

Research Article

Assiut University web-site: www.aun.edu.eg**DETECTION OF SOME PARASITES IN LIVERS AND SPLEENS OF SLAUGHTERED CATTLE**WAFAA G. MAHMOUD¹; NAGWA T. ELSHARAWY²; ALSHIMAA A. HASSANIEN³
AND ABEER A. KHEDR¹¹ Parasitology Department, Faculty of Veterinary Medicine, New Valley University, Egypt² University of Jeddah, College of Science, Department of Biology, Saudi Arabia
and Department of Food Hygiene, Faculty of Veterinary Medicine, New Valley University, Egypt.³ Department of Zoonoses, Faculty of Veterinary Medicine, Sohag University, Egypt.**Received:** 11 December 2019; **Accepted:** 29 December 2019**ABSTRACT**

Parasitic infections retarded growth rate, decrease milk and meat production, abortion, and mortality which leading to huge economic losses in addition to its zoonotic impacts. Slaughterhouses used to monitor the infectious disease among food animals and play an important role in eliminating various diseases and prevent the transmission of infectious and zoonotic diseases to humans. The study aimed to identify the protozoan and uncommon nematodes larvae that affect cattle in New-Valley Governorate, Egypt. All slaughtered cattle's slaughtered at El-Kharga abattoir were inspected for the presence of *Theileria spp.*, *Toxoplasma gondii* (*T. gondii*), and *Ascarid* larvae during the period from July 2017 to the end of November 2018. Eighty livers and twenty spleens were examined by tissue impression smear stained with Giemsa stain to detect the prevalence of the parasites that embedded in the liver and spleen tissue, the prevalence of *Theileria spp.* was 62.5% in liver samples and 60 % in spleen samples, while prevalence of *T. gondii* was (10%) in the spleen. However, the percentage of *T.gondii* in the liver was 1.25%. Five percent of examined cattle's have been infected by *Ascarid* larva, which was found mainly in spleen. More education programs to farmers, veterinarians and improved public awareness are recommended. Broad spectrum anthelmintic must be applied to the animals' pasture. Future molecular biological studies are recommended to find out if the *Theileria spp.*, *Toxoplasma spp.* and *Ascarid larvae* encountered in New-Valley cattle are pathogenic human genotypes will represent a serious health problem especially for populations who comes in contact with cattle.

Keywords: *Theileria spp.*, *T. gondii*, *Ascarid* larvae, edible offals, zoonoses, slaughterhouse.

INTRODUCTION

Cattle are one of great economic importance, which is considered the main source of meat, milk and hides in Egypt. Such meat is the most common animal protein for human consumption, so it should be free from diseases particularly parasites (Radwan, 2008). The rearing of cattle's is one of the most beneficial industries worldwide. Thus, the total number of cattle in 2013 represented 54 million heads all over the world while in Egypt had about 148 thousand local heads and 4 million 579 thousand foreign heads (ADOD, 2014).

Parasitism is the main cause of cattle production reduction globally. Parasites have been noted the

major constraints to ruminants' productivity in terms of pathology and economic importance. Several worms are found in the animal, such as trematodes, cestodes, nematodes, and protozoa (Pam *et al.*, 2013).

Nematodes (roundworms) are worms that infect the ruminants. Large numbers of roundworms can cause a nutritional disturbance. Developing and migrating larvae and feeding adult worms can cause sufficient damage to the animal edible organs. They also interfere with digestion and cause blood losses. Infections of protozoa are common and seen during microscopic examinations. Infected animals are considered carriers and possible sources of infection for susceptible animals (Kahn *et al.*, 2010).

The causative agent of East Coast Fever (ECF) is *Theileria Parva* which one of the tick-borne apicomplexan parasites of wildlife and most domestic animal species, predominantly ruminants, which cause acute lymphoproliferative growth which results in high mortality levels of cattle. Clinically evident as lymph node enlargement, and infiltration of other

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tissues; the lungs, gastrointestinal tract, by parasitized cells (Morrison, 2015).

T. gondii is an obligate intracellular tissue cyst-forming protozoan; the disease is affecting a wide range of different animal species in Egypt, causing serious economic losses all over the world (Abd El-Razik *et al.*, 2014). In addition, its zoonotic impact especially in pregnant women and immunocompromised persons causing a serious illness (leonard *et al.*, 2019).

Animal grazing habits, pasture management, nutritional deficiency, the immunological status of intermediate vector and host, climates, the number of infective larvae and eggs in the environment were the main routes in the transmission of the parasites. Mature worms' toxins' destroy Red Blood Cells, due to which unthrifty anemic conditions are generated. While immature worms migrating through the body tissues of animals open the way for bacteria and fungi to enter, which causes serious diseases (Raza *et al.*, 2013).

The losses caused by parasitic infections are in the form of lowered general health condition, retarded growth rate, diminishing the working efficiency, decrease milk and meat production, abortion, and mortality. On the other hand, the cattle may be infected by parasites for a longer period of time in milk or breeding production which resulting in enormous economic losses (Akanda *et al.*, 2014).

The infections are either clinical or subclinical, the latter being the most common and of great economic importance. Although clinical parasitism has received considerable attention as a result of obvious severity (Abouzeid *et al.*, 2010).

Slaughterhouses could be used to monitor the infectious disease status among food animals and play an important role in eliminating various diseases that are not fit for human consumption (Elmonir *et al.*, 2015).

Meat inspection also provides vital data and valuable information on the incidences and prevalence of animal diseases and conditions within any country to prevent the transmission of infectious and zoonotic diseases to humans. Well documentation and collection of this information will make changes in animal husbandry practices and disease control (Phiri, 2006).

The aim of the present work is to identify some parasites that affect cattle in New-Valley Governorate which is highly beneficial in planning control measures against these parasites. Hence, a survey of helminths and protozoan parasites is going to be done through; Examination of samples collected from cattle attended El-Kharga abattoir from different

localities in New-Valley Governorate, Studying the morphological features by these parasitic stages; Gross appearance and tissue impression smears of digestive system organs to detect different parasitic stages in cattle's.

MATERIALS AND METHODS

Ethical approval: This study has been approved by the Animal Rights and Ethical Use Committee of New Valley University, Egypt.

The study area: A cross-sectional study was conducted in El-Kharga abattoirs to detect the prevalence of *Theileria* spp., *T. gondii*, and Ascarid larvae infection in slaughtered cattle. El-Kharga city is the capital of New Valley Governorate. It is a part of the oasis, which is located to the west of the Nile Valley between 25.26°N latitude and 30.32°E longitude. El-Kharga abattoir slaughtered about 2540 bovine animals during 2017/2018. About 80 infected liver samples (75 cattle & 5 bovine) and 20 spleen samples (15 cattle & 5 bovine) samples were collected from all cattle and bovine which slaughtered at El-Kharga abattoir during daily inspection for the presence of *Theileria* spp., *T. gondii* and *Ascarid* larvae infection during the period from July 2017 to the end of November 2018.

Samples preparation:

Liver and spleen samples were collected randomly from slaughtered cattle's during the period of the experiment. Immediately after opening the carcass, liver and spleen samples were obtained with sterile single-use surgical blades and transferred into sterile plastic bags and examined for the purpose of studying liver and spleen parasites. Each sample was collected in a clean plastic cup and taken directly to the laboratory for examination.

1. Macroscopic examination of liver and spleen: (Griffiths 1978)

Examination performed by the naked eyes during the routine abattoir post mortem examination. The livers & spleen were palpated consistency, color, presence or absence of hemorrhage, presence of mucus or other unusual constituents, were noticed through the examination of samples by the naked eye and dissected for any parasites.

2. Microscopic examination:

2.1. Tissue impression smears: (Maslin and Latimer, 2009)

A total of 100 impression smears from internal organs were made from 20 spleens and 80 livers. These visceral smears were examined after staining with Giemsa stain for the detection of tissue stages of different parasites microscopically under the oil immersion lens.

2.2. Preparation of Giemsa stain (Lawrence and Thomas, 1987)

Stock Giemsa stain was prepared by mixing 3 gm of Giemsa powder with 250 ml Glycerin in a mortar until the powder was dissolved. The mixture was put in a flask and placed in a 60°C water bath for 2 hours, and then 250 ml absolute methyl alcohol was added. The stock solution was stored in a tightly stoppered dark bottle for two to four weeks.

RESULTS

1. Prevalence of parasitic infection in tissue impression smears:

The results of the examination of 100-impression smear of internal organs of cattle are presented in table (1) and figure (1); the total prevalence of *Theileria spp.* was 62.5% in liver samples and 60 % in spleen samples, while *T. gondii* was detected in spleen and liver with percentage 10% and 1.25% respectively. Ascarid larvae was detected in 5% of examined cattle only in spleen.

2. Microscopic examination of tissue impression smears:

The inspection of liver and spleen of cattle were found to be infected with a wide variety of protozoa (*Theileria spp.*, *T. gondii*) and Ascarid larva as following.

2.1. *Theileria spp.*

The prevalence of *Theileria spp.* in tissue impression smear tabulated in table (1) was about 62% from the inspected carcass, as following; 50/80 (65.2%) and 12/20 (60%) in liver and spleen respectively.

2.1.1. Morphology of schizonts: Schizonts were seen in smears made from livers (Figure 2; A, B, C & D) and spleens (Figure 2; E & F). Two types of schizonts

of *Theileria spp.*, small and large schizonts were observed in spleen and liver smears.

2.1.2. Small schizont: The small schizonts (Figure 2; A & B) in the liver and (Figure 2; E & F) in the spleen. Merozoites in the small schizonts were comma-shaped; the schizonts were not commonly seen inside the host cells. The merozoites in small schizont were varied.

2.1.3. Large schizont: The Large schizonts were located outside the host cell rarely observed inside the host cell. Scattered merozoites were also observed. The sizes of merozoites in these large schizonts were different, some of them being large and some small (Figure 2; C & D).

2.2. *Toxoplasma gondii*:

The prevalence of *T. gondii* in tissue impression smear tabulated in the table (1) was about 3% from the inspected carcass, as following; 1/80 (1.25%) and 2/20 (10%) in liver and spleen respectively. The size of tissue cysts was variable, measured from 50 to 70 µm and contains from 16–30 crescent-shaped tachyzoites. The cyst size inside the tissue is dependent on the host cell, cyst age, and the strain of *Toxoplasma gondii*. In small cysts detected inside host spleen tissue (Figure 3; A) and liver tissue (Figure 3; B) measuring with average 5µm and containing few tachyzoites with vacuoles of different sizes.

2.3. *Ascarid larva*:

The prevalence of *Ascarid larva* in tissue impression smear tabulated in the table (1) was about 1% from the inspected carcass, as following; 0/80 (0%) and 1/20 (5%) in liver and spleen respectively. The size of third stage larvae ranging from 0.56-1.07 mm in length and 0.03-0.05 mm in width (Figure 4; A). It is a cone-like structure with thick granular sheath (Figure 4; B).

Table 1: Prevalence of parasitic infection in tissue impression smear.

parasitic infection	Liver		Spleen	
	Examined no.=80		Examined no.=20	
	Infected no.	%	Infected no.	%
<i>Theileria spp.</i>	50	62.5	12	60
<i>Toxoplasma gondii</i>	1	1.25	2	10
<i>Ascarid larvae</i>	0	0	1	5
Total	51	63.8	15	75

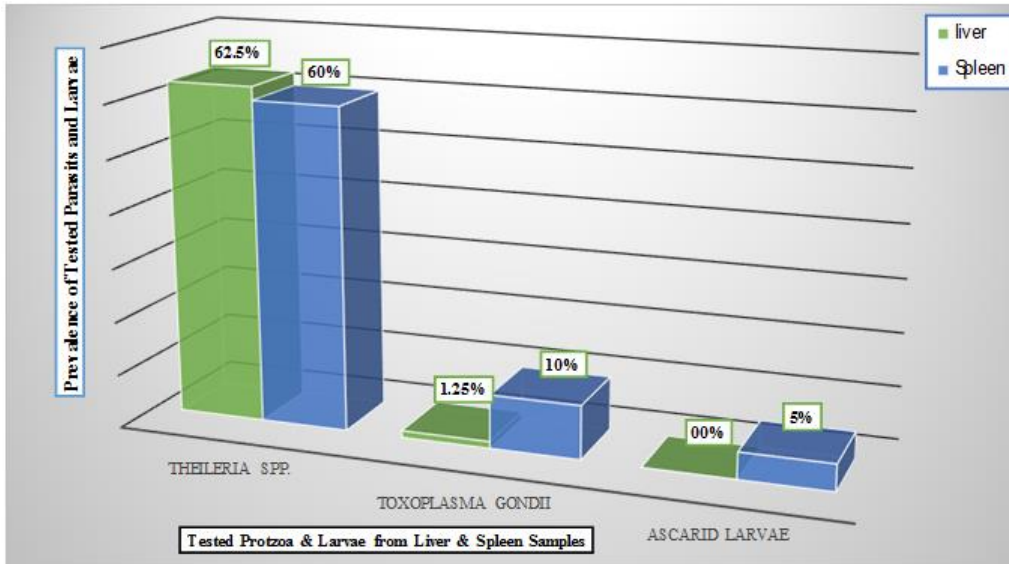


Figure (1): Histogram showing the prevalence of parasitic infection in tissue impression smear

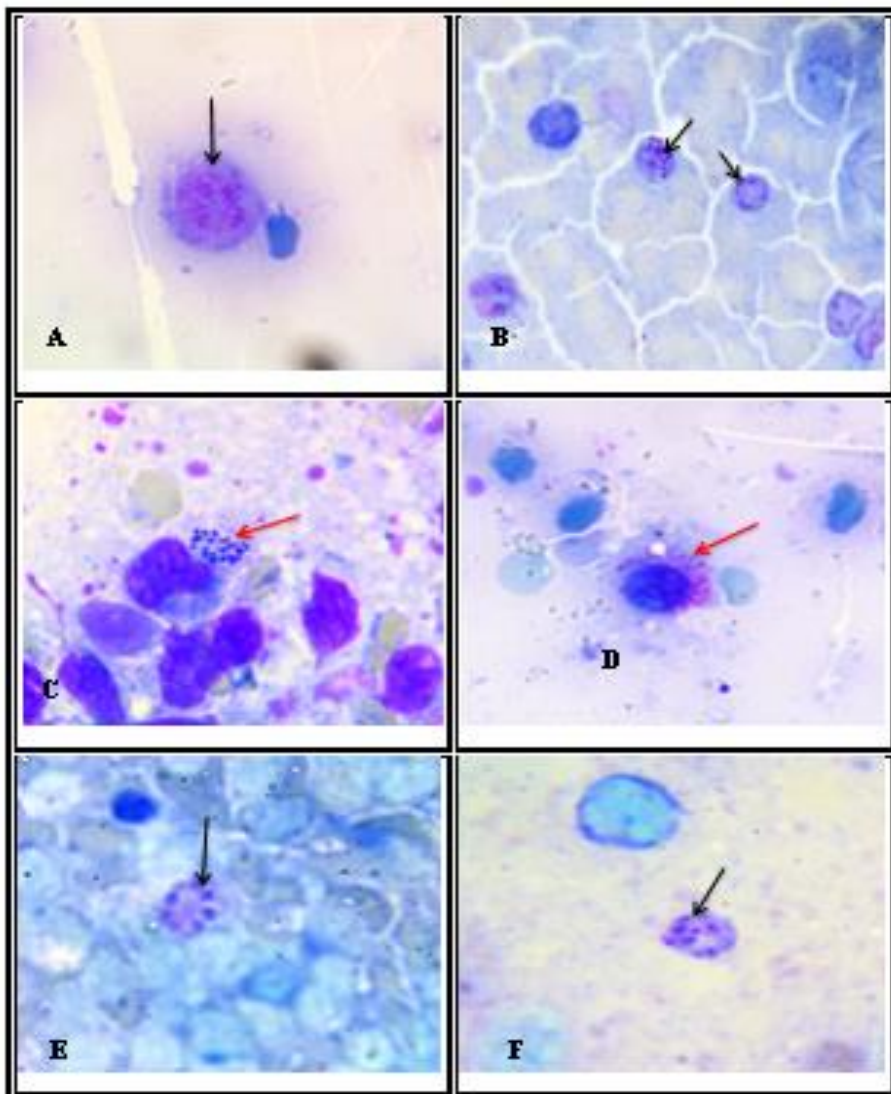


Figure (2): Microscopic view of smear made from Liver (Fig. A, B “intracellular schizont”, C, D “extracellular schizont” and Spleen (Fig. E, F “intracellular schizont” (x100).

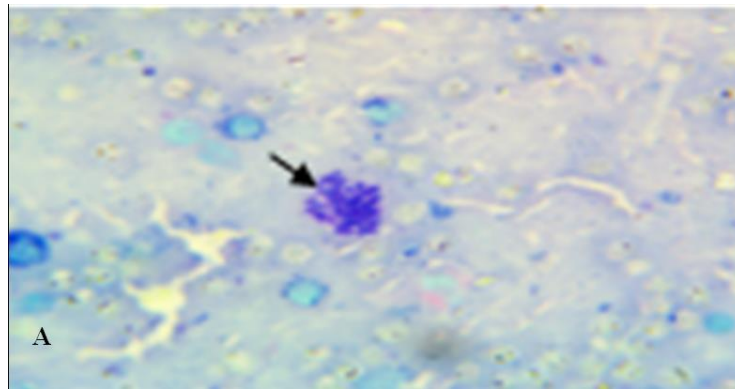


Figure (3): A) Tissue cyst of *Toxoplasma gondii* in spleen of infected cattle stained by Geimsa stain (X 100)

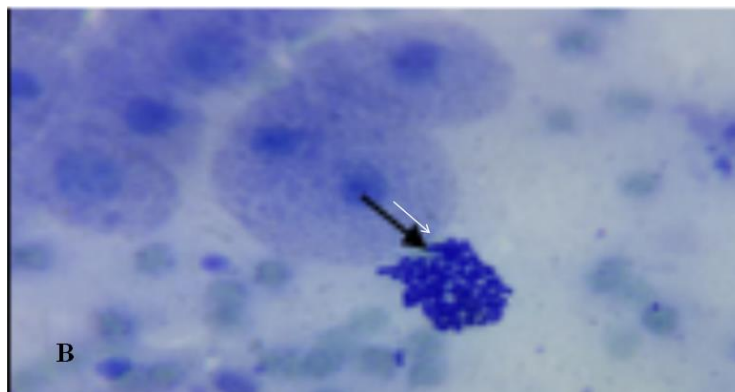


Figure (3): B) Tissue cyst of *Toxoplasma gondii* in liver of infected cattle stained by Geimsa stain (X100).

Figure (3): Impression smear of *Toxoplasma gondii*.

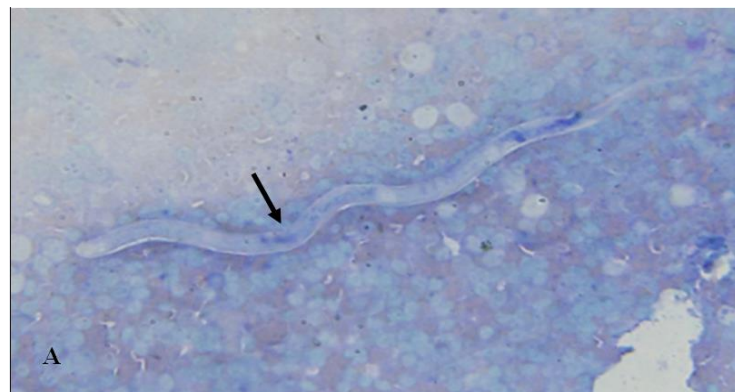


Figure (4): A) Microscopic examination of tissue impression smear showing Ascarid larva in the spleen (x100)

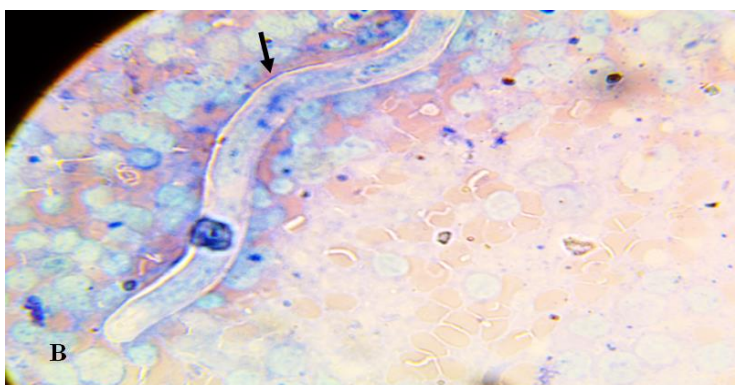


Figure (4): B) Ascarid larvae showing granular cone at the anterior end (x100).

Figure (4): Ascarid larvae in impression smear.

DISCUSSION

In this study, liver and spleen of inspected cattle were found to be infected with a wide variety of parasites such as; *Theileria* spp., *T. gondii* and Ascarid larva. As shown in table (1) and figure (1) the total prevalence rate of *Theileria* spp. infection inspected carcass was (50/80) 62% in liver & about (12/20) 60% in spleen. Similar result was observed by Usman *et al.* (2016) in Nigeria, who noticed that the infection rate of cattle was 60.4%. However, Yahaya and Tyav (2014) found that the infection of cattle was 34.9%. The protozoa infections were the most predominant parasites among cattle; the prevalence of identified protozoa was 59.3%. The prevalence rate of protozoa infection differed among studies were higher in cattle farms and villages of EL-Wadi EL-Gadid, Assiut, EL-Fayoum, EL-Minia and Sohage Governorates which were examined 150 cattle for theileriosis. The infection rates in blood and lymph samples were 65.6% and 45.3%, respectively, while PCR test showed that 58.3% were positive (Abdel-Rady *et al.*, 2010). In Taiwan, 81.3% for cattle (Huang *et al.*, 2014) and lower results evaluated in the different localities in Upper Egypt were; 17.5% in Assiut, 20% in Sohag and 40% in EL-Wadi EL-Gadid Governorates by Abdel-Rady *et al.*, (2008). A total of 405 cattle were randomly sampled in Menofia Province, Egypt the total prevalence of *Theileria* sp. in infected cattle was 65 (16.05 %) (Nayel *et al.*, 2012). In Pakistan, 13.62% for cattle (Rafiullah *et al.*, 2011), in India, 10.76% in cattle (Choubisa and Jaroli 2013), and in Cheyyartaluk, 16% (Vanisri *et al.*, 2016). In our results, schizonts were seen in smears made from liver 62.5% and spleen 60%. The presence of schizonts in liver and spleen have been recognized before by Yin *et al.* (2003) and Kaleibar *et al.* (2014) in Iran who found Koch's blue bodies (Macroschizont) on tissue sections of the bone marrow, liver, kidney, and heart.

Theileria spp. responsible for the economically important diseases of domestic and wild animals. *Theileria* spp. classification based on morphology, host cells in which schizogony occurs, the observation of piroplasms in the red blood cells associated with disease manifestation and host-vector specificity. The genus *Theileria* is distinguished by infection of leukocytes by sporozoites, maturation of schizonts into merozoites and subsequent infection of red blood cells to form piroplasms (Uilenberg, 2006). The infective stage called sporozoites during tick feeding into the feeding site. Sporozoites then infect leukocytes and multiply, larval or nymphal vector ticks ingest piroplasms and released it in tick gut, were forming a zygote, which divides into motile kinetes and infects the tick gut epithelial cells and migrates to the haemolymph and salivary glands (McKeever, 2009). *Theileria* epidemiology considers parasite and vector distribution mortality and

morbidity of disease outbreaks, disease outbreak risk assessment, and disease control measures, socio-economic factors, climate change, host resistance and susceptibility (Gachohi *et al.*, 2012). *Theileria* had a clinical disease characterized by theilerial parasitemia, macrocytic normochromic anemia with acanthocytosis and spherocytosis, lymphoid hyperplasia (lymphocytosis, edematous lymphadenomegaly), dysproteinemia, evidence of liver disease, and a low serum antibody titer against *Theileria* spp. which also can affect the spleen, which developed mild anemia while parasitemic (Alvarado-Equivel *et al.*, 2011).

As shown in table (1) and figure (1) *T. gondii* was detected in one liver and two spleens of cattle as following; 1/80 (1.25%) and 2/20 (10%) liver & spleen respectively, as the highest rate was observed in spleen. This also agree with Mohammed and Abdullah (2013) in Sulaimani Province, who recognized tissue cysts of *T. gondii* in liver and spleen stained by Geimsa stain by about 11%. Nearly similar results reported In Bangladesh 12% in spleen of cattle by Shahiduzzaman *et al.* (2011). Azizi *et al.* (2014) investigated *T. gondii* in cattle infection in Southwest of Iran by 12% from cattle spleen. A total of 30, 742 individual samples from 24 countries in Africa, the overall estimated prevalence of toxoplasmosis in cattle was 12% (8-17%) (Tonouhewa *et al.*, 2017). Higher results were detected from 88 cows in Egypt, seroprevalence of toxoplasmosis was 17 (19.3%) by Hassanain *et al.* (2013). In Brazil, about 26% of *T. gondii* was detected in beef cattle by Santos *et al.* (2009). Lower results estimated by Inpankaew *et al.* (2010) in Thailand who detected 9.4% of *T. gondii* infection in cows. *T. gondii* prevalence in New Caledonia Cattle was 3.3% by Roqueplo *et al.* (2011). Gharekhani (2013) determined 2.3% of the cattle samples infected with *T. gondii* in Western Iran. Ahmad and Qayyum, (2014) reported in northern Punjab, Pakistan, about *T. gondii* infection was 19.75% (79/400) from cattle samples. Matsuo *et al.* (2014) found *T. gondii* in 7.3% (31/422) of cattle in Japan. *T. gondii* is an intracellular protozoan parasite that can infect almost all warm-blooded animals, including humans because *Toxoplasma gondii* can infect a variety of intermediate hosts such as; warm-blooded animals and man. The definitive host for this parasite is feline species. Cat completes the coccidian life cycle with intestinal replication and passage of oocysts in the feces (Robert-Gangneux & Darde, 2012 and Lu, *et al.*, 2015). Humans infected primarily by intake raw or undercooked meat and offals containing viable tissue cysts, or by ingesting water or food contaminated with oocysts from infected cat feces. In healthy humans, the infection with *T. gondii* is usually asymptomatic. It causes severe encephalitis and neurologic diseases, and can affect the heart, liver, inner ears, and eyes (chorioretinitis) (Meireles,

et. al., 2015). Primary infections in pregnant women are normally asymptomatic in the mother; but the importance of estimating *T.gondii* infection in human has a great significance because of the risk of the infection of the fetus, immune compromised hosts and newborns (Baldursson, & Karanis, 2011 and Ahmad, & Qayyum, 2014).

This result showed that five percent of cattle using Giemsa stained impression smear have been infected by *Ascarid larva*, which was found mainly in spleen. These findings were confirmed by (Strube *et al.*, 2013) who estimated that the larvae do not develop into the adult stage, but rather migrate throughout the somatic tissue and persist as an infectious L3 stage for extensive periods. Those arrested larvae may lead to severe inflammatory reactions and consequently to a wide range of pathological and clinical manifestations. *Ascaris* has enormous reproductive potential and it has been estimated that a single female may produce more than 1 million eggs per day and remain viable in the soil for at least 6 years and contaminate the environment through fertilizer (Roepstorff *et al.*, 2011). Most *Ascarid larvae* are expelled from the hepato-tracheal migration animal tissue and probably human hosts and be unobservable by the naked eye which makes it hardly detected in medical and veterinary practice (Nejsun *et al.*, 2009 and Pinelli *et al.*, 2011). Zoonotic *Ascaris* infection is generally thought to be uncommon in humans in developed countries and associated with the previous residence in, or travel to, endemic areas (Maguire, 2005) or due to foodborne infection (Bendall *et al.*, 2011). Some studies report on *Ascaris* in lambs with affected livers (Criscione *et al.*, 2007) and lungs due to swine cross-infection migration (Lee *et al.*, 2010).

CONCLUSION

The effect of parasitic diseases remains one of the most important diseases of cattle in New-Valley Governorate and it is the main cause of reduced cattle productivity. In the present study, cattle may be considered as a reservoir host for *Toxoplasma* human infection which represents a serious human public health problem. The present results provide baseline data for future researchers and control strategies against parasites of cattle. More farmers, veterinarian, and public awareness, education programs, and improvement of post mortem inspection legislation and procedures are recommended. Strategic deworming of animals, when conditions are favorable for larval development on the pasture using broad-spectrum anthelmintic must be applied. All public health measures should be encouraged, including thorough handwashing with soap (particularly for children) and rinsing and cooking of vegetables. Future molecular biological studies are recommended to find out if the *Theileria* spp., *Toxoplasma* spp. and *Ascarid larvae* encountered in New-Valley cattle are

pathogenic human genotypes which will represent a serious health problem for populations who comes in contact with cattle.

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الكشف عن بعض طفيليات الكبد والطحال في مذبوحات المواشي

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تسبب الإصابات الطفيلية في تأخير معدل النمو وتقليل إنتاج الألبان واللحوم والإجهاض والوفيات مما قد يؤدي إلى خسائر اقتصادية هائلة. تستخدم المسالخ لرصد الأمراض المعدية بين ذبائح الحيوانات وله دور مهم في القضاء على الأمراض المختلفة ومنع انتقال الأمراض المعدية والأمراض الحيوانية المنشأ إلى البشر. تهدف الدراسة إلى التعرف على يرقات الديدان الخيطية الأولية وغير الشائعة التي تصيب الماشية في محافظة الوادي الجديد، بمصر. حيث تم فحص جميع الماشية المذبوحة في مجازر الخارجة بحثًا عن وجود الثيليريا وتوكسوبلازما جوندي (*T. gondii*) ويرقات أسكاريد خلال الفترة من يوليو ٢٠١٧ إلى نهاية نوفمبر ٢٠١٨. كما تم فحص ثمانين كبدًا وعشرين طحالًا. بواسطة فحص الأنسجة بصبغة جيمسا للكشف عن مدى انتشار الطفيليات التي تكمن في أنسجة الكبد والطحال، وسجلت النتائج انتشار ثيليريا بنسبة ٦٢,٥٪ في عينات الكبد و ٦٠٪ في عينات الطحال، في حين كان انتشار *T. gondii* (١٠٪) على الطحال اما في الكبد فكانت ١,٢٥٪. وكشفت الدراسة عن إصابة خمسة في المئة من الماشية التي تم فحصها بواسطة يرقات أسكاريد التي عثر عليها بشكل أساسي في الطحال. ينصح بتحسين البرامج التعليمية للمزارعين والأطباء البيطريين وتوعية الجمهور. يجب أن يستخدم مضاد ديدان واسع المدى على مرعى الحيوانات. كما يوصى بإجراء دراسات بيولوجية جزيئية مستقبلية لمعرفة الأنماط وراثية *Toxoplasma spp* ، *Theileria spp* التي تصيب أبقار الوادي الجديد وهل وتنقل للانسان وتمثل مشكلة صحية خطيرة للسكان الذين يتعاملون مع الماشية.