



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Original article doi:

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Isolation of aerobic bacteria from ticks infested sheep in Iraq

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ARTICLE INFO

Article history:

Received 27 Jul 2015

Received in revised form 10 Aug, 2nd

revised form 24 Aug 2015

Accepted 28 Sep 2015

Available online 23 Oct 2015

Keywords:

Ticks

Aerobic bacteria

Sheep

Iraq

ABSTRACT

Objective: To highlight the presence of aerobic bacteria in live ticks infested sheep, in Diyala Governorate, Iraq.**Methods:** One hundred and thirty adult alive ticks were picked up from sheep which were reared in different farms in Diyala Governorate, Iraq, during the period from November 2012 to May 2013. Ticks were classified in the Natural History Museum in Baghdad. They were dissected aseptically for extraction of the salivary gland and mid-gut. The removed tissue from each organ was inoculated in buffer peptone water (1%) and incubated for 2 h at 37 °C, to maintain weak and/or injured bacterial cells, then transmitted to nutrient broth incubated at 37 °C for 18 h. Culturing was done on three solid bacteriological media (nutrient, blood and McConkey agars), and then incubated at 37 °C for 24 h. Bacterial identification was performed by using multiple biochemical tests and API-20 strips. Data were analyzed by using Statistical Analysis System version 9.1, 2010. Chi-square test was used for comparison at significant level of $P \leq 0.05$.**Results:** Two species of ticks were identified [*Rhipicephalus (Boophilus) annulatus* and *Hyalomma turanicum*]. High bacterial isolation rate was observed (483 isolates). A significant high isolation rate was recorded from *Rhipicephalus annulatus* (63.14%). Six bacterial species were identified [*Escherichia coli* (28.36%), *Pseudomonas aeruginosa* (18.01%), *Bacillus cereus* (14.69%), *Staphylococcus aureus* (13.66%), *Citrobacter freundii* (13.04%), and *Enterobacter* species (12.21%)]. Also the high bacterial isolation rates were recorded in the temperate months (November, March and April); these coincided with high reproductive performance of ticks.**Conclusions:** The high isolation rate of aerobic pathogens from ticks might reflect the active contribution of this arthropod in environmental contamination and increase the probability of transmitting bacterial pathogens to their hosts.

1. Introduction

Ticks are the most harmful arthropod and infest different animal species and man[1]. They cause high economic losses among animal production[2]. The undesirable direct effects of tick's parasitism are anemia and skin lesions, resulting in damage of wool and leather, which ultimately interfere with their industries. Moreover, paralysis of different host species may occur due to tick's salivary toxins[3]. In addition, their serious role is transmitting many various pathogens via mechanical or biological ways[4]. Some of these pathogens are life-threatening to animals[5], whereas others have a great risk to human health[6,7]. Furthermore, it was believed that more than 100 000 diseased conditions in human being in the world belonged to the tick borne infection[8]. Few studies had been done to clarify the role of ticks in transmission of bacterial organisms. Several researches showed that

many bacterial species were isolated from salivary gland and mid-gut of various tick's species[9]. The incidence of tick borne diseases increased according to variation in weather of different environments, particularly when suitable conditions are provided for growth and reproduction of ticks[10]. It was reported an increment in infection rates of *Borrelia burgdorferi* and *Anaplasma phagocytophilum*, occurred in consequence to seasonal variations[11]. The current article is a preliminary study in Iraq, aimed to shed light on the extent of bacterial pathogens lurking in salivary glands and mid-guts of hard ticks infested sheep in Diyala Governorate, Iraq.

2. Materials and methods

2.1. Ticks

One hundred and thirty live adult ticks were collected from Awassi and Hamdani sheep, during the period from November 2012 to May 2013. The approval of this study had been taken from the Council of Veterinary Medicine College/University of Baghdad and the work was performed complied with the current Iraqi laws. Ticks were

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Table 1Bacterial isolates from salivary glands and viscera of tick. *n* (%).

Tick spp.	Tissues	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>C. freundii</i>	<i>Enterobacter</i> spp.
<i>R. annulatus</i>	Salivary gland	44 (9.12)	23 (4.76)	19 (3.93)	25 (5.17)	17 (3.51)	16 (3.31)
	Viscera	49 (10.14)	29 (6.00)	24 (4.96)	15 (3.10)	21 (4.34)	23 (4.76)
<i>H. turanicum</i>	Salivary gland	20 (4.14)	16 (3.31)	13 (2.69)	15 (3.10)	11 (2.27)	9 (1.86)
	Viscera	24 (4.96)	19 (3.93)	15 (3.10)	11 (2.27)	14 (2.89)	11 (2.27)
Total		137	87	71	66	63	59

C. freundii: *Citrobacter freundii*.

gently picked up by hands using wetted cotton piece with 70% ethyl alcohol[12]. Ticks were classified in the Natural History Museum in Baghdad[13]. Dissection of ticks was achieved under high aseptic condition (bacteriological hood)[14]. A sterile phosphate buffer solution was added to dissected tissues of ticks to avoid dryness.

2.2. Bacterial culture

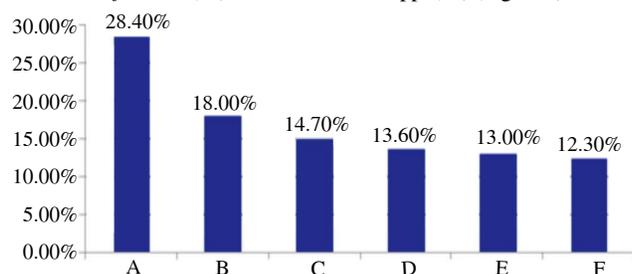
The removed salivary gland and mid-gut were inoculated in buffer peptone water and incubated for 2 h at 37 °C, in order to maintain and reactivate the weak microorganisms and repair the injured bacterial cells, then one full bacteriological loop was transmitted into nutrient broth and incubated at 37 °C for 18 h. Culturing was done on three solid media (nutrient, MacConkey and blood agars) and they were incubated at 37 °C[15]. The isolated bacteria were classified by using multiple biochemical tests and API-20 test strips (bioMerieux, Inc.).

2.3. Statistical analysis

Data were analyzed by using Statistical Analysis System (version 9.1, 2010) and *Chi*-square test was used for comparison at significant level of $P \leq 0.05$.

3. Results

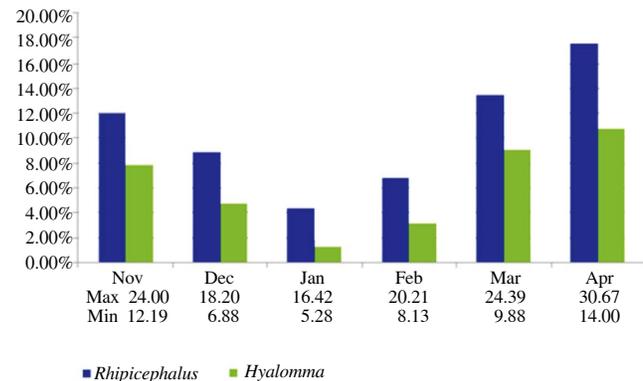
Two species of ticks were identified [*Rhipicephalus* (*Boophilus*) *annulatus* (*R. annulatus*) and *Hyalomma turanicum* (*H. turanicum*)]. Four hundred and eighty-three isolates were recorded from all collected ticks (130). The significant ($P \leq 0.05$) higher total isolation rate was recorded from *R. annulatus* (63.14%) whereas from *H. turanicum*, the total isolation rate was 36.85%. Moreover, the significant ($P \leq 0.05$) increases of bacterial isolation from *R. annulatus* were observed during all months of the study. Furthermore, a significant increase of *Escherichia coli* (*E. coli*) (137) isolates was recorded from both ticks species, followed by *Pseudomonas aeruginosa* (*P. aeruginosa*) (87), *Bacillus cereus* (*B. cereus*) (71), *Staphylococcus aureus* (*S. aureus*) (66), *Citrobacter freundii* (63) and *Enterobacter* spp. (59) (Figure 1).

**Figure 1.** Isolates of bacterial species from ticks.

A: *E. coli*; B: *P. aeruginosa*; C: *B. cereus*; D: *S. aureus*; E: *Citrobacter* spp.; F: *Enterobacter* spp.

The isolation rate increased significantly in April (28.36%) and decreased to a minimum value in January (5.28%) and the elevation of bacterial isolation rates seemed to coincide with temperate months of November, March and April (Figure 2). The insignificant higher total

isolation rates of bacterial species were recorded from viscera of both tick species: *R. annulatus* (161) and *H. turanicum* (94), whereas those from salivary gland (144, 84 in both species of ticks respectively) were recorded significant higher total isolation rates, with exception of *S. aureus* (Table 1).

**Figure 2.** Isolation rates of bacterial species from ticks according to months. Max: The maximum value rates of temperature (°C) during months; Min: The minimum rates of temperature (°C) during months.

4. Discussion

Ticks are important blood sucking ectoparasite causing anemia, skin lesion and they produce tick paralysis in hosts[3]. In addition, they play a serious role in transmission of many pathogens to various host species and some are life-threatening[16,17]. Few literatures were reported on the role of ticks in transmission of bacterial pathogens in animals. In fact, ticks are highly prevalent in Iraq, which hinder the development of farm animal production. This was a preliminary study aimed to highlight the extent of bacterial species residing within tissues of ticks infested sheep. The high isolation rate of bacterial organisms from both tick species (483 isolates), referred to the contribution of this arthropod in contamination of the environment, rather than transmission of these bacteria to their hosts. The different species of bacteria protect themselves from external harmful influences of the environment inside the body of tick and perpetuate their life in nature[18,19]. In the same instance, the ticks play a role in an evolutionary process of bacteria and might lead to create new bacterial strains or change their virulence[20,21]. The total isolation rate of bacterial organisms were significantly ($P \leq 0.05$) higher from *R. annulatus* (63.1%) than from *H. turanicum* (36.8%). This pointed to that tick species is a more serious reservoir for many pathogenic bacteria. It was also reported that certain tick species harbor pathogens while others do not[22]. The *E. coli* was an abundant (28.3%) bacteria, isolated from both tick species, which was in agreement with other researches[18]. Such a redundancy of *E. coli* isolates might be due to wide prevalence of this organism in the nature and the extensive fecal contamination among sheep farms might probably assist in increasing ticks infection (or harboring such pathogens). According to the increased isolation rate of *E. coli* bacteria reflected the unhygienic sanitation as well as the bad management, these ultimately increased the pollution of environment with *E. coli*. Six bacterial species were recorded in both tick species; some researches showed similar bacterial isolates

whereas others showed different isolates according to various regions and times[18,19,23]. However, other authors found many various species of bacteria in ticks related to certain ecological conditions[23,24]. Other different microbes including many bacterial species were isolated not only from adult ticks but also from their eggs, larvae and nymphs[24,25]. Moreover, it was reported that female ticks had less diverse microbiomes than males and nymph[26]. Indeed, these stages of life increase the probability of bacterial transmission and furthermore, the harboring of many different bacterial species by ticks reflects the variations in ecology and environments. A marked increment of bacterial isolation was observed in the temperate months: March (mean of minimum and maximum temperatures was 9.88 and 24.39 °C respectively) and April (14.02 and 30.67 °C respectively), which was coinciding with the reproductive activity of ticks. As the environmental temperature and rain fall[10,27], were highly suitable for optimum tick reproduction, ticks come out of their slumber and looked for suitable hosts. This gives an explanation for the presence of various bacterial species inside ticks in different areas. Ticks may take infection from environment or from contaminated wool or skin of the hosts. Simultaneously, this presence in the period is favorable for growth and multiplication of bacteria. The non-significant higher isolation rate from mid-gut (52.8%) of ticks, compared with salivary glands (47.2%), was recorded. Moreover, all isolated bacterial species showed higher isolation rates from viscera of ticks (except *S. aureus*), than that from salivary glands and on the contrary, others found higher infection rate in the salivary glands[9]. The residence of ticks in salivary gland increases the risk to their hosts and it was reported that tick's salivary secretions have roles in modulation of host defense mechanisms and pathogen transmission[28].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This work was performed as M.Sc. project in Baghdad University by Waleed Ibrahim Jalil under my supervision. It was supported by the College of Veterinary Medicine/University of Baghdad.

References

- [1] Reye AL, Arinola OG, Hübschen JM, Muller CP. Pathogen prevalence in ticks collected from the vegetation and livestock in Nigeria. *Appl Environ Microbiol* 2012; **78**(8): 2562-8.
- [2] Desta TS. Investigation on ectoparasites of small ruminants in selected sites of Amhara Regional State and their impact on the tanning industry [dissertation]. Addis Ababa: Addis Ababa University; 2004.
- [3] Hall-Mendelin S, Craig SB, Hall RA, O'Donoghue P, Atwell RB, Tulsiani SM, et al. Tick paralysis in Australia caused by *Ixodes holocyclus* Neumann. *Ann Trop Med Parasitol* 2011; **105**(2): 95-106.
- [4] Dennis DT, Piesman JF. Overview of tick-borne infection of humans. In: Goodman JL, Dennis DT, Sonenshine DE, editors. *Tick-borne diseases of humans*. Washington, D.C.: American Society for Microbiology Press; 2005, p. 3-11.
- [5] Heyman P, Cochez C, Hofhuis A, van der Giessen J, Sprong H, Porter SR, et al. A clear and present danger: tick-borne diseases in Europe. *Expert Rev Anti Infect Ther* 2010; **8**(1): 33-50.
- [6] Centers for Disease Control and Prevention. Tickborne diseases of the United States. Atlanta: Centers for Disease Control and Prevention; 2015. [Online] Available from: <http://www.cdc.gov/ticks/diseases/> [Accessed on 15th June, 2015]
- [7] Sonenshine DE, Roe MR. *Biology of ticks*. 2nd ed. New York: Oxford University Press; 2014.
- [8] de la Fuente J, Estrada-Pena A, Venzal JM, Kocan KM, Sonenshine DE. Overview: ticks as vectors of pathogens that cause disease in humans and animals. *Front Biosci* 2008; **13**: 6938-46.
- [9] El Kammah KM, Oyoum LM, Abdel-Shafy S. Detection of microorganisms in the saliva and midgut smears of different tick species (Acari: Ixodoidea) in Egypt. *J Egypt Soc Parasitol* 2007; **37**(2): 533-9.
- [10] Ramezani Z, Chavshin AR, Telmadarraiy Z, Edalat H, Dabiri F, Vantandoost H, et al. Ticks (Acari: Ixodidae) of livestock and their seasonal activities, northwest of Iran. *Asian Pac J Trop Dis* 2014; **4**: S754-7.
- [11] Estrada-Peña A, Ortega C, Sánchez N, Desimone L, Sudre B, Suk JE, et al. Correlation of *Borrelia burgdorferi sensu lato* prevalence in questing *Ixodes ricinus* ticks with specific abiotic traits in the Western Palearctic. *Appl Environ Microbiol* 2011; **77**(11): 3838-45.
- [12] Barmon SC, Paul AK, Dina MA, Begum N, Mondal MMH, Rahman MM. Prevalence of ectoparasites of sheep in Gaibandha District of Bangladesh. *Int J Biol Res* 2010; **1**(4): 15-9.
- [13] Wall RL, Shearer D. *Veterinary ectoparasites: biology, pathology and control*. 2nd ed. London: Wiley-Blackwell; 2011.
- [14] Edwards KT, Goddard J, Varela-Stokes AS. Examination of the internal morphology of the Ixodid tick *Amblyomma maculatum* Koch, (Acari: Ixodidae); a "How-to" pictorial dissection guide. *Midsouth Entomol* 2009; **2**: 28-39.
- [15] Quinn PJ, Carter ME, Markey BM, Carter GR. *Clinical veterinary microbiology*. London: Mosby-Year Book Europe Limited; 2004.
- [16] Blom K, Braun M, Pakalniene J, Dailidyte L, Béziat V, Lampen MH, et al. Specificity and dynamics of effector and memory CD8 T cell responses in human tick-borne encephalitis virus infection. *PLoS Pathog* 2015; **11**(1): e1004622.
- [17] Pferffer M, Dobler G. Tick-borne encephalitis virus in dogs-is this an issue? *Parasit Vectors* 2011; **4**: 59.
- [18] Rahman MH, Rahman MM. Occurrence of some bacterial isolates in ticks found in Madhupur Forest Area. *Bang Vet J* 1980; **14**: 43-7.
- [19] Amoo AO, Dipeolu OO, Akinboade AO, Adeyemi A. Bacterial isolation from and transmission by *Boophilus decoloratus* and *Boophilus geigy*. *Folia Parasitol (Praha)* 1987; **34**(1): 69-74.
- [20] Duron O, Noël V, McCoy KD, Bonazzi M, Sidi-Boumedine K, Morel O, et al. The recent evolution of a maternally inherited endosymbiont of ticks led to the emergence of the Q fever pathogen, *Coxiella burnetii*. *PLoS Pathog* 2015; **11**(5): e1004892.
- [21] Kang YJ, Diao XN, Zhao GY, Chen MH, Xiong Y, Shi M, et al. Extensive diversity of Rickettsiales bacteria in two species of ticks from China and the evolution of the Rickettsiales. *BMC Evol Biol* 2014; **14**: 167.
- [22] Pesquera C, Portillo A, Palomar AM, Oteo JA. Investigation of tick-borne bacteria (*Rickettsia* spp., *Anaplasma* spp., *Ehrlichia* spp. and *Borrelia* spp.) in ticks collected from Andean tapirs, cattle and vegetation from a protected area in Ecuador. *Parasit Vectors* 2015; **8**: 46.
- [23] Dietrich M, Gómez-Díaz E, McCoy KD. Worldwide distribution and diversity of seabird ticks: implications for the ecology and epidemiology of tick-borne pathogens. *Vector Borne Zoonotic Dis* 2011; **11**(5): 453-70.
- [24] Andreotti R, Pérez de León AA, Dowd SE, Guerrero FD, Bendele KG, Scoles GA. Assessment of bacterial diversity in the cattle tick *Rhipicephalus (Boophilus) microplus* through tag-encoded pyrosequencing. *BMC Microbiol* 2011; **11**: 6.
- [25] Olayide AJ, Bamidele AA. Transmission of bacteria isolates through all developmental stages of dog ticks (bacteriological evidence). *J Anim Vet Adv* 2008; **7**(8): 959-62.
- [26] Ponnusamy L, Gonzalez A, Treuren WV, Weiss S, Parobek CM, Juliano JJ, et al. Diversity of Rickettsiales in the microbiome of the lone star tick *Amblyomma americanum*. *Appl Environ Microbiol* 2014; **80**: 354-9.
- [27] Barandika JF, Berriatua E, Barral M, Juste RA, Anda P, García-Pérez AL. Risk factors associated with ixodid tick species distribution in the Basque region in Spain. *Med Vet Entomol* 2006; **20**(2): 177-88.
- [28] Kazimírová M, Štibrániová I. Tick salivary compounds: their role in modulation of host defences and pathogen transmission. *Front Cell Infect Microbiol* 2013; **3**: 43.