Molecular identification of Theileria species in cattle in Mosul city

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Abstract

This study showed that total percentage of infection with Theileria species in 100 blood samples of cattle in Mosul City was 38% with no significant differences between male and female of cattle. Cattle older than 1 year old had a high percentage of Theileria infection 40% compared to younger cattle 34.28%, with no significant variations in Theileria infection and age groups of cattle. Theileria measurement in red blood cells ranged from 0.5 to 2 microns, while parasitemia levels ranged from 0.2 to 15%, with an average of 6.572%. The results of molecular diagnosis confirmed the detection Theileria genus, Theileria annulata, and Theileria parva in blood samples of examined cattle and PCR revealed that the amplification products were 237bp, 690bp, and 700bp, respectively. In this study, we recorded new strains of Theileria in cattle of the Nineveh governorate by using the BLAST program consisting of three strains of T. annulata, one strain of Theileria ovis and two strains of Theileria parva which differs from the normal isolates in many nucleotides in different worlds. The phylogenetic tree showed the relative relation among the Theileria spp, the results matching species between 8 and 11, 1 and 4, 6 and 10, 3 and 9, and 2 and 12; the species 7 were more closely linked to 11 and 8, the species five similar to 6 and 10.

Introduction

Regarding meat and milk production, cattle and buffaloes are among the vital components of the livestock industry. Numerous infections that endanger these animals’ health are exposed to them, including theileriosis (1-4). Theileria is a genus of intracellular protozoan parasites in agricultural animals worldwide, particularly in the Middle East (5). Both domestic and wild cattle are affected by this substance. East Coast Fever (ECF) and Tropical Theileriosis (TT) are caused by the two most significant and pathogenic species, T. parva and T. annulata respectively (6). Thielarria can be transmitted trans-stadially by the tick vectors, mechanically through routine rearing practices, Rhipicephalus appendiculatus, a hard tick’s vector for Theileria parva, and Hyalomma ticks, a vector for Thielarria annulata. Both disseminate Thielarria species (7,8). Pasture tick infestations can last up to two years depending on the weather. Skin injury and blood loss are the results. Meanwhile, the parasite prevents the animal from receiving nutrients by infecting the red blood cells of vertebrates, including pets (9). Theileriosis in cattle typically manifests as pale mucous membranes, depression, lethargy, lack of appetite, fever, widespread lymphadenopathy, anorexia, loss of condition, collapse, and, in some cases, death if the animal is made to move or run. Pregnant cows frequently experience miscarriages and stillbirths, or their milk supply may decrease while their somatic cell count rises in dairy cows (9). Thielarria and Babesia are closely related. However Thielarria differs from them in that it has a leukocyte developmental stage profile before infecting erythrocytes (10). Thielarria infection decreases meat and milk production, causes several problems, and may increase mortality rates from an economic standpoint (11,12). For instance, 800 million dollars was expected to be lost annually in India’s economy (9). The definitive diagnosis is reached by combining of appropriate clinical signs and lab examinations. Thielarria parasites found in blood smears and a needle biopsy of a lymph node stained with the Giemsa stain are typically used to diagnose. Since most Thielarria form piroplasm is
physically identical and schizonts are not always present in the superficial lymph nodes during illness cycles, type-specific detection is challenging. Sometimes used to detect *Theileria* spp., PCR assays plus a DNA probe are highly sensitive for detecting even low infection levels (13-15).

The study aimed to use microscopic and molecular diagnosis (Polymerase Chain Reaction, Sequencing, Phylogenic study) to know *Theileria* species affecting cattle in order to control the disease due to the lack of studies on bovine *Theileria* parasite in Nineveh Governorate, as well as to find out, record the species of the genus, that microscopic differentiation is difficult between species, as well as with other species of genus *Babesia*.

**Materials and methods**

**Ethical approve**

This study has been approved by the scientific committee of department of Microbiology, Collage of Veterinary Medicine, University of Mosul at the first congress, dated 4/10/2021.

**Blood samples collection**

From cattle showing signs of theileriosis in various locations throughout the Nineveh Governorate, 100 blood samples were randomly selected from both sexes and different age groups. Blood samples were drawn from the jugular vein using sterile syringes and 70% ethyl alcohol to sterilize the region. The blood samples were kept in EDTA-containing tubes, and each sample's collection date, age, and sex were noted. The samples were then brought to the College of Veterinary Medicine/University of Mosul's Parasitological Laboratory for a laboratory analysis.

**Microscopic examination**

Thin blood smears were prepared and stained with Giemsa stain at a concentration of 5% for 30 to 60 minutes and were viewed under a light microscope. Thin blood smears were utilized to determine the shape and measurement requirements for laboratory evaluation to diagnose the *Theileria* parasite in cattle (16). The following formula was used to get the parasitism percentage: Number of affected RBCs/numbers of calculated RBCs*100.

**DNA extraction from blood**

DNA was extracted from blood samples containing *Theileria* using a DNA extraction kit (Qiagen). In order to rehydrate the DNA pellet, 100µl of rehydration solution was added, and it was then stored at -20°C until the next test.

**Polymerase chain reaction (PCR)**

PCR was done to confirm the diagnosis of *Theileria* spp. by using the primers (Table 1).

The PCR reaction mixtures were created in 20 µl containers with 10 µl of Master mix (Promega 2X), 1 µl of each primer, 4 µl of DNA template, and 4 µl of PCR-grade water. The multiplication reaction was performed using the custom program, as stated in table 2, after the PCR was completed using a thermocycler (Optimum 96 G Germany).

### Table 1: Types of primers and sequences of nucleotides of primers used for diagnosis of *Theileria* genus, *Theileria annulata* and *Theileria parva* by using PCR technique

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Target gene</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Theileria</em> genus 18s rRNA-F</td>
<td>GGTAATTCCAGCTCCAAATAG</td>
<td>18s rRNA</td>
<td>17</td>
</tr>
<tr>
<td><em>Theileria</em> genus 18s rRNA-R</td>
<td>ACCACCAAAATAGAACCAAGTC</td>
<td>Major merozoite</td>
<td>13</td>
</tr>
<tr>
<td><em>Theileria annulata</em>-F</td>
<td>GTAACCGTTAAAAACGT</td>
<td>surface antigen DNA</td>
<td>13</td>
</tr>
<tr>
<td><em>Theileria annulata</em>-R</td>
<td>GGTACGAACATGGGTTT</td>
<td>Bovine cytochrome b</td>
<td>18</td>
</tr>
<tr>
<td><em>Theileria parva</em>-F</td>
<td>ATTTAAGGAACCTGACGTGACTGC</td>
<td>gene</td>
<td>18</td>
</tr>
<tr>
<td><em>Theileria parva</em>-R</td>
<td>TAAGATGCCGACTATTAATGACACC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Steps of a conventional PCR program

<table>
<thead>
<tr>
<th>Stage</th>
<th>°C</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>95</td>
<td>6 min.</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95</td>
<td>45 sec.</td>
</tr>
<tr>
<td>Annealing</td>
<td>55</td>
<td>1.0 min.</td>
</tr>
<tr>
<td>Extension</td>
<td>72</td>
<td>1.0 min.</td>
</tr>
<tr>
<td>Final extension</td>
<td>72</td>
<td>5 min.</td>
</tr>
</tbody>
</table>

Electrophoresis separated the amplified products on 2% agarose gel in a 4 µl red safe. Each PCR result was placed into the agarose gel's well in a 4µl volume. The electrophoresis was performed using a power supply with 1X TBE buffer at 60 V for 45 minutes. The typical molecular marker was a 100 bp DNA marker (Biolaps), and 4µl of the gel was looked at using UV light (Gel Do cumintation).

**Determination the nucleotide**

*Theileria*’s nitrogenous base sequences were performed by the Hitachi Genetic Analyzer 3130 (Japan) and matched with NCBI using the BLAST tool for 12 positive samples.

**Results**

Giemsa staining 100 blood smears of cattle, and 38 (38%) tested positive for *Theileria* species (Table 3). Males exhibit
significantly higher positive blood test results for *Theileria* than females (40 versus 35), with no discernible variations between the sexes (Table 4). *Theileria spp.* infection in the same litters did not significantly differ between males and females. Cattle older than one year old had a steady percentage of *Theileria* infection 40% compared to younger cattle 34.28%, with no discernible variations in *Theileria* infection and age groups of cattle (Table 5).

Table 3: Number of cattle investigated, quantity of cattle infected with *Theileria* species, and proportion of infection determined by Giemsa stain

<table>
<thead>
<tr>
<th>Examined (n)</th>
<th>Infected (n)</th>
<th>% Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>38</td>
<td>38</td>
</tr>
</tbody>
</table>

Table 4: Interdependence of infection with *Theileria* and sex of animals

<table>
<thead>
<tr>
<th>Sex</th>
<th>Examined (n)</th>
<th>Infected [n(%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>40</td>
<td>14(35)a</td>
</tr>
<tr>
<td>Male</td>
<td>60</td>
<td>24(40)a</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>38</td>
</tr>
</tbody>
</table>

Similar letters mean that there is no significant difference in the percentage of infection with *Theileria* and the sex of the cattle.

Table 5: Correspondence of infection with *Theileria* and age of animals

<table>
<thead>
<tr>
<th>Age of animals</th>
<th>Examined (n)</th>
<th>Infected [n(%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less one years</td>
<td>35</td>
<td>12(34.28)a</td>
</tr>
<tr>
<td>1-2 years</td>
<td>40</td>
<td>16(40)a</td>
</tr>
<tr>
<td>&gt; 2 years</td>
<td>25</td>
<td>10(40)a</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>38</td>
</tr>
</tbody>
</table>

Similar letters mean that there is no significant difference in the percentage of infection with *Theileria* and the age of the cattle.

Blood smear microscopy analysis revealed the presence of intra-erythrocytic forms of *Theileria* species, including rod, round, oval, comma, ring-shaped, and organisms that resemble *Anaplasma* (Figure 1). *Theileria*’s length in red blood cells ranged from 0.5 to 2 microns, while parasitemia levels ranged from 0.2 to 15%, with an average of 6.572%.

The bands of DNA extracted from positive blood samples for *Theileria* species in a 25 ng/µl concentration. The concentration of extracted DNA was 50-100 ng with a purity of 1.7. The polymerase chain reaction results suggested that the DNA samples that were extracted and used in this reaction might be used to diagnose *Theileria* genus, *Theileria annulata*, and *Theileria parva*. PCR revealed that the amplification products were 237bp, 690bp, and 700bp, respectively (Figures 2-4). Ten *Theileria annulata* isolates and one *Theileria ovis* isolate’s genetic sequences were documented. According to table 6, the International Genbank’s website provided the sequence numbers (Table 6).

The six new isolates differ from the normal isolate in many nucleotides in different worlds, according to the sequencing results, and the new isolates of *Theileria* in cattle of the Nineveh governorate were reported using the BLAST tool consisting of three isolates of *Theileria annulata*, one isolate of *Theileria ovis* and two isolates of *Theileria parva* (Table 7).

Figure 1: *Theileria spp.* were found in intra-erythrocytic (rod-shaped, circular, comma-shaped) in thin blood smears (Giemsa-stained 100X by using a digital camera).

Figure 2: PCR reaction of *Theileria* genus with a reaction product of 237 bp.

Figure 3: The PCR reaction of the parasite, *Theileria annulata*, with a reaction product of 690 bp.

Figure 4: The PCR reaction of the, *Theileria prava*, with a reaction product of 700 bp.
Table 6: Determination of genetic diversity of *Theileria* spp. Parasite identified in bovine blood samples

<table>
<thead>
<tr>
<th>Name of gene</th>
<th>Genetic diversity</th>
<th>nucleotides</th>
<th>GenBank number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Theileria annulata</strong> clone Gxyl small subunit ribosomal RNA gene, partial sequence.</td>
<td>TTCTGCTGCAATGCTGTTGTCCTCTCGGAGTGCATGTGGCATTTCGCCAGGAGCTTTGCTGTTAGTGAATAAAGTGAGACATTTTGGTGTGTGTA</td>
<td>430</td>
<td>MK089831.1</td>
</tr>
<tr>
<td><strong>Theileria annulata</strong> isolate IQ-Camel NO.10 small subunit ribosomal RNA gene, partial sequence.</td>
<td>CTGCTGCAATGCTGTTGTCCTCTCGGAGTGCATGTGGCATTTCGCCAGGAGCTTTGCTGTTAGTGAATAAAGTGAGACATTTTGGTGTGTGTA</td>
<td>461</td>
<td>MT491139.1</td>
</tr>
<tr>
<td><strong>Theileria annulata</strong> isolate IQ-camel No.7 small ribosomal RNA gene - partial sequence</td>
<td>TTCTGCTGCAATGCTGTTGTCCTCTCGGAGTGCATGTGGCATTTCGCCAGGAGCTTTGCTGTTAGTGAATAAAGTGAGACATTTTGGTGTGTGTA</td>
<td>455</td>
<td>MT491136.1</td>
</tr>
<tr>
<td><strong>Theileria annulata</strong> isolates IQ-camel No.5 small subunit ribosomal RNA gene - partial sequence</td>
<td>TTGNNNTTCTCTGCTGCATTGCTGTTGTCCTCTCGGAGTGCATGTGGCATTTCGCCAGGAGCTTTGCTGTTAGTGAATAAAGTGAGACATTTTGGTGTGTGTA</td>
<td>461</td>
<td>MT491134.1</td>
</tr>
<tr>
<td><strong>Theileria annulata</strong> isolates IQ-camel No.4 small subunit ribosomal RNA gene - partial sequence</td>
<td>GGCTGANTTNGATT TTCTGCTGCATTGCTGTTGTCCTCTCGGAGTGCATGTGGCATTTCGCCAGGAGCTTTGCTGTTAGTGAATAAAGTGAGACATTTTGGTGTGTGTA</td>
<td>439</td>
<td>MT491133.1</td>
</tr>
<tr>
<td><strong>Theileria annulata</strong> small subunit ribosomal RNA gene - partial sequence</td>
<td>GGCTGANTTNGATT TTCTGCTGCATTGCTGTTGTCCTCTCGGAGTGCATGTGGCATTTCGCCAGGAGCTTTGCTGTTAGTGAATAAAGTGAGACATTTTGGTGTGTGTA</td>
<td>468</td>
<td>MT491131.1</td>
</tr>
<tr>
<td><strong>Theileria annulata</strong> small subunit ribosomal RNA gene - partial sequence</td>
<td>GGCTGANTTNGATT TTCTGCTGCATTGCTGTTGTCCTCTCGGAGTGCATGTGGCATTTCGCCAGGAGCTTTGCTGTTAGTGAATAAAGTGAGACATTTTGGTGTGTGTA</td>
<td>1679</td>
<td>MT26171.1</td>
</tr>
<tr>
<td><strong>Theileria annulata</strong> small subunit ribosomal RNA gene - partial sequence</td>
<td>GGCTGANTTNGATT TTCTGCTGCATTGCTGTTGTCCTCTCGGAGTGCATGTGGCATTTCGCCAGGAGCTTTGCTGTTAGTGAATAAAGTGAGACATTTTGGTGTGTGTA</td>
<td>1679</td>
<td>MT341858.1</td>
</tr>
<tr>
<td><strong>Theileria ovis</strong> 18S ribosomal RNA gene, complete sequence</td>
<td>GGCTGANTTNGATT TTCTGCTGCATTGCTGTTGTCCTCTCGGAGTGCATGTGGCATTTCGCCAGGAGCTTTGCTGTTAGTGAATAAAGTGAGACATTTTGGTGTGTGTA</td>
<td>1764</td>
<td>MT260171.1</td>
</tr>
<tr>
<td><strong>Theileria annulata</strong> isolate T178 small subunit ribosomal RNA gene - partial sequence</td>
<td>GGCTGANTTNGATT TTCTGCTGCATTGCTGTTGTCCTCTCGGAGTGCATGTGGCATTTCGCCAGGAGCTTTGCTGTTAGTGAATAAAGTGAGACATTTTGGTGTGTGTA</td>
<td>1056</td>
<td>MT31816.2</td>
</tr>
<tr>
<td><strong>Theileria annulata</strong> isolate T179 small subunit ribosomal RNA gene - partial sequence</td>
<td>GGCTGANTTNGATT TTCTGCTGCATTGCTGTTGTCCTCTCGGAGTGCATGTGGCATTTCGCCAGGAGCTTTGCTGTTAGTGAATAAAGTGAGACATTTTGGTGTGTGTA</td>
<td>1056</td>
<td>MT318159.1</td>
</tr>
</tbody>
</table>
Table 7: Thieleria new isolates from cattle blood samples diagnosed by (NCBI) according to the BLAST program

<table>
<thead>
<tr>
<th>No. of Thieleria isolate in NCBI</th>
<th>Name of isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC714837.1</td>
<td>Theileria annulata EM1 gene for 18S ribosomal RNA, partial sequence</td>
</tr>
<tr>
<td>LC714839.1</td>
<td>Theileria annulata EM3 gene for 18S ribosomal RNA, partial sequence</td>
</tr>
<tr>
<td>LC714842.1</td>
<td>Theileria ovis EM6 gene for 18S ribosomal RNA, partial sequence</td>
</tr>
<tr>
<td>LC714838.1</td>
<td>Theileria annulata EM2 gene for hypothetical protein, partial sequence GenBank</td>
</tr>
<tr>
<td>LC714841.1</td>
<td>Theileria parva EM5 gene for 18S ribosomal RNA, partial sequence:</td>
</tr>
<tr>
<td>LC211084.1</td>
<td>Theileria parva Ahlam-E-H gene for SSUrRNA, partial sequence</td>
</tr>
</tbody>
</table>

The phylogenetic tree showed the relative relation among the twelve Thieleria isolates from cattle, the results matching species between Thieleria annulata (8 and 11), Thieleria annulata (1 and 4), Thieleria annulata (6 and 10), Thieleria annulata (3 and 9), and Thieleria annulata 2 and Thieleria ovis 12, the isolate Thieleria annulata 7 were more closely linked to isolate Thieleria annulata 11 and 8, the isolate Thieleria annulata five similar to isolate Thieleria annulata (6 and 10) (Figure 5).

Figure 5: Phylogenetic tree analysis from Thieleria spp.

Discussion

Although the clinical indicators and blood smear examination are frequently accurate in detecting parasitic infection, the disease cannot be accurately diagnosed with these techniques due to their insufficient sensitivity. Using Giemsa-stained blood smears, the current investigation found that 38% of the investigated animals were positive for the Thieleria species. This result was in accordance with Kundave et al. (19), who discovered that the percentage of Thieleria infection by utilizing Giemsa staining thin blood smear was 30.98%, while Yaghfouri and Gh (8), El-Dakhly et al. (20), Rad et al. (21) and Tookhy et al. (22) recorded lower percentages of infection by Giemsa staining method, namely 22%, 12.93%, 9.31%, and 3.75%. Another study stated that 4% and 37% of cattle in the Herat region had Thieleria spp. (23). Higher infection rates may be connected to early acute stage microscopic examination making it simple to find both intracellular piroplasms and intralymphatic Koch's blue bodies (21,24).

In our study, the proportion of Thieleria infection appeared to be high prevalence in cattle aged 1-2 years and cattle aged two years was 40%. This result was consistent with findings from studies (20,25), which found that T. annulata infection was more common in adult cattle, while Yaghfouri and Gh (8), and Durrani (26) were concerned that the high occurrence of Thieleria was seen in animals under a year old, these findings conflicted with them. The physiological aspects, such as oestrus, were disclosed by Durrani (26) and Morzaria (27). Additionally, the antibodies to Thieleria that protect the calves against Thieleria infection may be the reason for the low incidence of disease in calves under one year old (28) due to pregnancy and lactation's temporary reduction of immunity and the increase in the percentage of infection in adult cattle.

A more significant percentage of Thieleria species infection was identified in the current study in female cattle than in male cattle, which was in keeping with Ayadi et al. (14), Inci et al. (29) and Kamani et al. (30). According to Al-Saeed et al. (13), females have higher hormonal swings and a relatively weaker immune system, which enhances the likelihood of disease. Additionally, because female cattle are kept for various functions, including reproduction and milk production, and because they spend much time in the meadows, Kamani et al. (30) found that tick-borne diseases in female cattle occur at a high incidence.

Thieleria annulata and Thieleria parva were diagnosed using primers developed based on Al-Saeed et al. (13), Odongo et al. (17), and Al-Hosary et al. (18) which yielded a base pair of 237, 690, and 700. This study's findings in diagnosing the genus Thieleria, Thieleria annulata, and Thieleria parva coincided with the investigations of Lempercur et al. (5), Yaghfouri and Gh(8), d'Oliveira et al. (12), Silatsa et al. (31), Khatoon et al. (32) and Al-Shabban and Alfatlawi (33). PCR technique is one of the most sensitive and specific methods for determining the pathogens (34-36) and using microscopic analysis of blood smears stained with Giemsa, is not sufficient for detecting species of Thieleria that are challenging to discriminate and define the species of Thieleria (37-39).

Phylogenetic research helps establish the basis for genetic differences and evolutionary relationships between
parasite species. Genetic diversity in parasites is crucial for establishing control strategies like medication treatment and immunization and parasite species (40). Each species might have a unique nucleotide, because the environment impacts it. As a result, the species can adapt to shifting environmental factors. The sequencing results demonstrated the discovery of new isolates, detected for the first time and differ from typical isolates in many nucleotides. The organisms adjust to this situation by mutating to build new genes for proteins, which helps them endure the harsh conditions and prepare for development. The nucleotides change may be caused by environmental causes. When an organism is isolated from its environment, it often responds to the conditions. In these cases, the organism may undergo mutations that affect one or more nucleotides, depending on the triple sequence of the protein molecule, or that affect amino acids with more than one code.

Conclusion

PCR technique is one of the most sensitive and specific methods for determining the *Theileria* and using microscopic analysis of blood smears stained with Giemsa, is not sufficient for detecting species of *Theileria* that are challenging to discriminate and define the species of *Theileria*. In this study new isolates of *Theileria* in cattle of the Nineveh governorate were reported by using the BLAST tool consisting of three isolates of *Theileria annulata*, one isolate of *Theileria ovis* and two isolates of *Theileria parva*

Acknowledgments

The authors like to thank department of microbiology, college of Veterinary Medicine, University of Mosul for their effort and support given to the current study.

Conflict of interest

The authors confirm no conflicts of interest in the publication of this paper.

Reference

التحديد الجزيئي لأنواع الثايليريا في الأبقار في مدينة الموصل

هيثم صديق البكري وإيمان غانم سليمان و أحلام فتحي الطائي

فرع الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

أظهرت النتائج الأولية أن نسبة الإصابة بثايليريا في الأبقار في مدينة الموصل كانت 27.8٪ مع عدم وجود فروق معنوية بين الذكور والإناث. الآفات التي يزيد عمرها عن سنة واحدة لديها نسبة أعلى من الإصابة بالثايليريا 40.4٪ مقارنة بالثوان البالغة 24.1٪، مع عدم وجود اختلافات كبيرة بين الانترتهايما الشمالية والشمال الشرقية. تراوحت كيماويات الثايليريا في خلال الدم الحمراء بين 2.0-0.02 ميكرون، بينما تراوحت مستويات التطفل بين 0.2-0.002 ميكرون، 15٪ من الأبقار كانت ضحايا للثايليريا. تمت تفتيشيا نتائج التشخيص الجزيئي تتشخيص كل جنس الثايليريا والثايليريا الحلقية وثايليريا بارفا في عينات الدم الأبقار المحفوظة، أظهرت نتائج الاستدامة تتشخيص تفاعلات الغرث للمستقبلات الفيروكسيما في الخلايا الوراثية:

1. في عينات الدم من البقار في مدينة الموصل كانت نسب الإصابة البالغة 24.0٪ والذي يزيد عمرها عن سنة واحدة. 2. تمت تفتيشيا نتائج التشخيص الجزيئي تتشخيص كل جنس الثايليريا والثايليريا الحلقية وثايليريا بارفا في عينات الدم الأبقار المحفوظة، أظهرت نتائج الاستدامة تتشخيص تفاعلات الغرث للمستقبلات الفيروكسيما في الخلايا الوراثية:

2. التحليل الجزيئي

3. تأثيرات على الأبقار في مدينة الموصل

4. ملاحظات

5. التوصيات

6. الإطار الزمني

7. التوصيات النهائية

8. استنتاج

9. الاتجاهات المستقبلية

10. مراجعات

التفصيل

11. تفاصيل

12. المستندات

13. المراجع

14. القواعد

15. التصنيف

16. الخاتمة

17. التأكيد

18. التطور

19. التحليل

20. التقييم

21. الاستنتاج

22. التحقيق

23. التأكيد

24. التحقيق

25. الاستنتاج

26. التحقيق

27. الاستنتاج

28. التحقيق

29. الاستنتاج

30. التحقيق

31. الاستنتاج

32. التحقيق

33. الاستنتاج

34. التحقيق

35. الاستنتاج

36. التحقيق

37. الاستنتاج

38. التحقيق

39. الاستنتاج

40. التحقيق

41. الاستنتاج

42. التحقيق

43. الاستنتاج

44. التحقيق

45. الاستنتاج

46. التحقيق

47. الاستنتاج

48. التحقيق

49. الاستنتاج

50. التحقيق

51. الاستنتاج

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