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Differentiation between Common Tick Species Using Molecular Biology Techniques in Saudi Arabia

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***Abstract*—**Protein and Esterase electrophoresis were used to genetically identify two Saudi tick species. Engorged females of the camel tick *Hyalomma dromedarii* (Koch) (Acari: Ixodidae) and the cattle tick *Boophilus annulatus* (Say) (Acari: Ixodidae) ticks collected from infested camels and cattle in the animals resting house at Hail region in KSA were used. The results showed that there are a variation in both of protein and esterase activity levels and a high polymorphism within and between the genera and species of *Hyalomma* and *Boophilus* . In conclusion, the protein and esterase electrophoretic analysis used in the present study could successfully distinguish among tick species, commonly found in Saudi Arabia.

***Keywords*—**Molecular biology, The camel tick *Hyalomma dromedarii*, The cattle tick *Boophilus annulatus* , Ticks**.**

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I. Introduction

CAMEL ticks *Hyalomma dromedarii* Koch (Acari: Ixodidae) and Cattle ticks *Boophilus annulatus* (Say) (Acari: Ixodidae) are the most serious common parasite of camel and cattle (respectively) in Saudi Arabia. They are disease vectors for different parasites and if uncontrolled, can cause serious losses to the livestock industry [1]. This investigation was aimed to identify these ticks species in Saudi Arabia by using Protein Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Esterase Isozyme Electrophoresis.

The identification of tick species has always been based on morphological key characters of the mouth parts and adjacent structures [2]. These methods cannot be applied to damaged specimens. Recently, protein electrophoresis and molecular genetic studies were introduced to differentiate among the different genera and/or species [3]-[4].

Molecular biology as a new approach helps to classify and control pests in a clear, easy and quick manner [5].

The objectives of this investigation were to assess the possibility of using molecular markers to identify tick species based on protein and esterase to estimate the similarity and difference between them.

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II. Materials and Methods

Collection of Ticks:

Engorged females of the camel tick *Hyalomma dromedarii* (Koch) (Acari: Ixodidae) and the cattle tick *Boophilus annulatus* (Say) (Acari: Ixodidae) ticks collected from infested camels and cattle in the animals resting house at Hail region in KSA.

Molecular Biology Techniques:

Protein Polyacrylamide Gel Electrophoresis (SDS-PAGE):

Preparation for total protein assay was carried out according to the method in [6]. Electrophoresis was carried out as described in [7] using pre-stained high molecular weight standard marker with molecular weight ranged from 200 KDa (KDa = Kilo Dalton) to 6.5 KDa.

After the electrophoresis process the gels were stained with silver stain and distained according to the method in [8]. The stained gels were photographed and examined for the presence and absence of visualized bands.

Esterase Isozyme Electrophoresis:

The same steps were followed for esterase electrophoresis using α – naphthyl propionate as substrate according to [9].

Concentration of protein and esterase bands (Conc. %), relative fragmentation and similarity coefficient (Sim co.) were calculated by following [10] and commonality percentage (Com. %) was calculated according to [11] .

$$\text{Conc. \%} = \frac{\text{O. D. of sample}}{\text{O. D. of standard}} \times \text{Conc. Of standard}$$

Where: O. D. = Optical density

$$\text{Rf value} = \frac{\text{Distance of migrated band}}{\text{Distance of migrated tracked gel}}$$

$$\text{Sim . co.} = 1 - \frac{NXY}{NX+NY}$$

Where:

NXY= The number of common bands in samples X and Y

NX= The number of bands in sample X

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NY= The number of bands in sample Y

Com. % = Number of common bands in samples X and Y / Number of total bands of both samples X and Y

III. RESULTS AND DISSCUSSION

Table 1 and Figs (1and 2) showed results of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for protein of two species of ticks collected from Saudi Arabia.

The camel tick *Hyalomma dromedarii* showed 16 visualization bands which: 1, 2, 3, 4, 5, 6, 7, 8, 11, 13, 14, 15, 17, 19, 20 and 22 . These relative bands ranged between 109.38 KDa and 5.96 KDa , have relative fragmentation (Rf) ranged from 0.012 and 0.917 and concentration varied between 24.44 and 1.23.

TABLE I
QUANTITATIVE PROTEIN PATTERN OF TWO SPECIES OF TICK FROM SAUDI ARABIA REGARDING THE TABLE: (+) PRESENT, (-) ABSENT, MOL.W.= MOLECULAR WEIGHT IN KDA, RF.= RELATIVE FRAGMENTATION, CONC.% = CONCENTRATION PERCENTAGE , SIM. %= 77.14% AND COM. %= 29.03%.

1	+	109.38	0.012		+	108.24	0.167	6.32
2	+	98.42	0.208		+	105.70	0.233	2.13
3	+	95.30	0.250		-	-	-	-
4	+	85.13	0.292		+	98.13	0.292	1.62
5	+	58.21	0.417		+	94.36	0.400	0.74
6	+	40.73	0.442		+	87.18	0.417	1.28
7	+	29.41	0.500		+	39.86	0.500	16.08
8	+	20.80	0.542		-	-	-	-
9	-	-	-		+	27.27	0.583	3.85
10	-	-	-		+	24.08	0.617	1.92
11	+	18.68	0.641		-	-	-	-
12	-	-	-		+	21.06	0.642	2.42
13	+	17.39	0.667		+	19.71	0.667	11.54
14	+	15.75	0.683		+	18.02	0.700	10.36
15	+	14.71	0.708		-	-	-	-
16	-	-	-		+	16.82	0.733	5.14
17	+	12.09	0.733		-	-	-	-
18	-	-	-		+	13.72	0.775	12.80
19	+	9.46	0.792		+	10.83	0.833	20.13
20	+	8.02	0.850		-			
21	-	-	-		+	9.31	0.875	3.44
22	+	5.96	0.917					

TABLE II
QUANTITATIVE ESTERASE PATTERN OF TWO SPECIES OF TICK FROM SAUDI ARABIA(+) PRESENT, (-) ABSENT, RF.= RELATIVE FRAGMENTATION , CONC.% = CONCENTRATION PERCENTAGE, SIM. % = 82.93% AND COM. %= 50.00%

Band number	The camel tick *Hyalomma dromedarii*			The cattle tick *Boophilus annulatus*		
	Band occurrence	Rf.	Con. %	Band occurrence	Rf.	Con. %
1	+	0.19	1	+	0.19	1
2	+	0.23	2	+	0.23	2
3	+	0.40	3	+	0.40	3
4	+	0.46	4	+	0.46	4
5	+	0.50	5	+	0.50	5
6	+	0.69	6	+	0.69	6
7	+	0.72	7	+	0.72	7
8	+	0.93	8	+	0.93	8

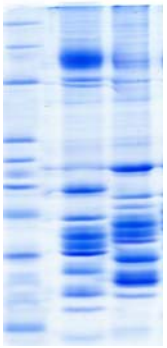


Fig. 1. SDS- polyacrylamide gel zymogram of denatured protein patterns in two species of ticks attacking domestic animals in KSA.1 and 2 represent lanes of tick samples, where 1 = the camel tick *Hyalomma dromedarii* and 2 = the cattle tick *Boophilus annulatus* and lane M represents the known molecular size marker.

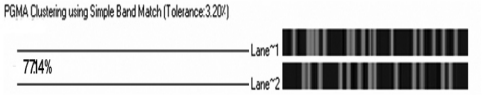


Fig. 2. Similarity relationships among protein bands in the camel tick *Hyalomma dromedarii* and the cattle tick *Boophilus annulatus* (Say) in KSA.

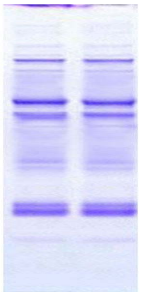


Fig. 3. Polyacrylamide gel zymogram of esterase isozyme patterns in two species of ticks in KSA. 1 = the camel tick *Boophilus annulatus* and 2 = the cattle tick *Boophilus microplus*.

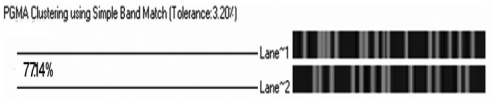


Fig. 4. Similarity relationships among esterase bands in the camel tick *Hyalomma dromedarii* and the cattle tick *Boophilus annulatus* in KSA.

On the other hands , the cattle tick *Boophilus annulatus* has 15 bands : 1, 2, 4, 5, 6, 7, 9, 10, 12, 13, 14, 16, 18, 19, 21. These bands were located between 108.24 KDa and 9.31 KDa , have Rf values ranged from 0.167 and 0.875 and concentration varied from 6.32 and 3.44. The common reactive bands between the camel tick *Hyalomma dromedarii* and the cattle tick *Boophilus annulatus* were nine bands : 1, 2, 4, 5, 6, 7, 13, 14 and 19 , with molecular weight ranged from 109.38 KDa and 9.46 KDa . Similarity % was 77.14% and the commonality % was 29.03% .

Data in Table 2 and figs (3 and 4) show esterase profile pattern of two different species of ticks in Saudi Arabia, the first species is the camel tick *Hyalomma dromedarii* and the second species is the cattle tick *Boophilus annulatus*. Both of species have eight different esterase bands , with Rf values ranged from 0.19 to 0.93 , respectively, in both two tick species and concentration ranged from 8.78 to 36.14 in the camel tick and from 8.59 to 35.04 in the cattle tick, respectively. Similarity % in esterase bands of ticks species was 82.93% and the commonality % was 50.00%.

In the same trend [13] detected fifteen negatively charged protein bands were found by acrylamide-gel electrophoresis to be present in the whole blood of the cattle tick *Boophilus microplus* in Australia. . The bands were further characterized into glycoproteins, haemoproteins, esterases, phosphatases, and an aminopeptidase. Reference [5] utilized SDS-PAGE and esterase profile patterns to discrimination between two species of fruit flies and in [14] used SDS-PAGE to detect midgut antigens of *Hyalomma anatolicum anatolicum* tick.

Isozyme was used for species identification of acarines. The most commonly studied enzymatic system in mites is that of the esterases [15].

In ticks, several enzymatic systems can be resolved from an individual. However, diverse studies have reported low polymorphism of the resolved loci [13] – [16]. Furthermore , Reference [17] used Esterase and RAPD-PCR analysis to differentiate among four species of ticks in Egypt, these tick species were: *Argas hermanni* (Audouin), *Argas persicus* (Oken), *Hyalomma dromedarii* (Koch), *Hyalomma anatolicum excavatum* (Koch).

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