) also transmitted by common red mite. *Agricultural Gazette of New South Wales*, 48(10): 591–592.

Kayedi, M.H., Taherian, M.R., Hosseini-Chegeni, A., Chegeni-Sharafi, A., Mokhayeri, H. & Khorramabad, I. (2016) The identification of genus, species and distribution of hard and soft ticks collected from livestock and fowl bodies in Aleshtar and Aligodarz counties, Lorestan Province, Iran. *Life Science Journal*, 13(1s): 1–6.

Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33(7): 1870–1874.

Kurtenbach, K. & Schäfer, S.M. (2001) Avian borreliosis. *In*: Service, M.W. (Ed.), *Encyclopedia of arthropod-transmitted infections of man and domesticated animals*. CABI Publishing, New York, USA, pp. 50–52.

Lisbôa, R.S., Teixeira, R.C., Rangel, C.P., Santos, H.A., Massard, C.L. & Fonseca, A.H. (2009) Avian spirochetosis in chickens following experimental transmission of *Borrelia anserina* by *Argas* (*Persicargas*) *miniatus*. *Avian Diseases*, 53(2): 166–168.

McNeil, E., Hinshaw, W.R. & Kissling, R.E. (1949) A study of *Borrelia anserina* infection (spirochetosis) in turkeys. *Journal of Bacteriology*, 57(2): 191–206.

Naddaf, S.R., Ghazinezhad, B., Bahramali, G. & Cutler, S.J. (2012) Phylogenetic analysis of the spirochete *Borrelia microti*, a potential agent of relapsing fever in Iran. *Journal of Clinical Microbiology*, 50(9): 2873–2876.

Quinn, P.J., Carter, M.E., Markey, B. & Carter, G.R. (1999) *Clinical veterinary microbiology*. Mosby, London UK., 648 pp.

Rafinejad, J., Choubdar, N., Oshaghi, M.A., Piazak, N., Satvat, T., Mohtarami, F. & Barmaki, A. (2011) Detection of *Borrelia persica* infection in *Ornithodoros tholozani* using PCR targeting rrs gene and xenodiagnosis. *Iranian Journal of Public Health*, 40(4): 138–145.

Rafyi, A. & Rak, H. (1985) *Parasitology of arthropods (entomology)*. Tehran University Perss, Tehran, Iran. 450 pp. (In Persian).

Rak, H. (1976) Tick borne diseases and their vectors in Iran. *Proceeding of International Congress on Tick-Borne Diseases and Their Vectors, Edinburgh, UK*, pp. 163–166.

Sambri, V., Marangoni, A., Olmo, A., Storni, E., Montagnani, M., Fabbi, M. & Cevenini, R. (1999) Specific antibodies reactive with the 22-kilodalton major outer surface protein of *Borrelia anserina* Ni-NL protect chicks from infection. *Infection and Immunity*, 67(5): 2633–2637.

Seneviratna, P. (1969) *Diseases of poultry (including cage birds)*. John Wright & Sons, Bristol, UK, 229 pp.

Shane, S.M. (1997) *Handbook on poultry diseases*. American Soybean Association, Singapore, pp. 191.

Sohrab, V., Maghami, G. & Bahar-Sefat, M. (1957) Fowl spirochaetosis in Iran with special reference to control of the disease by immunization with a formalized chick-embryo vaccine. *Archives of Razi Institute*, 10(1): 33–44.

Soni, J.L. & Joshi, A.G. (1980) A note on strain variation in Akola and Jabalpur strains of *Borrelia anserina*. *Zentralblatt für Veterinärmedizin Reihe B*, 27(1): 70–72.

Zaher, M.A., Soliman, Z.R. & Diab, F.M. (1977) An experimental study of *Borrelia anserina* in four species of *Argas* ticks. *Zeitschrift für Parasitenkunde*, 53(2): 213–223.

Zakian, N., Nayebzadeh, H., Dezfoulian, O. & Agha ebrahimi-Samani, R. (2014) Parasitic infections of local chickens from Lorestan province, Iran. *Veterinary Journal (Pajouhesh & Sazandegi),* 28 (4): 18–20 (In Persian with English abstract).

تشخیص مولکولی *Borrelia anserina* در کنه‌های *Argas persicus* (Acari: Argasidae) جمع‌آوری شده از استان لرستان، غرب ایران

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چکیده

عامل بورلیوز پرندگان، *Borrelia anserina*، مسبب ایجاد یک نوع بیماری خونی در طیفی از گونه‌های پرنده موجود در مناطق گرمسیری و نیمه معتدل دنیا است. بورلیوز پرندگان یکی از شایع‌ترین بیماری‌های طیور در ایران است که دارای اهمیت اقتصادی زیادی است. مطالعه حاضر به منظور شناسایی عامل بیماری در کنه‌های *Argas persicus* انجام شد. نمونه‌ها از شیارها و شکاف‌های اطراف لانه‌های مرغ در مناطق روستایی استان لرستان جمع آوری شدند. سپس غدد بزاقی، تخمدان‌ها و رحم کنه‌ها به منظور شناسایی عامل بیماری در اندام‌های خاص کنه با روش مولکولی، تشریح و جدا شدند. DNA با روش فنل-کلروفرم استخراج شد و سپس قسمتی از ژن فلاژلین (*fla*B) مربوط به *B. anserina* با واکنش زنجیره‌ای پلیمراز (PCR) تکثیر شد. بر اساس نتایج به دست آمده، ژن هدف *fla*B در کنه‌های *A. persicus* جمع‌آوری شده از استان لرستان شناسایی شد. به نظر می‌رسد *B. anserina* به طور گسترده‌ای در کنه‌های ناقل *A. persicus* وجود دارد. بر طبق نتایج، نژاد *B. anserina* بر اساس ژن *fla*B شبیه به بیشتر نژادهای دیگر مناطق دنیا است.

واژگان کلیدی: *Argas persicus*؛ بورلیای پرندگان؛ *Borrelia anserina*؛ ژن فلاژلین؛ ایران.

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