COMPARATIVE CHICKEN GENOME ANALYSIS OF EGYPTIAN LOCAL BREEDS AND DEVELOPED STRAINS

3. THE MICROSATELLITE DISCRIMINATION BETWEEN DOKKI-4, GOLDEN MONTAZAH AND SILVER MONTAZAH STRAINS

Kh. Roushdy¹, M.A.EL-Sayed² and A.A.Bakir³


ABSTRACT: This study represents the assessing the genetic characterization of three populations generated from Egyptian breeds Dokki-4, Golden Montazah and Silver Montazah. Hundred thirty-five genomic DNA were isolated from three chicken populations. Samples were used in a polymerase chain reaction (PCR) with Ten microsatellite markers. Genetic diversity measures considered were allele (bp), effective number of allele (ENA), observed and expected heterozygosity (H₀, Hₑ). A total of 138 alleles were detected averaged 13.8. Dendrogram was generated from estimates of genetic distance among chicken populations. Mean number of alleles per strains overall loci ranged from 4.1 (Dokki-4) to 5 (Golden Montzh), number of specific alleles ranged 5 (Dokki-4) to 14 (Golden Montazah) and effective number of allele (ENA) ranged from 1.58 (MCW0043) to 4.22 (ADL0176) with mean 2.90. Averages of observed (H₀) heterozygosity among loci were 0.56, 0.40 and 0.57 for Dokki4, Golden Montazah and Silver Montazah chicken populations, respectively. Averages of expected heterozygosity (Hₑ) were 0.62, 0.65 and 0.58 for Dokki4, Golden Montazah and Silver Montazah chicken populations, respectively. Dendogram Based Nei’s genetic distance revealed that Dokki4 and Golden Montazah chicken populations are closely related than that of Silver Montazah. The study revealed the existence of moderate genetic diversity in chicken populations studied and also showed that the markers used were highly informative and can be used in future studies involving chicken populations.

Key Words: Diversity, heterozygosity, alleles, microsatellite markers.
INTRODUCTION

Poultry biodiversity is considered one of the most endangered genetic resource. The determination of within-populations genetic characterization is a basic step in the evaluation of local breeds as genetic resources.

Dokki 4 strain was generated from a series of generations (4 generations) were maintained beginning as parents with cross between a Fayoumi cock and a number of Barred Plymouth Rock females (El-Itriby and Sayed, 1966). Silver Montazah has been developed from a cross between the Rhode Island Red males and Dokki 4 females for 3 generations (Mahmoud et al., 1974a). The same mating was done with different genotypes for five generations to produce Golden Montazah (Mahmoud et al., 1974b).

It can be assumed that Egyptian chicken breeds and developed strains contained the genes and alleles relevant to their adaptation to the particular environments and local breeding goals. Those chicken breeds and developed strains are needed to maintain genetic resources permitting adaptation to unforeseen breeding requirements in the future and a source of research material. Also, phenotypic and genotypic data is essential for the characterization of indigenous animal genetic resources (AnGR) for the effective conservation of useful gene pool for future generations.

In the process of evaluating genetic diversity to develop conservation measures in chickens, it is of special interest to assess genetic variation between different chicken breeds by utilizing modern molecular tools (Groenen et al., 1998). Several techniques like RAPD, AFLP and Microsatellite analysis are utilized to assess the population relationships. Microsatellites are the most molecular markers used in gene marker studies for their codominant, highly polymorphic nature, availability throughout the genome so the microsatellites are identified as reliable markers in chicken (Romanov and Weigend, 2001; Zhang et al., 2002 and Hillel et al., 2003).

Recent information in literature have revealed that microsatellite markers are useful in determining not only heterozygosity and estimating genetic distances among closely related species (Chen et al., 2004), it is also suitable for measuring important parameters such as effective number of alleles (ENA) in populations and can detect unique alleles (Bartfai et al., 2003 and Olowofeso et al., 2005). Thus, use of microsatellite markers has become a standard method to estimate genetic diversity indices in all livestock species. However, number of investigations have used microsatellites across Egyptian chicken populations (Roushdy et al., 2008, 2009 and 2012; El-Sayed et al., 2011; El-Tanany, 2011 and Ramdan et al., 2012).

The objective of this study was to investigate and compare genetic variance and homozygosity with 10 microsatellite loci in 3 chicken Egyptian populations.

MATERIAL AND METHODS

Chicken populations:

Three Egyptian local strains, Dokki-4, Golden Montazah (GM) and Silver Montazah (SM) were assayed in the present investigation. A total of 135 individuals were used from the three strains 45 each.

Blood sampling & DNA Isolation:

A half ml of blood sample was withdrawn from Jugular vein on EDTA tube as anticoagulant (0.2 ml of 0.5 M EDTA). DNA was freshly extracted from whole collected EDTA-blood. Two and half ml of lysis buffer TSTM (20 mM Tris-HCl pH 7.6, 640 mM sucrose, 2% Triton X-100, 10 mM MgCl2) was added to the aliquot. The mixture was centrifuged and the pellet suspended in 150 μl Proteinase K, 1.5 ml nuclei lysis buffer and 110 μl SDS 20%. After overnight incubation at 37° C,
the proteins were removed by NaCl 6M and the DNA were precipitated by ice cold absolute ethanol. Extracted DNA was diluted with sterilized water in ratio 5:495μl before the optical density (OD), that is, purity and concentration of each DNA was carried out with the use of ultra violet spectrophotometer.

**Microsatellite loci:**

Ten microsatellite loci (Table 1) were selected based on the degree of polymorphism and genome coverage have been recommended for the Measurement of Domestic Animals Diversity (MoDAD) (FAO, 2004), for application in diversity studies. Detailed informations about used microsatellites are available at the FAO website, (www.dad.fao.org/en/refer/library/guidelin-marker.pdf).

**Amplification, PCR procedure and programme:**

A set of ten microsatellite markers (Table 1) was used for the amplification of samples. The composition of the PCR reaction mixture with final volume of 10 μl in each PCR tube contained 1.5 μl DNA template, 2.5 μl Taq master mix (enzyme), 0.5 μl of primer (0.5 μl each of reverse and forward form) and 5 μl of double distilled water. PCR reaction was carried out in the PCR PC-200 under the following conditions: an initial denaturation step for 2 minute at 95°C, followed by 35 cycles of denaturation for 30 seconds at 94°C; annealing (47-52°C) for 60 seconds at optimized primer annealing temperature (Table 1), extension for 60 seconds at 72°C and final extension was at 72°C for 60 seconds. Following this, 5 μl of PCR products were loaded on to a 10% polyacrylamide gel with puc19 DNA marker and electrophorese were carried out in a double cassette plates. Gels were stained with ethidium bromide and visualization of samples were done under UV-trans-illuminator and photographed with a digital camera.

**Microsatellite and Genetic Analysis:**

All resulted gels were visualized and scored with Alphaimages 2200 software Version 4.0.1. All scored microsatellite data was firstly corrected to estimate each allele size according to its number of repeats for each marker. A Tandem Repeat Analyzer software package was adopted for this purpose. All possible extracted population figures were carried out employing a Arlequin 3.11 software package after data conversion using Convert program. It is common in such cases no amplicon is produced in certain samples for such primer rather than other. Thus, the absence of PCR product in these samples is manipulated as missing data. As a consequent, the analysis program accounts them as null (unknown) alleles not exceeded 0.1 of data as our default analysis.

**RESULTS AND DISCUSSION**

A set of ten highly polymorphic SSR markers were attended in the present investigation. All the characteristics of microsatellite loci are presented in Table (1).

The mean number of alleles per locus for the studied loci was 4.6 alleles ranged from 2.67 for MCW43 to 7.00 for ADL176 (Table 2). This average could be informative for such studies according to Barker (1994) who suggested that the average number of alleles per locus in studies of genetic distances must be >4 to reduce the standard error in the estimation of genetic distances. Only three loci had a number of alleles <4 as ADL171(3.00), MCW23 (2.67) and MCW51 (2.67) as shown in Table(2). Kaiser et al. (2000) reported mean number of alleles per locus 2.8 and 2.9 for two broiler populations L and C, respectively. Emara et al. (2002) reported values of 3.5, 2.8 and 3.1 allele per locus for three commercial broiler lines. In a study of genetic variation within and
between 52 populations from a wide range of chicken types mean number of alleles per locus was 3.5, 1.3 and 5.2 for the average, the least and the most polymorphic population, respectively (Hillel et al., 2003). Higher values for average number of alleles per locus was obtained 7.00 for ADL176. Similarly, Yu-Shi et al. (2005) reported, 9.2 alleles in 19 native Chinese chicken breeds and 11.4 in 64 population of chickens from different continents by Granevitz et al. (2007) and 7.5 for five sub-populations of Turkish native chicken breeds by Kaya and Yildiz. (2008); 4.9 for Egyptian native breeds (Fayoumi and Dandarawi) and commercial laying hens (brown Hy-line) by Roushdy et al. (2008) and 6.25 in 13 Spanish breeds of chickens by Davila et al. (2009). Finally, for 6 different populations of chickens from Ghana, Benin and Japan, three vulturine guinea fowls were included as outgroup, Kayan et al. (2010) noted average number of alleles per locus as 11.0.

Effective number of alleles (ENA) is a reciprocal of gene homozygosity (Hartl and Clark, 1997). Effective number of alleles used to corollary the HE (when heterozygosity is high, ENA will be highest). The lowest ENA was 1.58 for MCW43 when HE was 0.34 while, the highest ENA was 4.22 for ADL176 when HE was 0.77 (Table 2).

The mean observed Heterozygosity (H0) was 0.51 and ranged across loci from 0.20 for locus MCW43 to 0.79 for locus ADL136 whereas the mean expected Heterozygosity (HE) was 0.62 and ranged between 0.34 and 0.70 for the same loci.

The slight difference between mean observed compared to the expected heterozygosity probably due to one or more reasons. This might reflect slight inbreeding, selection against heterozygotes. Also, the nature of markers used in the current study might also contribute to the observed level of heterozygosity as a result of non-detection of homozygotes from heterozygotes due to presence of null alleles. The highest H0 was 0.9556 for Silver Montazah (SM) with ADL210 and MCW49 loci, and 0.9286 for Dokki4 with ADL176 locus. Whereas the lowest one was 0.02 for SM and Golden Montazah (GM) with MCW43 and ADL172, respectively. Average H0 in this study (0.51) was slightly lower than Wei et al. (2008) whose observed in a study on Xuen-feng black bone native chicken (0.6285) with 23 loci and Quain et al. (2006) in Wuding chicken with 25 markers (0.6382).

However, similar result of heterozygosity was reported by Zhen-hua et al. (2010) in Chai chickens with 10 microsatellite markers (0.5488). Also, Wimmers et al. (2000) found that the genetic variability of various local chicken populations derived from Bolivia, India, Nigeria and Tanzania was evaluated with 22 microsatellites, results showed that all populations showed high levels of heterozygosity with the lowest value of 0.45 for the population named Aseel from India and the highest value of 0.67 for Arusha from Tanzania (Wimmers et al., 2000). Lower values were reported for Chauha breeds (0.3514) studied by Ye et al. (2006) with 7 microsatellite markers. Thus, variations in heterozygosity may be due to differences in location, different sample sizes, different experimental chickens and number/sources of microsatellite markers as previously reported by Olowofeso et al. (2005).

The Polymorphic Information Content (PIC) is a good slandered for evaluated genetic markers. Among all loci PIC ranged between 0.271 for locus MCW73 and 0.7162 for locus ADL176 with general mean of 0.5545. The majority of the studied microsatellite loci used in this study was highly revealing (Table 2). According to classification of Botstein et al. (1980), the highly informative markers have PIC values >0.50, the
reasonably informative markers have PIC value between 0.25-0.50 and the slightly informative markers have PIC value <0.25. While two markers in the current study had reasonably informative PIC values 0.271 and 0.4628 for MCW43 and ADL171, respectively, the majority of markers had highly informative marker. Similarly, Yu-Shi et al. (2005) investigated that values of PIC varied in many studies on 19 native Chinese chicken breeds between 0.523-0.702. In 12 indigenous chicken populations in Southern Chins PIC was ranged from 0.560 to 0.641 as reported by Ya-Bo et al. (2006). Polymorphism information content in Turkish native chicken breeds was varied from 0.426-0.599 (Kaya and Yildiz. 2008).

Hardy-Weinberg equilibrium (HWE) test giving exact probability value per locus and per breeds. P-value indicate significance of deviation from HWE p <0.05 (table 2). The studied populations showed significant deviation from HWE except for Dokki4 with MCW49 and SM with ADL171 locus. These findings were in agreement with Davila et al. (2009) whose noted that some Spanish chicken breeds showed significant deviations from the Hardy-Weinberg equilibrium, suggesting that these breeds have been selected for years for morphological traits such as plumage, shank and egg colors and comb and earlobe sizes although the presence of null alleles or genotyping error could also be the reason. Furthermore, no departure from HWE was observed for 64 chicken populations from different continents (Granevitze et al., 2007).

The FIS is used to obtain a deeper insight to appraise the degree of inbreeding and endangerment potentiality and is considered as an important tool to judge the conservation priority (Simon and Bchenauer, 1993). Accordingly, when FIS is less than 0.05, the breeds are not in danger; between 0.05 – 0.15, they are potentially endangered; between 0.15 – 0.25, they are minimally endangered; between 0.25-0.40, they are endangered; and more than 0.40, they are critically endangered. Whereas, Liu et al. (2008) mentioned that FIS of the inbreeding coefficient, measure the relative heterozygote deficit and non-random mating in sample. Its value ranges between -1 (all individuals heterozygote), 0 (random association of alleles) and 1 (all individuals homozygote). If inbreeding is avoided, F=0; negative F indices are usually from selection in favor of the heterozygotes, positive values indicate that the considered population has an inbreeding system of mating. The mean values of FIS obtained estimate 0.36 for GM strain indicating the high level of inbreeding in GM strain confirming by IC which equal to 0.38 (Table 2). While, the mean values -0.04 and -0.07 for Dokki4 and SM strains, respectively, indicating high variability in these strains confirming by inbreeding coefficient (IC) values (0.10 and 0.02, respectively). These findings might be due to more recent divergence of Dokki4 and SM than GM one.

Common and specific alleles for Dokki4, GM and SM and band size range were illustrated in Table (3). A total of 43 common alleles were detected versus 10 microsatellite loci overall strains. Regarding specific alleles, a total of 30 out of 138 alleles (21.74 %) were noticed overall loci for the three strains studied. For Dokki4, 5 private alleles were observed while 14 and 11 ones were obtained in the case of GM and SM strains, respectively. Consequently, these private alleles would be utilized as breed fingerprint even one allele for one locus.

Analysis of molecular variance results estimated by Arlequine3.5 software package as standard genetic population input data is presented in Table (4) indicated that the majority of the genetic diversity obtained in the current study is presented by within individuals (66%) rather than others. Population fixation
indices give an idea about the population structure in terms of inbreeding coefficient and population differentiation. Fixation indices traced a 0.338 of variation referring to differences among individuals versus total variance ($F_{IT}$). While, among populations differences versus total variance was low fixation indices ($F_{IS}=0.164$) indicating low level of population differentiation. A pair wise difference among Dokki4, GM and SM breeds was 0.207 based on within breeds F index ($F_{ST}$).

The combining of generated molecular genotypic data (such as microsatellites) and appropriate statistical analysis program must be concerned to identify and interpret the relationships of genetic and phenotypic variations of the resource breeds. Ultimately, this approach can lead to the detection of DNA markers that can be applied for genetic improvement of populations in marker-assisted selection schemes of poultry breeding (Lamont, 2003).

Similarly, in 13 Spanish chicken breeds, a tester line and a White Leghorn population using 24 microsatellite markers, mean fixation index of each population ($F_{ST}$) was 0.244 (Davila et al., 2009). Furthermore, a total of 232 helmeted guinea fowls (Numida meleagris) sampled from three populations in Ghana, one population in Benin and two populations in Japan were genotyped across six autosomal microsatellite loci. The indigenous West African populations (Ghana and Benin) were more genetically diverse but less differentiated ($F_{ST} 0.162$) compared to the non-indigenous populations in Japan ($F_{ST} 0.389$) as reported by Kayang et al. (2010). On the opposite trend, Wright's $F$-statistics revealed negligible genetic differentiation ($F_{ST}$) in local Ghanaian chicken populations (Osei-Amponsah et al., 2010).

Cluster analysis based on Nei's genetic distance indicated that the studied populations formed two main groups (Fig. 1). The 1<sup>st</sup> group included Dokki4 and GM and the 2<sup>nd</sup> group harbored SM. Regarding to how the strains produced, Silver Montazah has been developed from a cross between the Rhode Island Red males and Dokki 4 females for 3 generations and the same mating was done with different genotypes for five generations to produce Golden Montazah. That is why GM more related with Dokki4 than SM.

The phylogenetic trees was constructed in many studies for different populations (Zhou and Lamont, 1999) for White Leghorn (Wimmers et al., 1999) for 22 local chicken populations derived from Bolivia, India, Cameroon, Nigeria and Tanzania (Yu-Shi et al., 2005) and (Ya-Bo et al., 2006) for Chinese chicken breeds (Kanginakudru et al., 2008) for Indian birds (Kay and Yildiz, 2008) for Turkish native chickens and finally by Davila et al. (2009) for 13 Spanish chicken breeds.

**CONCLUSION**

Microsatellite markers permitted the genetic characterisation of the three population of chickens (Dokki-4, Golden Montazah and Silver Montazah). The optimum use of such information can help to preserve allelic diversity and the existing genetic variation. The obtained results seem to be promising to define and control the ongoing animal genetic resources conservation program. The microsatellites panel adopted for this study could also be useful for genetic traceability purposes.
Table (1): Summarizes all information of tin microsatellites markers used and shows locus name, gene bank NCBI accession number, genome location, microsatellite repeat type, flanking sequences, annealing temperature, reported number of alleles and sequence tagged site (STS) size in base pairs.

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<th>Location2 Chr.No size range</th>
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2. Locations & Number of alleles listed as reported by US chicken genome project population tester kit#9.
4. STS: sequence tagged site size according to NCBI database.
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Mean (H0) 0.62 0.56 0.10
Mean (H1) 0.65 0.57 0.38
IC 0.02 0.02 0.02

GM: Golden Montazah , SM: Silver Montazah.
No: number of alleles, Ne: effective number of alleles, PIC: polymorphism information content.
Pv: indicate significance of deviation from Hardy-Weinberg equilibrium (p <0.05).
IC: inbreeding coefficient.

978
Table (3): Common and specific alleles for analyzed breeds Dokki-4, Golden Montazah and Silver Montazah

<table>
<thead>
<tr>
<th>Locus</th>
<th>Common alleles bp</th>
<th>Specific alleles</th>
<th>Band size range bp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dokki 4</td>
<td>GM*</td>
</tr>
<tr>
<td>ADL102</td>
<td>108, 126, 144, 162, 180</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>ADL136</td>
<td>132, 154, 176, 198, 220</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>DL171</td>
<td>090,108,126</td>
<td>--</td>
<td>144</td>
</tr>
<tr>
<td>ADL172</td>
<td>108,126, 144, 162</td>
<td>180</td>
<td>72, 90</td>
</tr>
<tr>
<td>ADL176</td>
<td>192,204,216,228,240,252</td>
<td>264,276,</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>282</td>
<td></td>
</tr>
<tr>
<td>ADL210</td>
<td>120,135,150,165,180</td>
<td>--</td>
<td>105,195</td>
</tr>
<tr>
<td>MCW48</td>
<td>190,208,226,244</td>
<td>--</td>
<td>108,126,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>136,154</td>
</tr>
<tr>
<td>MCW43</td>
<td>132,153</td>
<td>174</td>
<td>--</td>
</tr>
<tr>
<td>MCW49</td>
<td>118,130,142</td>
<td>--</td>
<td>106</td>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCW51</td>
<td>90,100,110,120,130,140</td>
<td>--</td>
<td>150,160,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>5</td>
<td>14</td>
</tr>
</tbody>
</table>

*GM: Golden Montazah , SM: Silver Montazah

Table (4): AMOVA analysis of Dokki-4, Golden M and Silver M breeds based on microsatellite DNA variation.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>S .S.</th>
<th>Percentage variation</th>
<th>Fixation indices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among populations</td>
<td>2</td>
<td>144.18</td>
<td>20.76</td>
<td>F_{IS}=0.16421</td>
</tr>
<tr>
<td>within populations</td>
<td>132</td>
<td>446.81</td>
<td>13.01</td>
<td>F_{ST}=0.20761</td>
</tr>
<tr>
<td>Within individuals</td>
<td>135</td>
<td>332.50</td>
<td>66.23</td>
<td>F_{IT}=0.33773</td>
</tr>
<tr>
<td>Total</td>
<td>269</td>
<td>923.494</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

F_{IS}: Fixation indices (Among populations)
F_{IT}: Fixation indices (Within individuals)
F_{ST}: Fixation indices (Among individuals within populations)
Figure (1): Dendrogram Based Nei’s (1978) Genetic distance of three chicken strain produced by UPGMA clustering based on Nei’s genetic distance using 10 microsatellite loci.

REFERENCES


FAO (2004). Secondary guidelines for development of national farm


Diversity, heterozygosity, alleles, microsatellite markers


الملخص العربي
مقارنة تحليلية لجينوم الدجاج المصرى المحلى والمستنبط
3- التمييز بين سلالات الدجاج دقيق; والمنتزه الذهبي والمنتزه الفضي بواسطة الواسمات الوراثية

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1. معهد بحوث الانتاج الحيواني والبنك القومي للجينات - مركز البحث الزراعي
2. البنك القومي للجينات - مركز البحث الزراعي
3. معهد بحوث الانتاج الحيواني - مركز البحث الزراعي

تمثل هذه الدراسة تقييم التوصيف الوراثي في ثلاثة من السلالات المصرية وهي دقيق-4، المنتزه الذهبي و المنتزه الفضي. تم عزل مائه وخمسون عينة من الحاملды النووي من سلالات الدجاج الثلاثة. استخدمت العينات في سلسلة من تفاعلات البلمرة من عشرون من الواسمات الوراثية وقدر التنوع الوراثي بعد الاليات المشاهدة وبلغ عدد الاليات التي تم الكشف عنها 138 أليل. وعدد الاليات الفعال والملاحظ والمتوافق من Heterozygote و بلغ عد الاليات لكل سلاله 54 أليل. وكان متوقط الاليات لكل سلاله في كل المواقيت المدروسة يتراوح بين 4.1 للدقيق-4 و 5 للمنتزه الفضي. وكان عدد الاليات المحددة تراوح بين 5 في الدقيق-4 إلى 14 في المنتزه الفضي. وعدد الاليات الفعال تراوح بين 108 في موقع Heterozygote إلي 324، لموقع ADL43 إلي 2.176، لموقع MCW43 إلي 3.57، لكل من الدقيق-4، المنتزه الفضي على التوالي. وكان متوقط الاليات المحددة إلى 108، لموقع Heterozygote إلي 324، لكل من الدقيق-4، المنتزه الفضي على التوالي. وكان متوقط الاليات المحددة إلى 108، لموقع Heterozygote إلي 324، لكل من الدقيق-4، المنتزه الفضي على التوالي. واستنادا إلى ال Dendogram قدرت المسافة الوراثية وكانت بين الدقيق-4 والمنتزه الفضي أقرب منها إلى المنتزه الفضي. كشفت هذه الدراسة عن وجود تنواع وراثي في سلالات الجامد المصرية وأوضحت أن الكافلات الجزئية المستخدمة بностью المعلومات ويمكن استخدامها في الدراسات المستقبلية في عشائر الدجاج.