Molecular detection of tick-borne pathogens in cattle from Saudi Arabia

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Abstract

Background: Babesiosis and anaplasmosis are tick-borne diseases that affect the health of cattle and may also be responsible for major economic losses. This study aimed to detect the presence of the causative agents of babesiosis and anaplasmosis among cattle in different provinces in Saudi Arabia and to characterise their species genetically.

Methods: A total of 362 blood samples were taken from cattle in four regions (Riyadh, Al-Kharj, Al-Hasa and Al-Qassim) of Saudi Arabia and were molecularly screened by polymerase chain reaction (PCR) of partial 18S rRNA and 23S rRNA genes to detect *Babesia* and *Anaplasma* species.

Results: The overall prevalence of *Babesia* and *Anaplasma* in cattle was 2.49% and 5.80%, respectively. *Theileria annulata* (*T. annulata*) and *Theileria ovis* (*T. ovis*) were found in seven (1.93%) and two (0.55%) of the 362 samples, respectively. *Anaplasma ovis* (*A. ovis*) was detected in 21 (5.80%) of 362 samples. Likewise, four of the cattle were found to be coinfectd with more than one pathogen (1.10%). All cattle samples tested negative for other species of *Babesia*, *Theileria* and *Anaplasma*.

Conclusions: The presence of *T. ovis* and *A. ovis* has been reported in Saudi Arabia. Here, the presence of two novel genotypes of *T. annulata* is also reported in Saudi Arabian cattle. Further molecular surveys on larger numbers of samples from the entire country are needed to correctly address the prevalence and geographical distribution of tick-borne disease.

Introduction

Tick-borne diseases (TBDs), including piroplasmosis and anaplasmosis, are mainly distributed in tropical and temperate regions [1, 2]. The main causative agents of bovine piroplasmosis belong to the genera *Babesia* (Piroplasmida; Babesiidae) and *Theileria* (Piroplasmida; Theileriidae). Furthermore, pathogens within the genus *Anaplasma* (Rickettsiales; Anaplasmataceae) cause bovine anaplasmosis [3]. TBDs affect the health of livestock and are responsible for heavy economic losses. The occurrence of these diseases also poses a potential risk regarding transmission of disease to humans [4]. Cattle production is severely affected by various diseases, especially in the absence of adequate veterinary services [5], and many endo- and ectoparasites, including ticks, affect the health, productivity and performance of the animals [6, 7].

The cattle population in Saudi Arabia numbered approximately 350,000 in 2015. The highest percentages were in the Eastern and Central regions of Saudi Arabia (<https://www.stats.gov.sa/en>). These regions also contain the majority of the cattle that is imported to Saudi Arabia from Europe [8]. In Saudi Arabia, cattle are infested by several species of hard ticks (Acari, Ixodidae), including *Hyalomma anatolicum anatolicum*, (*Hy. a.anatolicum*), *Amblyomma variegatum* (*A. variegatum*), *Rhipicephalus praetextatus* (*Rh. praetextatus*) and *Hyalomma marginatum rufipes* (*Hy. m. rufipes*) [9-11]. These ticks can act as vectors for a variety of pathogens of veterinary importance, and several tick-borne diseases are known to be endemic in Saudi Arabia. However, knowledge of tick-borne pathogens (TBPs) in cattle of this country is very limited. A few molecular epidemiological studies have been conducted on a limited number of animals [12-14]. Therefore, the present study aimed to determine the prevalence of ixodid ticks and investigate their associated pathogens at the molecular level among cattle in Saudi Arabia.

Methods

Ethical approval

This study was revised and approved by the Ethical Research Committee, Deanship of Scientific Research, King Khalid University, according to the ethical principles of human and animal research (Approval no. (ECM#2019-74) - (HAPO-06-B-001)).

Sampling procedures

A cross-sectional study was conducted from May to October 2018 in four regions of Saudi Arabia: Riyadh (24°0'N, 45°30'E); Al-Kharj (24°08'N, 47°18'E); Al-Hasa (25°23'N, 49°36'E) and Al-Qassim (25°48'N, 42°52'E) (Figure 1). All cows were apparently healthy at the time of sampling and were screened for tick infestation. Ticks found within 15 min were collected (2–5 ticks/infested animal) and placed in labelled tubes containing 70% ethanol that were individualised per cow. Ticks were identified to the species level by examination of their morphological features and use of morphological keys [15-17]. Each cow was categorised according to age and sex.

Blood samples were collected from each animal (2–5 ml) from the jugular vein into vacutainer tubes with ethylenediaminetetraacetic acid (EDTA) (BD Vacutainer® Tube, Gribbles Pathology, VIC, Australia) and transported in ice boxes to the molecular laboratory, Department of Biological Sciences, Faculty of Science and Humanities, Shaqra University, for DNA extraction.

Molecular procedures

Total genomic DNA (gDNA) was isolated from the blood samples using a Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the manufacturer's instructions. Aliquots of between 50 and 100 µl of gDNA from each sample were stored at –20 °C prior to molecular analysis.

The primers were designed to target a conserved region that encoded ribosomal 18S rRNA (969 bp) and 238S rRNA (485 bp) to detect the DNA of *Babesia* [18] and *Anaplasma* [19], respectively (Table 1). Polymerase chain reaction (PCR) assays were performed in an automated BIO-RAD Thermal Cycler (BIO-RAD, Singapore) using one PCR master mix (GeneDireX, Taiwan). The PCR conditions for the *Babesia* amplification were as follows: incubation at 95 °C for 15 min; 35 cycles of 1 min at 95 °C; 30 s of annealing at 58 °C and 1 min at 72 °C; followed by a final extension for 5 min at 72 °C. Positive and negative controls were included. The PCR conditions used for *Anaplasma* amplification were as follows: an initial denaturation step at 95 °C for 15 min; then 40 cycles that consisted of 1 min of denaturation at 95 °C, 1 min of annealing at 55 °C, a 1 min extension at 72 °C, a final extension cycle at 72 °C for 7 min and cooling of the reaction mixtures at 15 °C. Distilled water samples were used as negative controls. Positive genomic controls of *T. annulata* and *Anaplasma marginale* were included. The amplifications were confirmed by electrophoresis on a 1.5% agarose gel, stained with Red Safe and examined by ultraviolet transillumination. A DNA molecular weight marker (100 bp DNA Ladder H3 RTU, GeneDireX, Taiwan) was used to estimate the size of the products. The PCR products of the positive samples were purified using a PCR Clean-Up & Gel Extraction Kit (GeneDireX, Taiwan) according to the manufacturer's instructions.

Sequencing and phylogenetic analyses

The purified PCR products were sequenced at Macrogen Lab Technology, Korea. The obtained sequences were assembled using ChromasPro software (ChromasPro 1.7, Technelysium Pty Ltd., Tewantin, Australia). The obtained sequences of *Babesia* and *Anaplasma* were submitted to GenBank and then compared with those available in the GenBank database by the US National Centre for Biotechnology Information Basic Local Alignment Search Tool (NCBI BLAST) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Moreover, the sequences obtained from positive samples and sequences of validated species already available in GenBank were aligned using Bioedit software version 7.0.5.3 (Clustal W multiple alignment) [20]. For taxonomic analyses, maximum-likelihood phylogenetic trees were constructed using Molecular Evolutionary Genetics Analysis (MEGA) software version X [21] with 500 bootstrap replications.

Statistical analysis

The significant differences regarding the prevalence of infections with *Babesia* and *Anaplasma* and their risk factors, such as sex and age of the cattle and their levels of tick infestation, were calculated by χ^2 tests using a program in Statistical Package for the Social Sciences (SPSS), version 20.0, at $P < 0.05$.

Results

Of the 362 cattle examined, nine (2.49%) were found to be infected with *Babesia*, 21 (5.80%) with *Anaplasma* and four (1.10%) were found to be coinfecting with more than one pathogen (Table 2). *A. ovis* was the most prevalent pathogen (5.80%), followed by *T. annulata* (1.93%) and *T. ovis* (0.55%) (Table 2). All the samples tested negative for other species of *Babesia*, *Theileria* and *Anaplasma*. Among the four provinces, the highest prevalence of infection was observed in Riyadh (12.14%), and the lowest was recorded in Al-Qassim (3.03%). In the region of Al-Kharj, no TBDs were detected (Table 2).

Of the 362 cattle examined, 245 (67.7%) were infested by ticks ($n=541$). Of these, 328 (60.6%) were males and 213 (39.4%) were females. The most representative tick species were *Hy. a. anatolicum* (207, 38.3%), *Hyalomma dromedarii* (*Hy. dromedarii*) (116, 21.4%), *Hyalomma impeltatum* (*H. impeltatum*) (97, 18%), *Rhipicephalus* (*Boophilus*) *annulatus* (*Rh. annulatus*) (71, 13.1%), *Rhipicephalus turanicus* (*Rh. turanicus*) (47, 8.7%) and *Hyalomma excavatum* (*Hy. excavatum*) (three, 0.5%). Data regarding the sex and age of the sampled cattle along with the number and percentage positivity for TBPs are reported in Table 3.

The PCR products showed the expected band size for each pathogen (Figures 2 and 3). By BLAST analysis, *T. annulata* and *T. ovis* were identified. Two novel genotypes of *T. annulata* were identified in this study (GenBank: MN826137 and MN826138) that were 99.8% identical to *T. annulata* in *H. anatolicum anatolicum* from Hofuf, Saudi Arabia (GenBank: MK300062) and 99.2% identical to *T. annulata* in cattle from Egypt (GenBank: MN625888 and MN625889). Moreover, a new genotype of *T. ovis* (GenBank: MN826139) was detected in cattle with 98% similarity to those in sheep from Egypt (GenBank: MN625886). In addition, in the present study, a novel *A. ovis* genotype was detected in cattle (GenBank: MN880224) with 99% similarity to those of *A. ovis* detected in cattle from Egypt (GenBank: MN625933). The phylogenetic trees of these genotypes are shown in Figures 4 and 5.

Discussion

The high prevalence of tick infestation (67.7%) and ticks borne pathogens (TBPs) (8.3%) among cattle in Saudi Arabia represents a potential threat to the health and welfare of these animals as well as to livestock production. The most prevalent tick species was *Hy. a. anatolicum*, which is considered to be the main species that parasitizes cattle [10, 22, 23]. Camels are the main host for the species *Hy. dromedarii*, although these ticks can also infest cattle, sheep, goats, horses and donkeys [24]. The species *Hy. impeltatum* infests a wide range of mammalian hosts, including cattle [25]. These tick species are the main vectors of *Babesia* spp., *Theileria* spp. and *Anaplasma* spp. of domestic and wild ungulates in Saudi Arabia [12, 13, 26-30]. *Rh. annulatus* has been reported from cattle in Jazan in the southwest and from animals in central parts of Saudi Arabia [31, 32]. This tick species is an efficient vector of *Babesia bovis* globally [33]. The other two species herein identified in cattle, *Rh. turanicus* and *Hy. excavatum*, usually infest sheep, goats and horses [10, 11, 31] but are reported herein for the first time on cattle, which might be due to husbandry practices in desert areas, where livestock share the same environment.

The overall TBPs prevalence in cattle reported in this study was 8.3%, which is in agreement with previous findings [13, 26]. Additionally, the findings of this study indicate that *T. annulata* is present in the central and eastern parts of Saudi Arabia, which overlaps with the distribution of *H. anatolicum anatolicum*, the putative vector of *T. annulata* [8, 31].

T. ovis is a nonpathogenic parasite that is widespread in Saudi Arabia, particularly in sheep and goats [34]. Moreover, according to experimental transmission studies [35], *H. anatolicum anatolicum* could be the vector of this parasite in Saudi Arabia. This tick has been found to infest sheep and goats in Saudi Arabia [10, 31].

This study reports two novel *T. annulata* genotypes (GenBank: MN826137 and MN826138) that are 99.8% identical to *T. annulata* in *Hy. a. anatolicum* from the Hofuf region of Saudi Arabia (GenBank: MK300062) and 99.2% identical to *T. annulata* in cattle from Egypt (GenBank: MN625888 and MN625889). The results are in agreement with previously published data on the occurrence of *T. annulata* in cattle in Saudi Arabia [8, 13, 14]. Moreover, a new genotype of *T. ovis* (GenBank: MN826139) has been detected in cattle blood with 98% similarity to those of *T. ovis* detected in sheep from Egypt (GenBank: MN625886). A novel *A. ovis* genotype has been identified in Saudi cattle (GenBank: MN880224) with 99% similarity to those of *A. ovis* detected in cattle from Egypt (GenBank: MN625933). While this study did not cover all Saudi Arabian regions, it provides a clear picture of TBP distribution and prevalence in Saudi cattle; however, further epidemiological and molecular studies are required to elucidate better strategic control plans and assess the risks to cattle health and productivity.

Conclusion

This is the first study to report *T. ovis* and *A. ovis* in cattle from Saudi Arabia. Furthermore, two new genotypes of *T. annulata* in cattle were recorded, and therefore, cattle may be considered carriers for *Babesia* and *Anaplasma* spp. of other ruminants in Saudi Arabia. Further extensive molecular surveys on a large number of animals from the whole country are needed to correctly address the prevalence and geographical distribution of TBPs. This in turn will help in the design and implementation of effective preventive and control measures.

List Of Abbreviations

TBDs = Tick-borne diseases.

TBPs = Ticks borne pathogens.

PCR = Polymerase chain reaction.

Declarations

Ethics approval and consent to participate

The blood sampling performed in this study was approved by the Ethical Research Committee, Deanship of Scientific Research, King Khalid University, according to the ethical principles of human and animal research (Approval no. (ECM#2019-74) (HAPO-06-B-001).

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors shared in the design of this study. MNA, MSA, ASA, and MYA collected blood samples from cattle and extracted DNA from the blood samples. ADA, HHAMA, AMA, BSME and SA participated in the performance of PCR protocols, purification of PCR products, sequence analyses, construction of phylogenetic trees and analyses of the data. ADA, SA, HHAMA and AMA cowrote the manuscript. ADA, MSA and SA reviewed and wrote the final draft of the manuscript. All authors revised and approved the final version of the manuscript.

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Tables

Table 1. Primers and target genes of pathogens investigated.

gens	Target gene	Primer Name	Primers (5'-3')	Annealing temperature (°C)	Reference
ia	18S rRNA	piro18S-F1	GCGAATGGCTCATTAAACA	58	Dahmana et al. 2019
		piro18S-F4	CACATCTAAGGAAGGCAGCA		
		piro18S-F3	GTAGGGTATTGGCCTACCG ^a		
		piro18S-R3	AGGACTACGACGGTATCTGA ^a		
ataceae	23S rRNA	Ana23S-212f	ATAAGCTGCGGGGAGTTGTC	55	Dahmani et al. 2017
		Ana23S-753r	TGCAAAAGGTACGCTGTCAC		

Table 2. Prevalence of infection among cattle with tick-borne pathogens according to regions of Saudi Arabia.

Region	Examined animals	<i>Theileria annulata</i>		<i>Theileria ovis</i>		<i>Anaplasma ovis</i>		Co- infection		Overall prevalence		c ²	Sig
		Positive	%	Positive	%	Positive	%	Positive	%	Positive	%		
Riyadh	173	6	3.5	1	0.6	16	9.25	2	1.16	23	13.3	12.080	0.002
Al-Kharj	101	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	-	-
Al-Hasa	55	1	1.8	1	1.8	4	7.27	2	3.64	6	10	1.000	ns
Al-Qassim	33	0	0.00	0.00	0.00	1	3.03	0	0.00	1	3.03	-	-
Total	362	7	1.93	2	0.55	21	5.80	4	1.10	30	8.3	13.471	0.001

ns= not significant

Table 3. Risk factors associated with PCR detection of *Babesia* and *Anaplasma* from cattle in different provinces of Saudi Arabia.

Factor		Total animals	Bebesia		Anaplasma		c ²	Sig.
			Positive	%	Positive	%		
Age (year)	≤1	225	5	2.22	17	7.56	6.545	0.011
	>1	137	4	2.92	4	2.92	0.000	ns
	c ²		0.111		8.049			
	Sig.		ns		0.005			
Gender	Male	201	7	3.48	16	7.96	3.522	ns
	Female	161	2	1.24	5	3.11	1.286	ns
	c ²		2.778		5.762			
	Sig		ns		0.016			
Housing	Yes	245	8	3.27	19	7.76	4.481	0.034
	No	117	1	0.85	2	1.71	0.333	ns
	c ²		5.444		13.762			
	Sig.		0.020		<0.001			

Sig= significant , ns= not significant

Figures

Figure 1

Map showing the location of the study areas in Saudi Arabia.

Figure 2

Image of agarose gel electrophoresis (1.5%) showing the 1100-bp band for the amplification of the 18S rRNA gene of *Piroplasma*. Lane M: DL 2000 marker; Lane P: positive control; Lane N: negative control; Lanes 1-11: negative samples; Lane 12: positive sample.

Figure 3

Image of agarose gel electrophoresis (1.5%) showing a 520-bp band for the amplification of the 23S rRNA gene of *Anaplasma*. Lane M: DL 2000 marker; Lane P: positive control; Lane N: negative control; Lanes 1-8, 11, 12: negative samples; Lanes 9, 10: positive samples.

Figure 4

18S rRNA-based phylogenetic analysis of genotypes identified in this study. Phylogenetic tree highlighting the position of *Theileria* sp. in the present study (bold) related to other *Theileria* sp. available in GenBank. The sequences of 18S rRNA were aligned using CLUSTAL W, and phylogenetic inferences were constructed in MEGA X using maximum likelihood based on the Tamura-Nei model for nucleotide sequences with 500 bootstrap replicates. There was a total of 491 positions in the final dataset. The scale bar represents a 5% nucleotide sequence divergence.

Figure 5

23S rRNA-based phylogenetic analysis of genotypes identified in this study. Phylogenetic tree highlighting the position of *Anaplasma* sp. in the present study (bold) related to other *Anaplasma* sp. available in GenBank. The sequences of 23S rRNA were aligned using CLUSTAL W, and phylogenetic inferences were determined in MEGA X using maximum likelihood based on the Tamura-Nei model for nucleotide sequences with 500 bootstrap replicates. There was a total of 435 positions in the final dataset. The scale bar represents a 5% nucleotide sequence divergence.

Supplementary Files

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