Clinical and molecular detection of *Sarcoptes scabiei* in the Iraqi camels

M.H. Al-Hasnawy¹, H.A. Aljabory² and L.S. Waheed²

¹Department of Parasitology, ²Department of Internal Preventive Medicine, College of Veterinary Medicine, Al-Qasim Green University, Babylon, Iraq

**Abstract**

*Sarcoptes scabiei var cameli* is the most frequent zoonotic species of mites causing mange in camels worldwide. The prevalence of camel’s mange in Iraq is still little studied. Thus, this research is conducted to detect *S. scabiei* in camels in the four provinces of the Middle-Euphrates area: Al-Muthanna, Al-Diwaniyah, Najaf, and Babil, from January 2020 to December 2020. The Molecular technique depending on the conventional polymerase chain reaction (cPCR) is performed for the direct detection of *S. scabiei* based on the mitochondrial cytochrome oxidase subunit 1 (*COX1*) gene from skin scrape lesion samples. The results reveal that 125 out of 425 samples (29.41%) of the examined camels are infested with *S. scabiei*. According to the sex of the infested animals, the infestation rate was higher in females than in males, 85 (30.91 %) and 40 (26.67%) respectively. In addition, the 1.5 year age shows the highest number of infestation (83 out of 85) with a percentage of 97.65%, but the percentages are 21 out of 60 (35%) and four out 68 (5.88%) in 2 and 7 years old animals, respectively. The results also record that infested animals found in Najaf and Al-Diwaniyah have the highest number of infestations, with of 36% and 35%, respectively. The findings also demonstrate that the highest infestation percentage is during the winter months (January and February), with of 92.31% and 80%, respectively. The sequencing and phylogenetic analysis shows that the local isolates of the Iraqi camels are consistent with the isolates recorded in China.

**Introduction**

One humped-camel (*Camelus dromedarius*) is one of the domestic animals with have economic importance in the world, including Iraq (1). They can be infected with various zoonotic parasitic diseases such as mange which considers a contagious skin disease (2). Some species of *Sarcoptus*, *Psoroptus*, *Chorioptus*, and *Demodex* can cause mange in camels (3,4). Sarcoptic mange (*Sarcoptes scabiei var cameli*) is considered the most frequently identified zoonotic species (5) and can commonly spread among animals, particularly in tropical and subtropical areas via direct physical contact with an infested animal and indirectly through physical objects in farms (3,6). *S. scabiei* is an obligate ectoparasite taxonomically belongs to the class Arachnida, subclass Acari, order Astigmata, and family Sarcoptidae (7,8).

This ectoparasite burrows into the skin and consumes the host epidermis, leading to itching, which is the main clinical sign accompanied by mange infestations (9). Other symptoms can also be observed in camel mange, such as: intense pruritis, dermatitis, and parakeratotic scaly crust formation (10,11). In some cases, mange causes fissures underlying the epidermis, leading to allergy-like reactions, intensely pruritic lesions and probably secondary bacterial infections (12,13). According to the documented data, the prevalence of mange mite infestation geographically differs from one country to another, where the percentage ranges...
between 2.3% to 97.4% (14). Previous studies using molecular tools have already been conducted to understand the epidemiology and molecular characteristics of mange isolates in different hosts (15,16). Nevertheless, the epidemiology and molecular identification of mange in Iraqi camels are still incompletely understood. This research has focuses on the clinical isolation and molecular detection of sarcoptic mange isolated from infestation camels.

Materials and methods

Ethical Approval
The ethical approval letter (No.11 in 04/12/2019) is given by Department of Internal Preventive Medicine/ College of Veterinary Medicine/Al-Qasim Green University to conduct this scientific work, and based on the present research design, data collection permit is orally taken from all owners before collection the samples during each visit to their living areas in /and around the four provinces covered in this study.

Animals of study
In order to investigate mange mites on local camels (S. scabiei) in the Middle- Euphrates area which includes Al-Muthaana, Al-Diwaniyah, Najaf, and Babil, 425 samples were collected from (males: 150, females: 275) frequent visits beginning from January 2020 until December 2020. The collecting samples included age groups ranging from 1.5 to 7 years.

Clinical inspection, sample collection, and isolation
During each visit, animals living in that areas under investigation were clinically examined, and then samples were taken from animals that suspect to be infested by sarcoptic mange. The collecting samples were kept in sterile containers and then transferred to a laboratory for further examination according to (17,18).

Molecular identification using the conventional polymerase chain reaction technique (cPCR)
This technique is performed for direct detection of S. scabiei based on mitochondrial cytochrome oxidase subunit 1 (COXI) gene from skin scrape lesion samples. This method is carried out at the College of Veterinary Medicine, Al-Qasim Green University, and carried out according to the method described by (19) as following steps:

DNA extraction
Genomic DNA from skin lesion samples were extracted by using gSYAN DNA mini kit extraction kit (Tissue protocol) Geneaid. U.S.A., and done according to company instructions

Genomic DNA estimation
The extracted genomic DNA from fecal samples is checked by using a Nanodrop spectrophotometer (THERMO, U.S.A.) in order to check and measure the purity of DNA through reading the absorbance at (260 /280 nm)

Specific primers preparation
A 570bp DNA fragment of S. scabiei COX-1 gene is designed in this study based on NCBI-Genbank (MF083742.1) (19) and primer 3 plus. The primers were provided by (Scientific Reseracher. Co. Ltd / Iraq) as follows; the forward primer is 5’-TCAGTTGTAACCAGCCATGC-3’ and the reverse primer is 5’- AATGTAAACTTCGGGTTCCA -3’.

PCR master mix preparation
The PCR master mix was prepared by using (GoTaqTM Green PCR Master Mix kit, Promega, U.S.A.). This master mix was used according to company instructions. The PCR master mix tubes were transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes, then placed in the PCR thermocycler. The PCR thermocycler conditions were as follows steps; initial denaturation at 95°C for 5min, followed by 35 temperature cycles, each cycle consists of denaturation at 95 °C for 30sec, annealing at 58 °C for 30sec, and extension at 72 °C for1min. After 35 cycles, there was a final extension at 72 °C for 5min. The amplified PCR product was analyzed by 1.5% agarose gel electrophoresis using ethidium bromide stain (10mg/ml) and then visualized by using U.V. Transilluminator.

DNA sequencing method
DNA sequencing method was performed for the genetic relationship analysis of COXI gene in local S. scabiei isolates as follows; the PCR product of COXI gene was shipped to Macrogen Company in Korea by DHL for performing the DNA sequencing using AB DNA sequencing system. The DNA sequencing analysis was done by the NCBI BLAST analysis, and the Phylogenetic tree analysis was conducted using Molecular Evolutionary Genetics Analysis version 6.0. (Mega X version) and Multiple sequence alignment analysis based on the ClustalW alignment analysis and the evolutionary distances were computed using the Maximum Composite Likelihood method by the phylogenetic tree UPGMA method, the identified S. scabiei isolates were submitted into NCBI-GenBank to get the Genbank accession number.

Statistical analysis
The data was statistically analyzed using the SPSS program (version 25). The chi-square test was applied to compare categorical variables, and the P Values < 0.05 were considered to be statistically significant.
Results

Gross inspection
The main clinical signs shown on the infested animals were itching, scratching the affected areas, and hairless spots on the infested skin. In severe cases, the skin became dry, gray, and thick, giving a wrinkled and crusted appearance. More importantly, all infested camels had weight loss and anemia.

Molecular study
The molecular diagnosis (cPCR) based on COX1 gene revealed that 125 out of 425 samples (29.41%) of examined camels were infested with *S. scabiei*. As shown in (Figure 1), the agarose gel electrophoresis technique clearly demonstrates the bands of the amplified DNA of *S. scabiei*.

![Figure 1: Agarose gel electrophoresis (1.5%) image based on the ethidium bromide shows the PCR product analysis of the S. scabiei COXI gene from the skin lesion scrappy camel’s samples. The Lane (M): DNA marker ladder (1500-100bp) and the Lane (1-8) show some positive PCR amplification of S. scabiei at 570bp PCR product size.](image)

Infestation rates according to sex
According to table 1, the infestation rate is high in females compared to males, 85 (30.91 %) and 40 (26.67%), respectively. The statistical analysis shows that there are no significant differences between infected males and females.

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. examined</th>
<th>No. (%) infested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>150</td>
<td>40 (26.67 %)</td>
</tr>
<tr>
<td>Females</td>
<td>275</td>
<td>85 (30.91 %)</td>
</tr>
<tr>
<td>Total</td>
<td>425</td>
<td>125 (29.41)</td>
</tr>
</tbody>
</table>

$\chi^2$ = 0.841  
$P$ value = 0.359

Infestation rates according to age
According to table 2, the results found that infested animals with age 1.5 years shows the highest number of infestation (83 out of 85) with of 97.65%. The rates of infestation gradually decrease in animals with age 2 and 7 years, recording 35% and 5.88% respectively. Statistically, there is significant differences among infected camels according to age.

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>No. examined</th>
<th>No. (%) infested</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>85</td>
<td>83 (97.65)</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>21 (35)</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>7 (12.73)</td>
</tr>
<tr>
<td>4</td>
<td>55</td>
<td>5 (9.09)</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>4 (7.41)</td>
</tr>
<tr>
<td>6</td>
<td>68</td>
<td>4 (5.88)</td>
</tr>
<tr>
<td>7</td>
<td>48</td>
<td>1 (2.08)</td>
</tr>
<tr>
<td>Total</td>
<td>425</td>
<td>125 (29.41)</td>
</tr>
</tbody>
</table>

$\chi^2$ = 14.702  
$P$ value = 0.002

Infestation rates according to area
In the table 3, the results illustrated that animals found in Najaf and Al-Diwaniyah had the highest number of infestations, followed by Al-Muthaana, recording 36%, 35%, and 28%, respectively. In contrast, the lowest number of infestations is in Babil with of 8%. Statistically, significant differences are noticed in the infected camels based on the examined areas. The current finding is associated with the presence of large number of camels living in desert areas of Al-Muthaana and Al-Diwaniyah, suggesting that crowding and climate may play a vital role in the high prevalence of infestation.

<table>
<thead>
<tr>
<th>Area</th>
<th>No. examined</th>
<th>No. (%) infested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Muthaana</td>
<td>250</td>
<td>70 (28)</td>
</tr>
<tr>
<td>Al-Diwaniyah</td>
<td>100</td>
<td>35 (35)</td>
</tr>
<tr>
<td>Najaf</td>
<td>50</td>
<td>18 (36)</td>
</tr>
<tr>
<td>Babil</td>
<td>25</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Total</td>
<td>425</td>
<td>125 (29.41)</td>
</tr>
</tbody>
</table>

$\chi^2$ = 14.702  
$P$ value = 0.002

Infestation rates according to months of the year
The table 4 reveals that the highest infestation percentage is in the winter months (January and February) with of 92.31% and 80%, respectively compared with other months.
of the year. At the same time, the lowest is in October 2.27%. The finding also reports that no infestation during the months (July, August, and September), suggesting that the infestation with mange could increase with low temperatures and high humidity. The statistical analysis shows significant differences among the infected camels according to months of the year.

Table 4: The number and percentage of S. scabiei -infested camels according to months of the year 2020

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>No. examined</th>
<th>No. (%) infested</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>65</td>
<td>60 (93.31)</td>
</tr>
<tr>
<td>February</td>
<td>50</td>
<td>40 (80)</td>
</tr>
<tr>
<td>March</td>
<td>25</td>
<td>12 (48)</td>
</tr>
<tr>
<td>April</td>
<td>25</td>
<td>4 (16)</td>
</tr>
<tr>
<td>May</td>
<td>30</td>
<td>2 (6.67)</td>
</tr>
<tr>
<td>June</td>
<td>25</td>
<td>1 (4)</td>
</tr>
<tr>
<td>July</td>
<td>30</td>
<td>0 (0)</td>
</tr>
<tr>
<td>August</td>
<td>30</td>
<td>0 (0)</td>
</tr>
<tr>
<td>September</td>
<td>32</td>
<td>0 (0)</td>
</tr>
<tr>
<td>October</td>
<td>44</td>
<td>1 (2.27)</td>
</tr>
<tr>
<td>November</td>
<td>30</td>
<td>2 (6.67)</td>
</tr>
<tr>
<td>December</td>
<td>39</td>
<td>3 (7.69)</td>
</tr>
<tr>
<td>Total</td>
<td>425</td>
<td>125 (29.41)</td>
</tr>
</tbody>
</table>

\[
\chi^2 = 277.344 \\
P \text{value} = 0.000^* 
\]

Sequencing and phylogenetic analysis

The comparative analysis for the nucleotides sequence of COXI gene for local S. scabiei isolates with the S. scabiei isolates existed in GenBank database was constructed using the ClustalW Alignment tool of MEGA-X software. Two out of 125 S. scabiei positive samples were sequenced with specific primers using AB DNA sequencing system. The sequence representatives for each identified isolate were submitted to the GenBank/EMBL/DDBJ database under the accession numbers OK510217.1 and OK510218.1 (Figure 2).

Figure 2: Multiple sequence alignment analysis of mitochondrial cytochrome oxidase subunit I (COXI) gene partial sequence in local S. scabiei (IQ-No.1 and IQ-No.2) and NCBI-Genbank related S. scabiei related isolates. The multiple alignment analysis is constructed using the NCBI BLAST alignment tool and shows the nucleotide alignment similarity as (*) and substitution mutations in mitochondrial cytochrome oxidase subunit I (COXI) gene.

Table 5: the NCBI-BLAST Homology Sequence identity (%) between local S. scabiei isolates and NCBI-BLAST submitted related S. scabiei China isolate

<table>
<thead>
<tr>
<th>Sarcoptes scabiei</th>
<th>Genbank Accession number</th>
<th>NCBI-BLAST homology sequence identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IQ-No.1</td>
<td>OK510217.1</td>
<td>No. Mutation Type of mutation Polymorphism Identity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>IQ-No.2</td>
<td>OK510218.1</td>
<td>2</td>
</tr>
</tbody>
</table>
Figure 3: Multiple sequence alignment analysis S. scabiei (COXI) gene in local S. scabiei camel isolate IQ-No.1 (OK510217.1) and NCBI-Genbank S. scabiei genotypes.

Figure 4: Multiple sequence alignment analysis S. scabiei (COXI) gene in local S. scabiei camel isolate IQ-No.2 (OK510218.1) and NCBI-Genbank S. scabiei genotypes.

Discussion

The gross and clinical examination of infested camels show that the animals were uncomfortable, suffering from itching and scratching accompanied with other clinical pathogenic signs such as weight loss and anemia (20). In addition, the infested skin was hairless spots, gray and thick in severe cases. Interestingly, it is mentioned that the mange of camels can predominantly be identified based on clinical signs (21). Therefore, these observations are consistent with the clinical signs of mange caused by S. scabies, where they agreed with other previous studies (21-24). The molecular diagnosis (cPCR) based on COXI shows that 29.41% of the examined camels were infested with S. scabiei. This gene is previously used in some studies to identify the S. scabiei parasite in different animals (23-25). The current finding agrees with a study done in Najaf abattoir and nomadic areas for detecting sarcoptic mange in camels, where the percentage was 25.9% (25). It is also similar to an epidemiological study in Ethiopia which recorded 31.5% of mange in camels caused by sarcoptic mites (26). Compared to the current findings, small increase in infection rates is shown in camels infected by Sarcoptes scabiei var. camel in Pakistan, recording 42.22% (27). Most recently, this parasite is also found in cows of Anbar province, Iraq, recording 37.5% which tends to be in agreement with the present study (28). However, the current percentage is high compared to a previous study which are carried out in three governorates;
Al-Qadisiya, Al-Najaf and Al-Muthanna in Iraq (29) and Saudi Arabian camels (30). This variation in infestation rates can be attributed to the virulence of mange type infested camels. Thus, the current study focused on scabetic mange, which is the most prevalent and serious mange infests camels (31).

Regarding the sex of infested camels, the infestation rate is high in females compared with males. This finding is in agreement with previous studies (32-35). However, other studies show that the highest infestation rate is in males compared to females (36,37). This variation may be ascribed to the number of examined males in the current study or breeding behavior in different areas. It is known in our area that owners have used a small number of males in the herd compared to females. Additionally, it was suggested that females might be more susceptible to infestation due to some hormonal influences such as: prolactin and progesterone (38). According to the age of the infested animals, the results find that infested the highest infestation rate is in young camels while the percentages rates gradually decrease in old animals. These results agree with the previous studies, which show that young camels are more susceptible to infestation than older camels (26,39,40). However, the current study does not agree with a previous study which found that the high infestation rates of Demodex mites were in the ages between 5 to 10 years (29), as well as in Egypt (34) who showed that the highest rate of infestation in camels was in the age over 2 years. This variation with the current findings is attributed to be ascribed to the number of examined samples of each age and the technique used for detecting the infestation. As for the infestation rates according to the area, the findings illustrate that camels live in Najaf and Al-Diwaniyah had the highest number of infestations, followed by Al-Muthanna. In contrast, the lowest number of infestations was in Babil. The current findings might be associated with the presence the large number of camels living in the desert areas of Al-Muthanna and Al-Diwaniyah, suggesting that crowding and climate could play a vital role in the high prevalence of infestation. When looking at the prevalence of the infestation rates throughout the year, the highest infestation percentage was in winter months while no infestation was recorded during the summer months, suggesting that the infestation with mange could increase with low temperatures and high humidity. The current result is consistent with a recent study in Egypt which found that the highest infestation rates were recorded in the winter season (34). Other previous studies also agree with our finding, showing that the infestation was prevail more in winter than in summer months (39). Nevertheless, an acute form of Sarcoptes infestation in the camels of Cholistan Desert, Pakistan, was observed in the hot and rainy periods (40). This difference with the present findings may be associated with climate circumstances and the topography of each area.

Furthermore, sequencing and phylogenetic analyses were carried out in the current study based on the comparative analysis of the nucleotides sequence of COXI gene for local S. scabiei isolates with the S. scabiei isolates GenBank database. Notably, the sequencing of this gene was previously used for detecting Sarcoptes spp and analyzing the phylogeny of the parasite (23, 25). The NCBI-BLAST Homology Sequence Identity (%) of local S. scabiei isolates IQ-No.1 (OK510217.1), and IQ-No.2 (OK510218.1) reveals that the nucleotide alignment similarity is identical to the available GenBank sequences for S. scabiei China isolates with the accession number (KJ499544.1). Nevertheless, the NCBI-BLAST Homology Sequence Identity between local S. scabiei isolates and NCBI-BLAST submitted related S. scabiei China isolates show the substitution mutations in COXI gene. Thus, further research can be useful to analyze other isolates in our areas in order to identify and understand the relationship among them and their similarity or differences with the local or international isolates.

Conclusion

To sum up, the gross observations recorded that infested animals were uncomfortable, suffering from itching and scratching the affected areas with metallic parts. Their infested skin showed hairless spots and pain. In severe cases, the skin becomes dry, gray, and thick, giving a wrinkled and crusted appearance. The molecular technique based on the conventional PCR shows that 29.41% of the examined camels were infested with S. scabiei. The infestation rate was high in females compared with males. In addition, the young camels showed the highest number of infestations, while the lowest percentages were in old animals. The results also record that the infested animals in Najaf and Al-Diwaniyah had the highest number of infestations. The finding also demonstrates that the highest infestation percentage was in the winter months, while no infestation was found in the summer months. The sequencing and phylogenetic analysis based on the COXI gene identifies that the local isolates of the Iraqi camels are consistent with isolates recorded in China. However, two substitution mutations were also noticed in these isolates, suggesting that more studies are needed to investigate the phylogeny of S. scabiei and other species in the Iraqi camels.

Acknowledgment

The authors are thankful to College of Veterinary Medicine at Al-Qasim Green University for the facilities provided to complete this work.

Conflicts of interest

The authors declare no conflicts of interest.
References


الكشف السريري والجزيئي لطفيلي القارمة الجربية في الجمال العراقية

محمد هادي الحسناوي١، حامد عيسى حسن الجبوري٢
ولينا شهيد وحيد٢

١ فرع الطفيلي، ٢ فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة القاسم الخضراء، بابل، العراق

الخلاصة

يعتبر طفيلي القارمة الجربية أو سوس الحكة من أكثر أنواع الطفيليات شيوعاً بالعالم والتي تسبب الجرب في الجمال. ولا تزال إحصائية انتشار جرب الحمام في العراق غير مروسة بشكل كامل، لذا اجريت الدراسة الحالية للكشف عن طفيلي الساركتي في الجمال المنتشر في أربع مراكز في منطقة الفرات الأوسط وهي: المثنى والقادسية والنجف وبابل، ابتداء من كانون الثاني 2020 إلى نهاية كانون الأول 2020. اعتمدت الدراسة التقنية الجزيئية المعتمدة على تفاعل سلسلة البلمرة التقليدي للكشف المباشر عن الطفيلي المعزول من مسحات جلد الحمام واعتماداً على جين يدعى الورقة الفرعية (كوكس 1). تظهر النتائج ان 125 حيوان (29.41%) كان مصاباً بطفيلي ساركتي. كما تبين النتائج حسب جنس الحيوان أن معدل الإصابة كان مرتفع في الإناث مقارنة مع الذكور حيث سجلت نسب اصابة 30.91% و 26.67% على التوالي. بالإضافة إلى ذلك، تشير النتائج أن نسبة الإصابة كانت أيضاً مرتفعة في الأعمار سنة ونصف 37.45%، بينما كانت النسبة في الأعمار سنين وسبع سنوات هي 35.88%، على التوالي. كما توضح الدراسة الحالية أن أعلى نسبة كانت في مدينة النجف والديوانية 36% و 35% على التوالي بالنسبة إلى انتشار الإصابة حسب الوقفة الجغرافية. كما تبين النتائج أن أعلى نسبة أصابة سجلت في شهر قيفر الشاه (كانون الثاني وشباط) 19.73% و 80% على التوالي. كذلك تشير نتائج تقنية التسلسل وتحليل الشيوع والتطور لجنس الطفيلي المستخدمة في هذه الدراسة إلى أن العزلات المحلية الحالية كانت متوافقة ومتماثلة مع العزلات الموجودة في الصين.