

CLINICAL AND PATHOLOGICAL STUDY OF THEILERIOSIS IN CATTLE AND BUFFALOES IN AL-GHANIEM REGION, ASSIUT GOVERNORATE, UPPER-EGYPT

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ABSTRACT

A detailed Clinico-pathological profile of clinically diseased cattle and buffaloes with Theileriosis located in Al-Ghaniem region, Assiut Governorate, was aimed. *Theileria annulata* was confirmed by the presence of *T. annulata* piroplasms in blood smears and/or lymph smears followed by polymerase chain reaction (PCR). During the period of investigation (April 2015 to August 2018), out of the clinically inspected cattle (n= 300) and buffaloes (n= 100), 80 (26.67%) and 15 (15 %) cases were clinically suspected to have Theileriosis, respectively. The positive cases were molecularly identified (PCR). The general observed signs were anorexia, fever, swelling of superficial lymph nodes. Ocular lesions were white cloudiness were more obvious in the center of cornea rather than the borders (yellowish colored corneoscleral opacity surrounded by hyperemic band). A watery discharge from the eyes. Serous ocular discharge (watery lacrimation) was remarkable, however in severe cases the ocular discharges was accumulated in the medial canthus. Some newly born calves of less than one month exposed to ocular symptoms mainly protruding of eye ball with icteric conjunctiva. The clinical examination of conjunctivae of the clinically suspected cases with Theileriosis indicated that icteric appearance of conjunctivae in some cases. Three cases showed petechiated conjunctivae. In our study some animals showed up-word visible bulging of temporal fossa. Visible protrusion of hemorrhagic conjunctiva with apparently exophthalmia (ocular edema) were observed. Bloody diarrhea and tarry like diarrhea, change in feeding behavior or habit like depraved appetite by eating mud ,soil were noticed. On the other side, the most prominent necropsy features the recently succumbed animals: Gross changes in various organs including heart lungs, trachea, stomach, liver, spleen, kidneys superficial lymph nodes, mesenteric lymph nodes, small and large intestine. All mucous membranes and conjunctivae, peritoneum and abdominal fatty tissues were icteric. On external observation. Jaundice, petechial hemorrhages involving mucosal and serosal surfaces of many organs as well as body fat. In the thoracic cavity, the most prominent autopsy findings were obviously extra edematous swelling of all lobes of the lung, hydrothorax and the lung was distended, discolored, solid in texture, and filled with exudate by palpation, The liver was friable, yellowish, and larger than normal, with the gall bladder being markedly distended with dark olive-green or brownish green bile. The abomasum was the most severely affected organ in the alimentary canal, it contains numerous ulcers about 3 mm. in diameter .a few linear ulcers were present on the leaves. There were prominent hemorrhagic ulcers and petechial hemorrhages were seen in the abomasum of the most cases. There were remarkable enlargement of spleen (splenomegaly) were also recorded. The kidneys were congested or dark brown in color and their perirenal fat were yellowish in color. The heart had petechial and hemorrhages on the outer and inner surface of the auricles.

Key word: Cattle, Buffaloes, Theileriosis, polymerase chain reaction, autopsy finding

INTRODUCTION

Explanation of the severity of symptoms and lesions of theileriosis in nature from mild to severe started by (Mehlhorn *et al.*, 1994). Gill *et al.* (1977) indicated that the clinical signs and severity of

theileriosis depend on pathogenicity of the strain of *Theileria*, the quantum of infection and the susceptibility and age of the host The work described here was a part of a study undertaken to determine the incidence, distribution, epizootiology and pathogenicity of tropical theileriosis in Al-Ghaniem region-Assiut Governorate, Egypt. The present investigation was conducted to study Clinical and necropsy findings of *Theileria* infection in Cattle and

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Buffaloes in Al-Ghaniem region, Assiut Governorate, Egypt.

Cattle and buffaloes play a pivotal role in Egyptian's economy by uplifting the socioeconomic condition of resource-poor farming communities and alleviating poverty. They are the principal animals for milk and meat production in Egyptian land. *Theileriosis* is an outstanding endemic vector-borne hemoprotozoal disease of ruminants particularly cattle and buffaloes caused commonly by two hemo-protozoal agents, *Theileria parva* and *Theileria annulata*. (Kundave *et al.*, 2017). *Theileria annulata* infection appears to be more prominent in large ruminant population in tropical, subtropical, and Mediterranean including southern Europe and Middle East countries; from Morocco, Algeria to India and China, causes Bovine Tropical Theileriosis (BTT). Such distribution may be attributed to the distribution of suitable tick vector (Neitz., 1959; Flach and Ouheli., 1992, Tuli *et al.*, 2015, Gharbi *et al.*, 2017). Tropical theileriosis imposed serious constraints on the health and productivity of the infected animals and the economic impacts of the disease in both cattle and buffaloes population and there are enormously reviewers reviewed that tropical theileriosis acts as a terrifying enemy for the productive capacities and reproductive performance of beef and dairy cattle and buffaloes, and it causes morbidity and mortality in calves and non-indigenous (exotic) cattle rather than native (local) breed. (Kivaria *et al.*, 2007, Gharbi and Darghouth, 2015 and Gharbi *et al.*, 2017). Moreover control of theileriosis is costly precious and the highest outcome is mostly associated with the intensive tick control rather than the diagnostic procedures and therapy of the infected animals (Decastro *et al.*, 1997). Seriously, it is matter of common fact that following recovery from primary *Theileria annulata* infection, the infected animals become persistent carriers (latent form of bovine theileriosis) and act as reservoirs of infection. Thereby, playing a critical role in disease epidemiology (Gharbi *et al.*, 2017). Practically, diagnosis of Tropical Theileriosis in large and small ruminants is primarily based on Giemsa-stained blood smear in association with detection of schizonts in Giemsa-stained smears from lymph node fine needle aspiration, which is a fast and low cost tool, but it is not suitable for epidemiological studies because of its low sensitivity (Uilenberg 2004). Moreover, by microscopic examination, it is difficult to discriminate different species of *Theileria* that may occur either as a single or mixed form within the same bovine host (OIE, 2014). On the other side, serodiagnosis was also utilized (Bilgic *et al.*, 2016). The serological tests are also not suitable due to cross-reactivity with other *Theileria* species (Dolan 1986). Inability to distinguish between active cases and animals with antibodies due to prior infection (Roy *et al.*, 2000). To overcome the above mentioned difficulties in laboratory diagnosis of *Theileria* infection, Ilhan

et al. (1998), Roy *et al.* (2000) and Sahoo *et al.* (2017) corroborated that the polymerase chain reaction (PCR) method allows the detection of a single piroplasm in extra little amount of blood sample.

MATERIALS AND METHODS

Area

The current study was undertaken in Al-Ghaniem region- Assiut Governorate, Egypt. Al-Ghaniem is one of the big centers of Assiut Governorate about 40 kilometers away from Assiut city. It is considered as a desert rural city and It is divided into an agricultural part and consists of the eastern half. The second half is desert,. It also divided into four areas, northwest, southwest, northeast and southeast that include different villages like Dir Elganadla, Almashayaa, Ghaniem Bahary, Ghaniem Sharq and Ghaniem qibly. The above mentioned villages are the biggest villages of Al- Ghaniem center.

Animals

During the period of investigation (April 2015 to August 2018), a total number of randomly selected 300 cattle were clinically inspected. Out of the 300 cattle, 80 cattle (*Bos Taurus*) were clinically suspected. These animals were hematologically examined, A total number of 100 Buffloes were clinically inspected .Out of them 15 Buffaloes (*Bubalus bubalis*) were clinically suspected and were hematologically examined. The clinically examined animals were in different ages and sexes. All the animals were randomly collected from different villages in Al-Ghaniem extreme south-west of Assiut Governorate.

Parasitological study

Blood samples were collected from the ear veins in vacutainers containing EDTA for the preparation of thin blood smears. Kelly (1979) and (Chaudhri and Gupta, 2003). All smears were then fixed with absolute methanol, stained with Giemsa, and examined by light microscopy at $\times 1,000$ under oil immersion for the presence of *Theileria* sp. piroplasms. Blood samples were also processed for DNA extraction. Lymph node biopsy smears from the enlarged lymph nodes were collected or carried out by using 18 gauze needle air dried which rapidly inserted into the substance of swollen lymph node (usually pre scapular) the hub of the needle then covered with a finger and the needle withdrawn. (Van Amstel, 1982).

Clinical and necropsy examinations

A thorough clinical examination was performed on all animals (Jackson G.G. Peter. & Peter D. Cockcroft, 2002). The signs in clinical cases of *T. annulata* infection were observed and recorded. Thin blood smears were also prepared from the ear veins of all animals. Lymph node aspirates were prepared from

suspected cases of tropical theileriosis Kelly (1979). Animals which died from infection (six) were examined post-mortem. Six animals (four females and two males) were recently succumbed subjected to postmortem examination according to Jubb *et al.* (1993).

DNA extraction and PCR assay

This test is designed to include the examination of 20% of clinically suspected cases of cattle (n=16) and 50% of clinically suspected buffaloes (n= 7), so large ruminant tested by PCR were 20 samples .Out of 20 tested samples 10 (50%) tested by *Theileria spp*

primer and all 20 (100%) tested by *T. annulata* primer according to D’oliveira *et al.* (1995). and concerning small ruminants. By amplification of *Theileria spp.* DNA from blood sample were extracted and obtained from both clinically suspected and infected animal and then tested by using PCR technique depending on two types of primers as shown in table (1). The first was derived from the gene encoding the SSU r RNA gene used to diagnose *Theileria spp.* The second primer was derived from the gene encoding the 30-kDa major *Theileria annulata* merozoite surface antigen according to D’oliveira *et al.* (1995).

Table 1: Primers sets used in PCR according to D"oliveira *et al.* 1995.

Primer	Sequence	Product	Characteristic
Forwarded	GTAACCTTTAAAAACGT	Pb	T.annulata annulata
Reserve	GTTACGAACATGGGTTT	721	
Forwarded	AGTTTCTGACCTATCAG	1098	Theileria species
Reserve	TTGCCTTAAACTTCCTTG		



Fig.1: Yellowish corneo-scleral opacity surrounded by hypermic zone in cattle



Fig.2: Severly congested eye with lacrimation



Fig.3: Eye lesion of animals infected with *Theileria annulata* (icteric condition suggesing hepatic insufficiency) A; Ocular lesion of eye of unweaned cattle acalf with exophthalmia and petechae (highly degree of icteric conjunctiva)B; adult cattle with icteric mucous membrane of eye.



Fig.4: Severly congested buffalo eye with lacrimation



Fig.5: Corneal opacity of young unweaned cattle calf



Fig.6: Out-bulging (edematous swelling) of infra orbital fossa



Fig.7: Prescapular lymph node enlargement in young cattle calve 3 month



Fig.8: Prefemoral lymph node enlargement (cattle)



Fig.9: Petechiae of vulvar mucous membrane



Fig. 10 Tarry feces of clinically confirmed case of *Theileria annulata*.

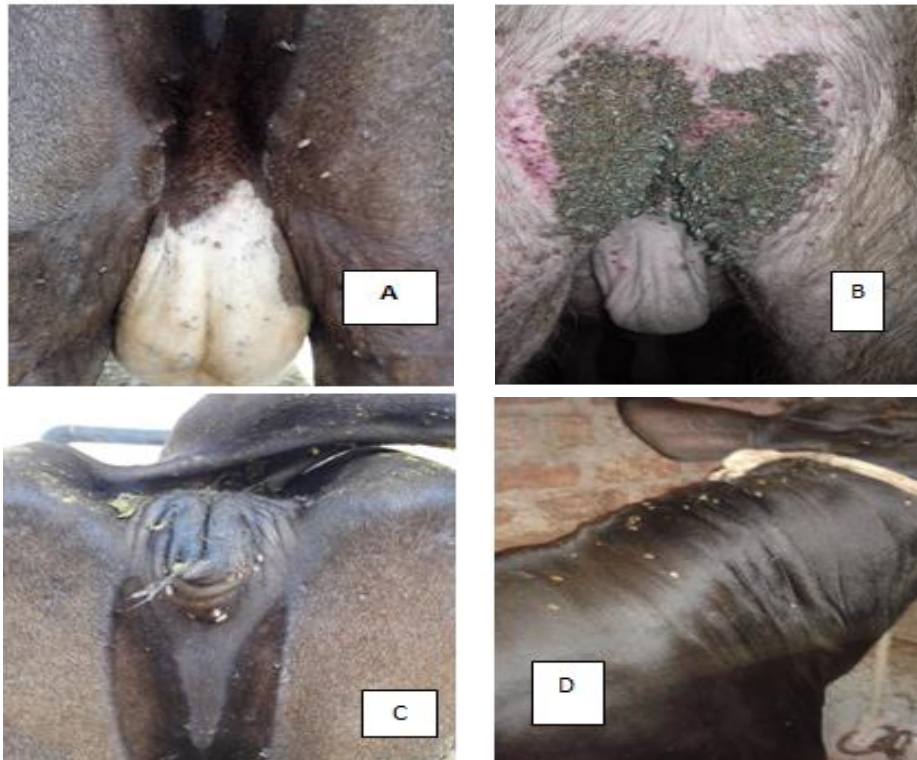


Fig.11: Tick infestation in cattle and buffaloes at different body parts.

- A, tick infestation in inner side of the thigh in cattle
- B, highly infested male buffalo ox at perineum region.
- C, presence of the tick under tail and vulva at cattle
- D, presence of tick on weather and neck region at male cattle.



Fig.12: Remarkable wide spread yellowish coloration in the viscera of infected cattle with *Theileria* infection note: icterus in rumen abdominal viscera and some of internal organ



Fig.13: Prescapular lymph node enlargement edematous, hemorrhagic



Fig.14: Tenacious fluid (arrow) from lung when palpated or compressed by hand (Lung edema)

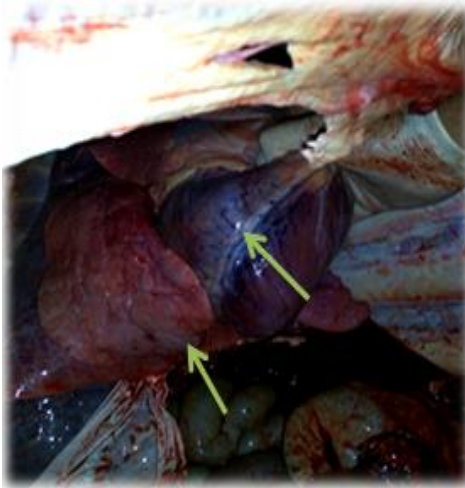


Fig. 15: Remarkable petechiae of heart and extra -edematous swelling of lung (arrow).



Fig. 16: Obviously distention of gall bladder (arrow) of infected cow with Theileriosis with hepatic enlargement note: falaby icteric liver with icteric fat adjacent to it.

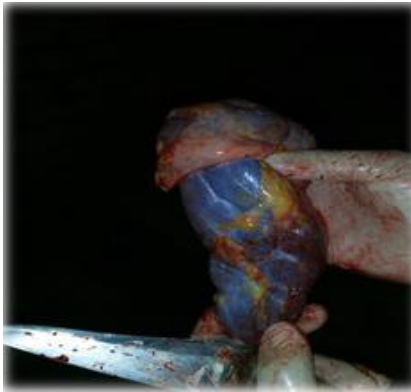


Fig. 17: Kidney edematous with yellowish discoloration of perirenal fat.



Fig. 18: Splenomegaly of cattle infected with Theileriosis.



Fig. 19: Highly congested abomasum before opening.(arrow)

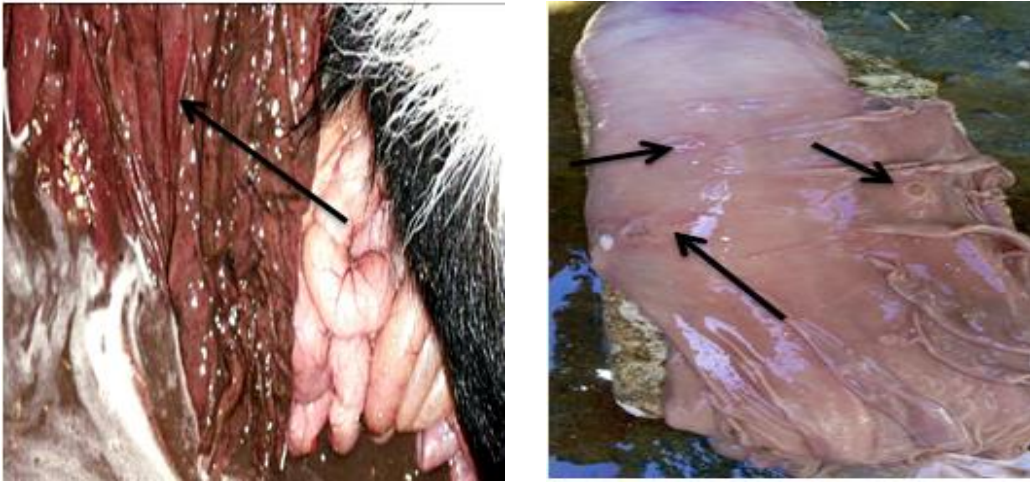


Fig.20 Abomasal ulceration arrows

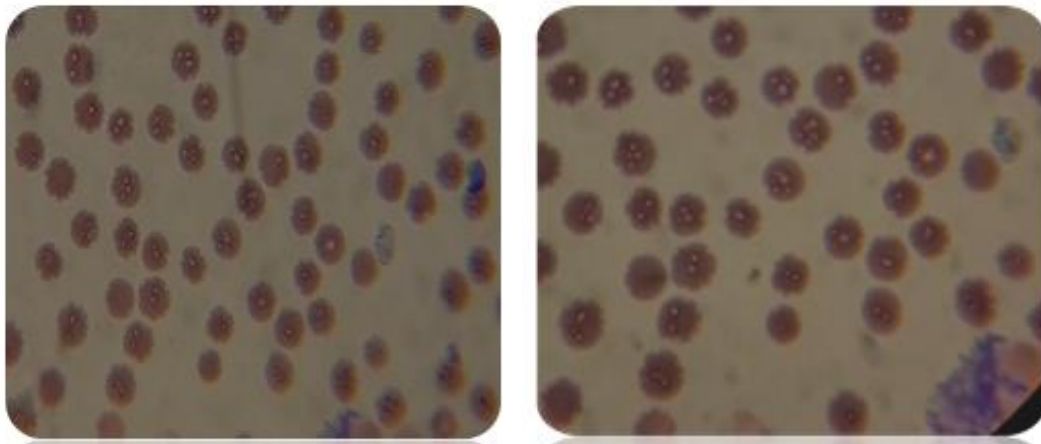


Fig.21: Giemsa stained thin blood film showing the intra-erythrocytic stage of Theileria annulata.x1000

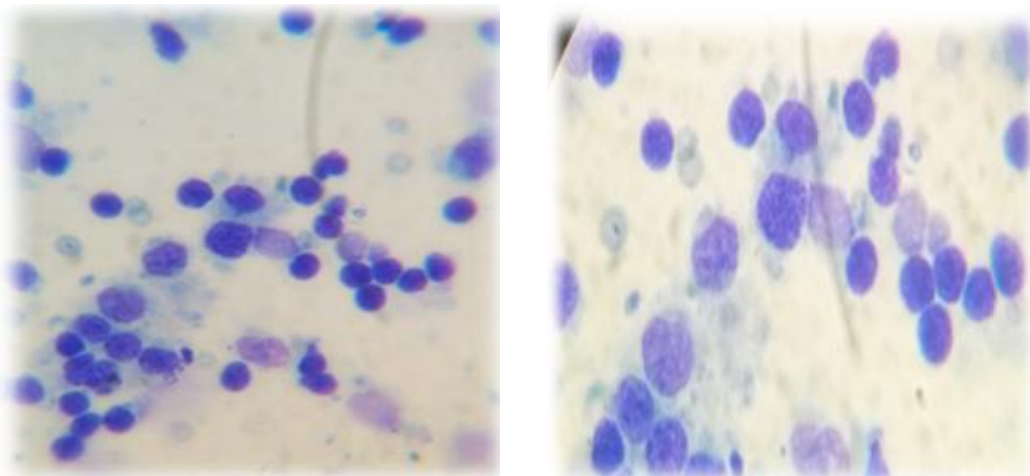


Fig. 22: Lymph smear showing Koch's blue bodies. X 1000

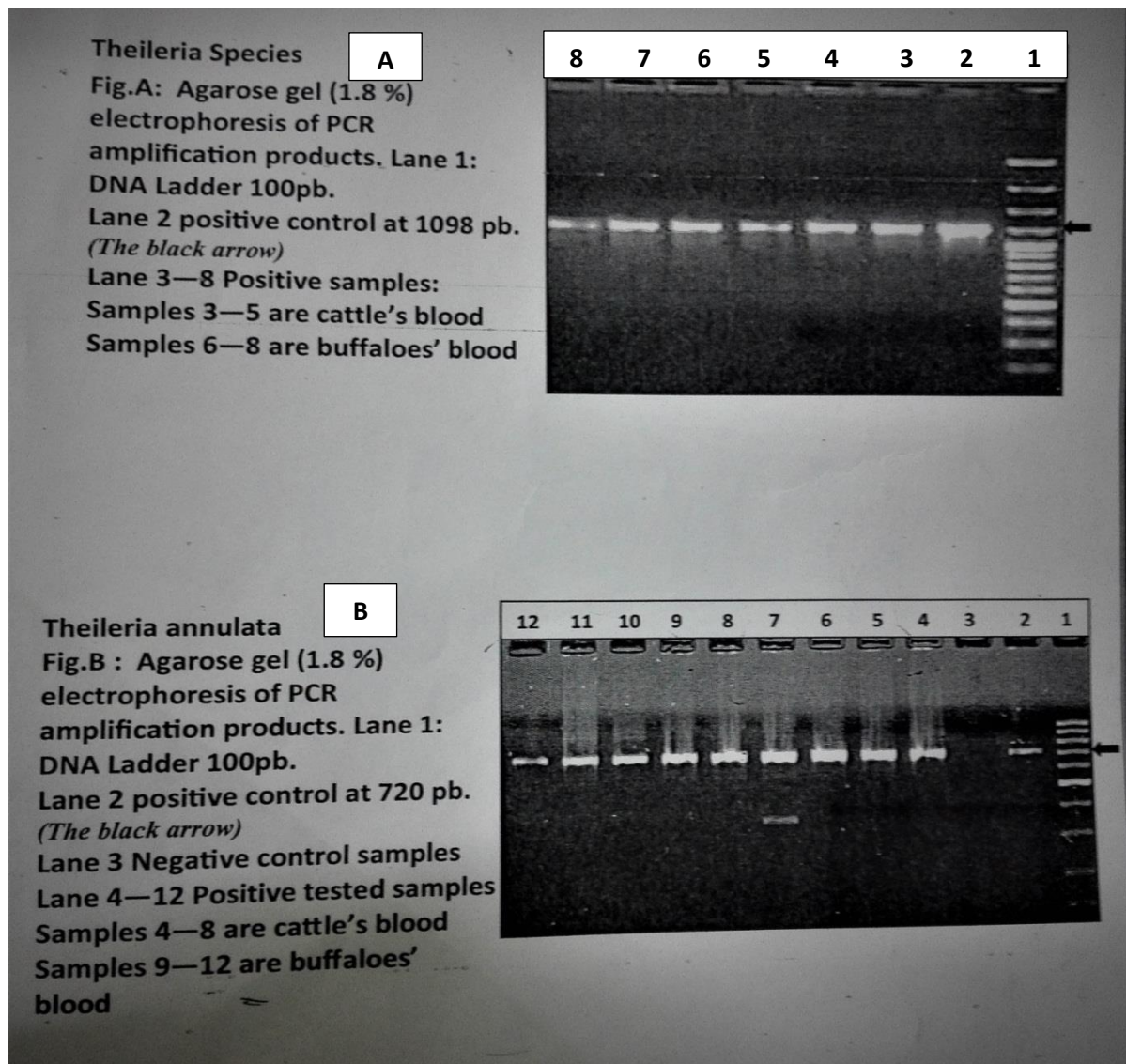


Fig. 23: Agar gel electrophoresis of PCR products of *Theileria* positive samples DNA extracts with primers specific for *Theileria species A*, and *Theileria annulata B*.

RESULTS

Parasitological findings

Total 400 large ruminants (300 cattle and 100 buffalo) with or without clinical signs were screened clinically and parasitologically for theileriosis 95 (23.75%) cases of them were found clinically suspected with *Theileria* parasites.

In case of cattle:

Out of the clinically inspected cattle (n= 300), 80 (26.67%) were clinically suspected to have *Theileriosis*. The clinically suspected cases were haematologically (blood film) and lymphatologically (lymph smear) tested for the presence of *Theileria* infection.

In case of buffaloes:

A total of 100 buffaloes were clinically inspected for *Theileriosis*, out of them, 15 (15%) were clinically suspected.

Diagnosis (Hematological examination results):

On basis of blood film:

Cattle: A total 300 clinically inspected cattle. Out of them, 80 clinically suspected cattle 26.67% (80/300) On the basis of blood smear examination (Giemsa stain) 60 cases out of 80 (75%) were showed a *Theileria annulata* piroplasm. (Fig, 21)

Buffaloes:

A total 100 clinically inspected buffaloes. Out of them, 15 clinically suspected buffaloes (15%). On the basis of clinical symptoms and blood smear examination (Giemsa stain) 10 cases out of 15 (66.67%) were showed a *Theileria annulata* piroplasm.

Lymph smear:

In present study, total 60 animals (cattle) clinically suspected and haematologically positive were found

positive for *Theileria piroplasm* 30 animal 50% (30/60) with lymphadenopathy. Animals were found with enlargement of lymph nodes either unilateral or bilateral enlargement. Out of 30 animals lymph node smears 13 cases with were *Theileria schizont* positive (43.33%). (Fig.22). In current study, there is no enlargement of lymph nodes recorded in buffaloes.

Specific PCR for examination of 20% of clinically suspected cases of cattle (n=16) and 50% of clinically suspected buffaloes (n= 7), so large ruminant tested by PCR were 20 samples. Out of 20 tested samples 10 (50%) tested by *Theileria spp* primer and all 20 (100%) tested by *Theileria annulata* primer according to D'oliveira *et al.* (1995) By amplification of *Theileria spp.* DNA from blood sample were extracted and obtained from both clinically suspected and infected animal and then test by using PCR technique depending on two types of primers as shown in table (1). The first was derived from the gene encoding the SSU r RNA gene used to diagnose *Theileria spp.* The second primer was derived from the gene encoding the 30-kDa major *Theileria annulata* merozoite surface antigen according to D'oliveira *et al.* (1995). The majority of the molecularly tested samples of large ruminant were *Theileria annulata* positive. By another meaning out 10 tested samples 6 samples (60%) were positive by *Theileria spp* primer. And out of 20 samples tested by the second primer of *Theileria annulata* 15 (75%) were positive to *Theileria annulata* (Fig.23).

Clinical findings

In addition to pyrexia and swelling of superficial lymph nodes (Fig.,7,8). Which were used as indicators for clinical disease, inappetance, ocular lesion were white cloudiness was more obvious in the centre of cornea rather than the borders (yellowish coloured corneo-scleral opacity surrounded by hyperemic zone) (Fig.,1). a watery discharge from the eyes. Serous ocular discharge (watery lacrimation) was remarkable, however in severe cases the ocular discharges was accumulated in the medial canthus (Fig.,1,2,4,5). Some newly born calves of less than one month exposed to ocular symptoms mainly protruding of eye ball with ictric conjunctiva (icteric condition suggessting hepatic insufficiency or co-infection with other hemopathogen like *Anaplasma spp.*) (Fig.,3). The clinical examination of conjunctivae of the clinically suspected cases with *Theileriosis* indicated that icteric appearance of conjunctivae in some cases. Some few cases showed petechiated conjunctivae and or vulva (Fig.,9). In our study some animals showed upword visible bulging of temporal fossa (Fig.,6). Another cases showed outword visible protrusion of haemorrhagic conjunctiva with exophthalmia (ocular oedema) (Fig.,3). Others showed bloody and tarry like diarrhea (Fig.,10). Change in feeding behavior or habit like deprarved appetite by eating mud ,soil were observed. Co-infection or concurrent infection with other

infectious disease like lumpy skin disease. Tick infestation all over the body also recorded (Fig.,11).

Necropsy findings

The most prominent features: Gross changes in various organs including heart lungs, trachea, stomach, liver, spleen, kidneys superficial lymph nodes mesenteric lymph nodes, small and large intestine. All mucous membranes and conjunctivae, peritoneum and abdominal fatty tissues were icteric with their intensity varying from case to case. On external observation jaundice, petechial and ecchymotic haemorrhages involving mucosal and serosal surfaces of many organs as well as body fat. In the thoracic cavity, the most prominent autopsy findings were obviously extra edematous swelling of all lobes of the lung, hydrothrox and the lung were distended, discolored, solid in texture, and filled with exudate by palpation (Fig.,14). The liver was friable, yellowish, and larger than normal, with the gall bladder being markedly distended with dark olive-green or brownish green bile (Fig.,16). The abomasum was the most severely affected organ in the alimentary canal, it contains numerous ulcers about 3 mm. in diameter a few linear ulcers were present on the leaves. There were prominent hemorrhagic ulcers and petechiae seen in abomasum in most of cases (Fig.,20). There were remarkable enlargement of spleen recorded (Fig.,18,19). The kidneys were pale, congested or dark brown in color and their perirenal fat were yellowish in color (Fig.,17). The heart had petechial and ecchymotic haemorrhages on the outer and inner surface of the auricles. (Fig.,15).

DISCUSSION

Tick-borne diseases hamper the progression of the livestock industry and *Theileriosis* is, unfortunately, an endemic disease among cattle and buffalo population in our country (Mason, 1922 and Daubney and said., 1951). At the beginning of the last century, since 1922 Mason gave a loud-shout about the importance of the disease. The settlement of *Theileria* infection may due to the continuously existence of the ticks responsible for life-saving and transmission of *Theileria* infection from invertebrate to vertebrate hosts, without strategic program of eradication, which is the cornerstone in preventive measures of *Theileriosis*.

T. annulata infection in this study was diagnosed by observing the intraerythrocytic forms (Fig.,21). Koch's blue bodies in the lymphocytes of the lymph nodes (Fig.,22), and Molecular examination revealed that these animals were infected with *T. annulata*.

The clinical signs and the pathological changes reported in the present wrok in cattle and buffaloes were similar to previous studies (Gill *et al.*, 1977; Uilenberg 1981; Omer *et al.*, 2002). In almost of the

animals (six animals) necropsied, the gross lesions was similar to work of Panda *et al.* (2011) and Mahajan *et al.* (2013).

The necropsy lesions including hepatomegaly, splenomegaly, and abomasal ulcers are in agreement with those reported by Sandhu (1996), Oryan *et al.* (2012), Sandhu (1996) who indicated severe damages to the hepatobiliary system due to hypoxia that resulted from hemolytic anemia and jaundice. The gross pathological lesion in infected animals were represented by general emaciation and other or some cases of icteric skeletal muscles, abomasal ulcers, the necrotic foci in different organs. Some investigators claimed the immunomorphological changes Fedorov and Karput (1970) due to a toxin excreted by the protozoan itself Hooshmand Rad (1976), enlargement of body lymph node especially superficial lymph node were enlarged with edema and congestion, liver was enlarged, flabby and pale yellow in colour with gall bladder fully distended by bile juice, enlargement of spleen punched out haemorrhagic ulcers in large numbers. The small intestine was markedly distended with congestion. Abomasal ulcer is of diagnostic significance for Theileriosis which was consistently evident in all the cases, These ulcers were also varying in size and color, probably due to the formation of petechiae that causes ischemia or anemia (anoxemia) locally resulting in the death of tissues in this foci, or due to the digestion of local epithelial cells affected by excreted enzymes from the present glands. The necrotic epithelium then was exfoliated by the friction of the content into the stomach lumen to leave an ulcer behind. These ulcers were surrounded by a hyperemic, intensely edematous and inflamed zone due to the secondary infections by the present flora or by other agents might come. (Dschunkowsky and Luhs, 1904). The heart showed redness (hemorrhage on pericardium) the animals showed congestion and edema of the lungs particularly the diaphragmatic lobes. There were frothy exudates in the trachea and bronchi. Lung edema may be explained due to effect of disturbance in fibrin and fibrinogen level in blood (Shitakha *et al.*, 1983). Another opinion explains the results of lung edema due to releasing of the serotonin substance produced from massive destruction of platelets act as vascular dilator which leads to increasing the permeability and producing edema (Kimeto, 1978) the myocardium was comparatively enlarged and congested Kidneys showed enlargement Perirenal fat was slightly icteric. The pathological changes reported in the present investigation were similar to those described for *T. annulata* infections in the previous studies More or less similar gross findings were described by various workers Sivaseelan (2003), Ahmad *et al.* (2007), Panda *et al.* (2011) and Mahajan *et al.* (2013).

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دراسة اكلينيكية وتشريحية عن مرض الثيلريا الحلقية في الابقار والجاموس بمنطقة الغنايم -
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استهدف هذا العمل دراسة مرضية إكلينيكية تفصيلية للماشية والجاموس المريضة سريريًا بمرض الثيلريا في منطقة الغنايم بمحافظة أسيوط- مصر في الفترة من شهر ابريل ٢٠١٥ حتى أغسطس ٢٠١٨ مع تقييم العوامل المهمة والتي تؤثر علي وبائية المرض في المجترات كما شملت الدراسة بعض التغييرات المرضية التي تطرأ علي الحيوان المصاب مع دراسة تشخيصية لطفيل الثيلريا باستخدام تقنية تفاعل البلمرة المتسلسل. استغرقت الدراسة ثلاث سنوات حيث شملت فحص ٣٠٠ بقرة اكلينيكية من مختلف الاعمار والسلالات من كلا الجنسين حيث جمعت بصورة عشوائية من منطقة الغنايم - محافظة اسيوط - مصر كما تم فحص ١٠٠ من الجاموس ايضا اكلينيكية من مختلف الاعمار والاجناس سواء حالات فردية او مخالطة للابقار في نفس مكان التربية. خلال فترة الدراسة (من أبريل ٢٠١٥ إلى اغسطس ٢٠١٨) ، من بين الماشية التي تم فحصها سريريًا (ن = ٣٠٠) والجاموس (ن = ١٠٠) ، ٨٠ حالة (٢٦.٦٧٪) و ١٥ (١٥٪) يشتبه إصابتهم سريريًا ، على التوالي. تم اختبار الحالات الإيجابية جزئيًا باستخدام تفاعل البلمرة المتسلسل. اظهرت ٨٠ بقرة و ١٥ من الجاموس علامات سريرية متمثلة في الحمى مع وجود القراد الناقل علي الحيوان مع تضخم الغدد الليمفاوية السطحية وعتامة بالعين متصاحبة في بعض الحالات بعلامات تنفسية واسهال مدمم والبعض الاخر اسهال اسود وبعض الحالات بها اصفرار (اليرقان) بالاغشية المخاطية للعين والملتزمة مما يوكد اصابة الكبد وتأثرة بالعدوي بطفيل الثيلريا وحالات اخري اظهرت وجود بقع نزفية علي العين والاغشية التناسلية تم عمل تشريح لستة حالات بعد النفوق اربعة اناث واثنين ذكور وتبين ان الحيوان النافق نتيجة هزال عام وبة تضخم للغدد الليمفاوية وتجمع سوائل رشحية بالرئة وتضخم الطحال وقرح بالمعدة والرابعة وتضخم الكبد في بعض الحالات مع امتلاء المرارة بالعصارة الصفراوية. رغم اهمية مسحات الدم للكشف عن الحالات الحادة ولكن تظهر اهمية اختبار تفاعل البلمرة المتسلسل في الكشف عن الحالات المزمنة والتي يصعب كشفها بمسحات الدم. ومن الدراسة إتضح ان التخلص من القراد هو بيت القصيد للقضاء على الثيليريا وطفيليات الدم الأخرى وبوصي الرش بالمبيدات الحشرية دوريا للقضاء علي ناقل المرض وسد الشقوق والفتحات بحظائر الحيوانات.