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Molecular Characterization of *Borrelia Anserina* of Chicken Using RAPD Technique in Egypt

Eskander, N.B.; El-Khatib, R.M.; Gamal El-Din, H.Y. and Hegazy, N.A.M.
Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo

ABSTRACT

Random amplified polymorphic DNA (RAPD) technique provided characteristic fingerprints for *Borrelia anserina* isolates. The isolates of *Borrelia anserina* were collected from 3 different localities in Egypt (El-Giza, New Valley province and Wady AlNatron), six oligonucleotides primers reflected different phylogenetic relationship among the 3 *Borrelia anserina* isolate. The degree of similarity among the three isolates reflected the presence of both species-specific and even strain specific, the statistical analysis suggested a closer phylogentic relationship between El-Giza and New Valley province, while *Borrelia anserina* isolates is distantly related to Wady Al-Natron. RAPD technique could easily differentiate with a great potential the minor change in the genomes of the related genotypes of *Borrelia anserina* isolates in Egypt.

INTRODUCTION

Avian spirochaetosis caused by *Borrelia* (spirochaeta) *anserina* is an acute febrile highly fatal disease of chickens, turkeys, geese and other fowls. The organism is transmitted by fowl ticks (*Argus persicus*) and other arthropods ***(1).***

The disease causes great morbidity and mortality rates ***(2,3).*** The rate of infection in Egypt was 23.7% in chicken, 67.4% in ducks, 5.8% in geese and 3% in turkeys ***(4).***

The disease causes diarrhea, restlessness and marked decrease in red blood cell count leading to severe anaemia and emaciation as well as heart enlargement with petechial haemorrhage and congestion ***(5)***. Liver, spleen and kidney were enlarged. congested and showed mottled appearance ***(2).***

The random amplified polymorphic DNA (RAPD) technique, which is based on the amplification of anonymous targets by the use of arbitrary primers. This generates fingerprint of multiple bands. Because no previous knowledge of nucleotide sequence is known RAPD has been used for discriminating genotypes of microorganisms populations, such as *Borrelia anserina* ***(6-9).***

The objectives of this study was to isolate and characterize the different *Borrelia anserina* isolates from 3 different Egyptian localities, El-Giza i.e. (isolate A), New Valley province (isolate B) and Wady Al-Natron (isolate C). RAPD was the techniques of choice to accurately describe the homogenicity and heterogenicity among the studied *Borrelia anserine* isolates.

MATERIAL AND METHODS

Borrelia anserina isolates

Three *Borrelia anserina* (field isolates) were isolated from blood of naturally infected chicken following the previously applied technique ***(10, 11)*** from different localities El-Giza (isolate A), New Valley province (isolate B) and Wady Al-Natron (isolate C). The infected blood was collected on sterile citrated solution. The isolates were maintained by serial passage in susceptible fowl every 28 days.

The isolates were kept at 4°C until used for separation of Borelia anserina pellets .

Separation of Borrelia anserina pellets

The *Borrelia anserina* pellets were prepared from different isolates as the previously described by several authers ***(2, 12, 13)***.

Preparation of PCR reactions

Random amplification of DNA from 3 isolates was performed ***(6)***. The reactions were

carried out in a volume of 50µl containing 250ng of genomic DNA as a templete.

Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR)

This procedure allows rapid detection for the presence or absence of a target DNA sequence in any genetic material. In this technique, DNA is amplified *in vitro* by a series of polymerization cycles consisting of three temperature-dependent steps (denaturation, annealing and extension) resulting in target DNA amplification ***(14, 15).***

RAPD-PCR

Reactions were performed in a total volume 50µl reaction buffer (100mM KCl, 100mM tris HCl pH 8.3) 3.0mM $MgCl_2$, 200mm dNTPs (Promega Biotec. Inc) 50 p/mole primers and 0.2µl taq polymerase (hot start). This reaction was added to 0.1µl genomic DNA. All reactions tubes, pipette tips, micropestles and water were irradiated with UV light as tamplate containing 250 mg to destroy possible contaminated with RNase ***(16).*** Irradiation treatment was 20 minutes at 2.5cm from the bulbs of a gene linker (Biorad Inc). Tubes contain mixtures were placed in a thermcycler (Perkin-Elmer 2400) and DNA was amplified ***(17)***.

Primers

Six 6-mer oligonucleotide primers (Z-01, Z-14, A-1. A-10, C-20 and A-03) were synthesized by Agricultural Genetic Engineering Research Institute. Each primer was used with each of the isolate for random amplification of genomic DNA ***(6, 18).*** The 6 primers used in this study are as follows:

Primer description	Base sequence
Z-01	5` - TCTGTGCCAC - 3`
Z-14	5` - TCGGAGGTTC - 3`
A-1	5` - CAGGCCCTTC - 3`
A-10	5` - GTGATCGCAG - 3`
C-20	5` - ACTTCGCCAC - 3`
A-03	5` - AGTCAGCCAC – 3`

RESULTS

Primer Z-01 as shown in Fig. 1 and Table 1 revealed a total number of 6 different amplified DNA bands for the three isolates under study. The electrophoretogram of the isolates displays 5 different polymorphic bands of molecular size 600, 500, 490, 410 and 400 bps. Different diagnostic bands characterizing specific isolate(s) were recorded, isolate (A) displays one positive diagnostic band of molecular size 490 bps, isolate (B) shows diagnostic band of molecular size of 500 bps, but isolate (C) exhibits one diagnostic band of molecular size of 600 bps. Only one monomorphic bands appears at molecular size of 710 bps.

Primer Z-14 as shown in Fig. 1 and Table 1 shows a number of 6 different amplified, polymorphic bands of molecular sizes 1000, 700, 625, 600, 390 and 300 bps. Isolate (C) only shows two positive diagnostic bands of molecular sizes 1000 and 625 bps.

Primer A-1 as shown in Fig. 1 and Table 1 revealed a total number of 7 different amplified DNA bands for the 3 isolates under investigation. The electrophoretogram of the isolates displays a number of 4 polymorphic bands of molecular sizes 1250, 1000, 800 and 600bps. The 3 remaining bands are monomorphic exhibiting the molecular sizes of 500, 400 and 300 bps. Close inspection of the data shows 4 different diagnostic bands characterizing specific isolate. Isolate (B) displays 3 diagnostic bands of molecular sizes 1250, 1000 and 800 bps. Isolate (C) shows one diagnostic band of molecular size 600 bps.

Primer A-10 as shown in Fig. 1 and Table 1 revealed three polymorphic bands of molecular sizes 1350, 1000 and 430 bps and three monomorphic bands of molecular sizes 920, 600 and 520 bps. Isolate B shows two diagnostic bands of molecular sizes 1350 and 430, while isolate A and C showed no diagnostic band.

Primer C-20 as shown in Fig. 2 and Table 2 revealed a total number of 8 different amplified DNA bands for the three isolates under study. The electrophentogram of the isolates displays 6 different polymorphic bands of molecular sizes 1580, 1480, 900, 700, 400 and 310 bps. The data showed 3 different diagnostic bands of molecular sizes 1580, 900 and 400 bps characterized isolate (C). Two monomorphic bands appear at molecular sizes of 1050 and 550 bps.

As far as primer A-03 is concerned the electrophoretogram of the 3 isolates displays the highest number of bands (12 bands).

As shown in Fig. 2 and Table 2, this primer shows 10 polymorphic bands and two monomorphic bands. The polymorphic bands are of molecular sizes 1350, 1050, 980, 870, 730, 620, 500, 460, 250 and 220 bps. Isolates (A), (B) and (C) each shows one positive diagnostic band of 980, 870 and 460 respectively. The two monomorphic bands display molecular sizes of 1490 and 800 bps.

Table 1. RAPD analysis using 4 different primers of three *Borrelia anserina* isolates

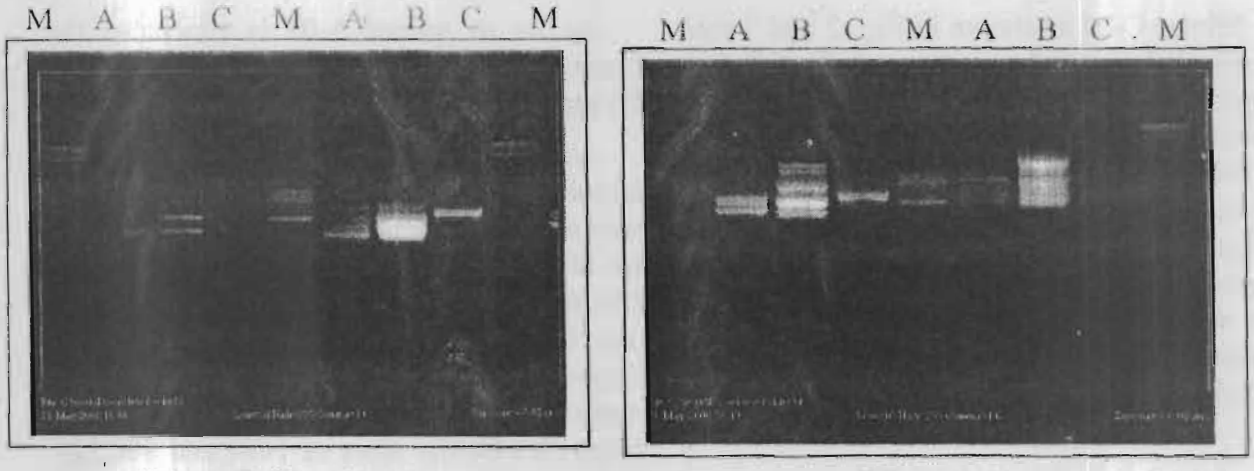
Z-01				Z-14				A-1				A-10			
5` - TCTGTGCCAC - 3`				5` - TCGGAGGTTC - 3`				5` - CAGGCCCTTC - 3`				5` - GTGATCGCAG - 3`			
Molecular size of polymorphic and monomorphic band (bps)	*Borrelia anserina* isolates of			Molecular size of polymorphic and monomorphic band (bps)	*Borrelia anserina* isolates of			Molecular size of polymorphic and monomorphic band (bps)	*Borrelia anserina* isolates of			Molecular size of polymorphic and monomorphic band (bps)	*Borrelia anserina* isolates of		
	A*	B**	C***		A	B	C		A	B	C		A	B	C
710	1	1	1	1000	0	0	1	1250	0	1	0	1350	0	1	0
600	0	0	1	700	1	1	0	1000	0	1	0	1000	1	1	0
500	0	1	0	625	0	0	1	800	0	1	0	920	1	1	1
490	1	0	0	600	1	1	0	600	0	0	1	600	1	1	1
410	1	1	0	390	1	1	0	500	1	1	1	520	1	1	1
400	1	1	0	300	1	1	0	400	1	1	1	430	0	1	0
								300	1	1	1				

* A : isolate of El-Giza ** B : isolate of New Valley province. *** C : isolate of Wady Al-Natron.

Table 2. RAPD analysis using two different primers of three *Borrelia anserina* isolates

C-20				A-03			
5` - ACTTCGCCAC - 3`				5` - AGTCAGCCAC – 3`			
Molecular size of polymorphic and monomorphic band (bps)	*Borrelia anserina* isolates of			Molecular size of polymorphic and monomorphic band (bps)	*Borrelia anserina* isolates of		
	A*	B**	C***		A	B	C
1580	0	0	1	1490	1	1	1
1480	1	0	1	1350	0	1	1
1050	1	1	1	1050	1	1	0
900	0	0	1	980	1	0	0
700	0	1	1	870	0	0	1
550	1	1	1	800	1	1	1
400	0	0	1	730	0	1	1
310	0	1	1	620	1	0	1
				500	0	1	1
				460	0	1	0
				250	0	1	1
				220	1	1	0

* A : isolate of El-Giza ** B : isolate of New Valley province. *** C : isolate of Wady Al-Natron.

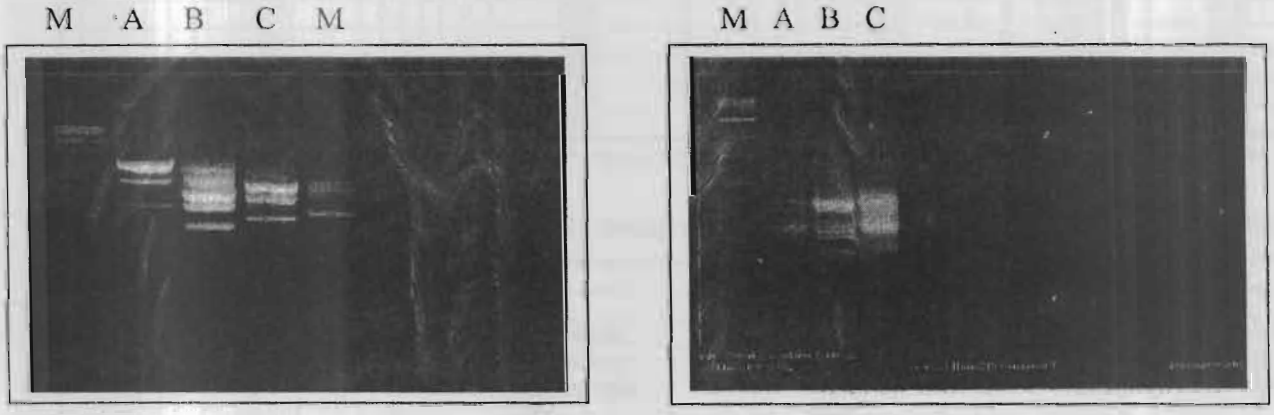


Primer Z-01 and Primer Z-14

Primer A-1 and Primer A-10

Fig. 1. RAPD profiles of *Borrelia anserina* genomic DNA isolates amplified by 4 random oligonucleotide primers

(Z-01, Z-14, A-1 and A-10)
Lane A: Isolate of El-Giza
Lane B: Isolate of New valley province
Lane C: Isolate Wady Al-Natron
M: Hae III DNA Digest, Biotool, Spain



Primer C-20

Primer A-03

Fig. 2. RAPD profiles of *Borrelia anserina* genomic DNA isolates amplified by 2 random oligonucleotide primers (C-20, A-03)

Lane A: Isolate of El-Giza
Lane B: Isolate of New valley province
Lane C: Isolate Wady Al-Natron
M: Hae III DNA Digest, Biotool, Spain

Table 3. Degree of similarity (homology) among *Borrelia anserina* isolates using Dice coefficient of PCR-RAPD amplified bands.

	El-Giza	New Valley province	Wady Al-Natron
El-Giza	100.0	70	51
New Valley province	70	100.0	56.7
Wady Al-Natron	51	56.7	100.0

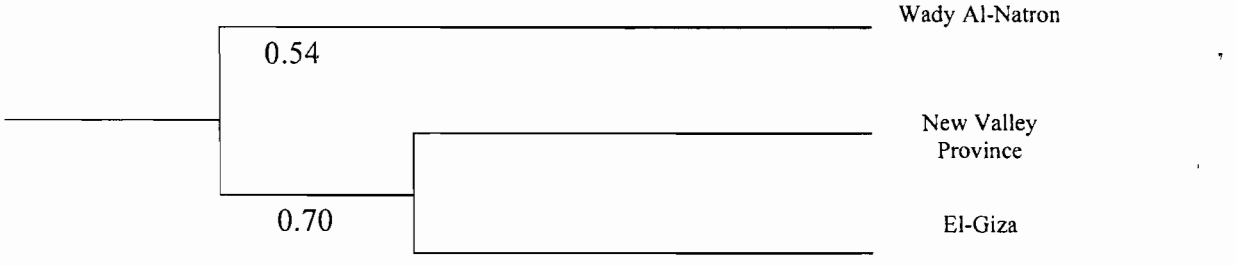


Fig. 3. The dendrogram showing the phylogenomic relationship among the 3 *Borrelia anserina* isolates

DISCUSSION

Spirochaetosis caused by *Borrelia anserina* is a highly fatal to chickens and transmitted by fowl ticks (*Argus persicus*), and other arthropods also transmit it ***(1).*** The disease cause great morbidity and mortality rates ***(3,6).***

The 6 different decamr primers used in PCR-RAPD study revealed genotype-specific DNA fragment. These DNA fragments or bands can be used as a diagnostic feature to characterize a given isolate (genotype) for a given primer.

In this respect, the most indicative used primers are A-03 (12 bands) followed by C-20 (8 bands) and then A-1 (7 bands) and the primers Z-01, Z-14 and finally A10 (6 bands).

The degree of similarity among the 3 *Borrelia anserine* isolates using 6 oligonucleotide primers was tested and the results were scored as shown in Tables 1, 2 and 3 and figures 1, 2 and 3.

The similarity between the 3 species revealed a great similarity between New Valley province and El-Giza to a proportion of 70% while New Valley province and Wady Al-Natron revealed a proportion of 56.7% while El-Giza and Wady Al-Natron revealed similarity to 51%.

The present results revealed that the isolates of El-Giza and New Valley province are closely related to each other, but Wady Al-Natron strain was distantly related to the other 2 strains. This may refer to trials for vaccine preparation which should consider the phylogenetic relationship in order to avoid vaccination failure.

The obtained data appears to be in a consistent with the previously cited reports ***(7-9).***The investigators used RAPD method to compare between different strains of *Borrelia anserina.* They mentioned that the degree of relationship might vary according to the strains within aspects and probably according to the

used primer. This method is quick and easy to perform and differentiate among the different genomic groups of *Borrelia anserina* isolates.

This finding confirmed the results obtained by williams and his collegues *(6)* who mentioned that RAPD technique could differentiate and clarify even the minute difference between the isolates of the same species.

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الملخص العربى

التوصيف الجزيئ لعترة البوريليا انسيرينا للدجاج باستخدام أختبار التكبير العشوائى المتعدد الاوجة فى مصر

نشأت بطرس اسكندر ، رجب محمد الخطيب ،

حامد يوسف جمال الدين ، نصر أحمد حجازى

معهد بحوث الأمصال واللقاحات البيطرية-العباسية-القاهرة

تم استخدام اختبار التكبير العشوائى المتعدد الأوجه للحامض النووى DNA (PCR-RAPD technique) لتحديد البصمة الوراثية لعترة البوريليا انسيرينا المعزولة من ثلاث محافظات بمصر (الجيزة – الوادى الجديد – وادى النطرون) وقد تم التصنيف الوراثى باستخدام ٦ بوادئ (6 primers) لتحديد أوجه التشابه والاختلاف بين العترات الثلاثة المعزولة. وقد وجد ان هناك العديد من الحزم المختلفة للحامض النووى DNA المشتركة بين المعزولات الثلاثة وقد تم تحديد الحزم النووية الخاصة لكل واحدة من المعزولات الثلاث وقد كان التشابه كبير بين عترة البوريليا المعزولة من محافظات الجيزة والوادى الجديد واقل بين البوريليا انسيرينا المعزولة من محافظات الجيزة ووادى النطرون. وهذا يدل على وجود تقارب كبير فى التركيب الوراثى بين عترات البوريليا انسيرينا المعزولة من محافظة الجيزة والوادى الجديد وابعد نسبياً عن العترة المعزولة من محافظة الجيزة ووادى النطرون. وعلى ذلك يعتبر اختبار (RAPD technique) له قدرة فعالة وعالية لتحديد الاختلافات الوراثية فى الحامض النووى لعترات البوريليا انسيرينا.