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PARASITES TRANSMITTED TO HUMAN BY INGESTION OF DIFFERENT TYPES OF MEAT, EL-MINIA CITY, EL-MINIA GOVERNORATE, EGYPT

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Abstract

Meat-borne parasites are *Sarcocystis species, Toxoplasma gondii*, *Taenia saginata, Taenia solium* and *Trichinella spiralis*. A total of 300 animals including 100 cattle, 100 goat, and 100 pigs, slaughtered in El-Minia governmental slaughterhouses. From each animal, five samples were taken from different muscles (esophageal, tongue and cardiac) and different organs (liver and brain). Meat samples were examined macroscopic and microscopic (direct, homogenization and H&E staining) for detection of the above-mentioned parasites. Serum samples were subjected to IHA for detection of *T. gondii* specific antibodies. This study revealed that *Sarcocystis* species were the highest parasites that could be detected, with overall prevalence of 80%, which was statistically significant (P≤0.001). The digestion method was more sensitive than direct method for detection of *Sarcocystis* species. On the other hand, *T. gondii* was only diagnosed by using IHA test as 50.9% serum samples were positive, which was statistically significant (P≤0.004). Besides, 20% of examined cattle were infected by *Cysticercus bovis*, and 12% of pigs were infected with *C. cellulosae*, but without statistical significant (P≤0.5).

Key words: Meat, *Sarcocystis spp., Toxoplasma gondii*, *Taenia saginata, Taenia solium* and *Trichinella spiralis.*

Introduction

Ggenerally, food-borne parasitic diseases are under recognized, though becoming more common (Dorny *et al*, 2009). Meat-borne parasite diseases was *Sarcocystis* species, *Toxoplasma gondii* (Cook *et al*, 2000), *Taenia saginata and T. solium* (Somers *et al*, 2007) and *Trichinella spiralis* (Gottstein *et al*, 2009). The *Sarcocystis* species are obligatory intracellular parasites (Dubey *et al*, 1989). Eating raw or undercooked beef and pork containing viable sarcocysts of *S. hominis* and *S. suihominis*, respectively, resulted mostly asymptomatic human intestinal sarcocystosis (Bunyaratvej *et al*, 1982). *Sarcocystis* can be detected in meat by direct observation of macroscopic sarcocysts or by optic microscopy of microscopic sarcocysts (Fayer, 2004).

Toxoplasmosis is widespread in man and many animals, by ingesting the infective stage (Dubey, 1996). Its diagnosis is either direct detection of *T. gondii* cysts in tissues or indirect detection of serum-specific antibodies (Bayarri *et al*, 2012). *Taenia saginata* infection results from eating raw or undercooked beef (Hird and Pullen, 1979) or frozen ones (Hilwig, 1978) containing viable *cysticerci.* Its diagnosis is by detecting *cysticerci* in muscles and organs by macroscopic and microscopic examination. (Abdo *et al*, 2009)**.** *T. solium* is an important zoonosis in pork-eating countries and associated with low economic development (Murrell, 2005), which is quite asymptomatic (Farrar *et al*, 2013). Cysticercosis is a risk condition, which most commonly occurs in the central nervous system causing neurocercosis. It is also frequently found in muscles and subcutaneous tissues. The globe of the eye is also a common site (Schantz, 1989). Meat inspection is the only diagnostic method carried out on large scale in slaughterhouses for the post-mortem detection of pig cysticercosis (Wanzala *et al*, 2003).

Trichinellosis results from eating raw or undercooked pork. It has declined significantly as a zoonotic disease, particularly in the developed countries where a reduction of the domestic cycle was observed in the last decades (Bruschi, 2012). *Trichinella* infection rarely causes clinical signs in the parasite's

natural hosts, unless they are infected with a very large number of larvae (Bruschi and Murrell, 2002). *T. spiralis* is diagnosed by detecting *T. spiralis* larvae in muscle samples or by testing for the presence of anti-*Trichinella* antibodies in the serum or in the meat juice (Gottstein *et al*, 2009).

This study aimed to detect the prevalence of different parasites in cattle, goats and pigs slaughtered in El-Minia governmental slaughterhouse and to evaluate the sensitive diagnostic method.

Materials and Methods

This study was done from September 2014 to May 2015, samples were collected from 300 animals including; cattle, goats and pigs, 100 each, in five slaughterhouses in El-Minia city.

The predilection sites to watch out for these parasites immediately after slaughtering the animal are esophageal muscle, tongue muscle, cardiac muscle, liver and brain (Latif *et al*, 1999). Furthermore, blood samples were collected from neck veins of these animals in clean tubes before they were slaughtered. Meat samples were put in ice container after removal immediately. Samples were transported to the Parasitology Department to be examined for parasitic infections.

Macroscopic examination: Fresh samples were examined by naked eye for the presence of *Sarcocystis species* cysts, *Taenia saginata* and *T. solium cysticerci* (Abuseir *et al*, 2006).

Direct microscopy: Small pieces of fresh muscle were prepared by muscle squash method and examined for sarcocysts of *Sarcocystis* species, *Trichinella spiralis* coiled larva (Gamble, 1998) and *Toxoplasma* cysts (Dubey, 1998). Small pieces of liver and brain tissues were cut and compressed between two slides to detect cysts of *Toxoplasma gondii* (Dubey, 1998). The viable *cysticerci* were carefully removed from the connective tissue capsule, compressed between two glass slides and examined (Gracey *et al*, 1999).

Homogenization: A total of chopped 15 grams of muscle were suspended in 50 ml 0.85 % NaCl solution and vigorously shaking with glass beads for 10 minutes. The contents were poured and washed with saline and allowed to stand for 30 min. The sediment material was transferred to slides then examined microscopically.

Histopathological examination (Myerowitz, 1983): The muscle and organs samples were fixed in 10% formo-saline, stained with Erlich's hematoxylin and eosin. Serological test (For detection of anti-*Toxoplasma* antibodies in sera)

Blood samples were left at 37°C for 20 minutes. The samples were centrifuged at 1500 g for 10 minutes to separate sera. Sera were kept at -20°C until used (Behymer *et al*, 1973).

Indirect Haemagglutination test (IHAT): Specific anti-*Toxoplasma* antibodies, IgG & IgM were quantitatively detected in animals' sera using commercially available Toxo-HAI kit (Toxo-Hai Fumouze® CE 0459) following the manufacturer's instructions.

Interpretation: Negative reactions: presences of a more or less wide sedimentation ring in the well bottom. Positive reactions: presences of an agglutination mat or reddish brown film covering at least half of the bottom of the well with or without a thin peripheral ring of sedimentation.

Statistical methods: Data entry and analysis were all done with I.B.M. compatible computer using Statistical Package for the Social Sciences (SPSS) for windows version 13. Graphics were done by Excel. Data were presented by frequency distribution. Chi square test, Fisher exact and Z test (test of proportions) were used to compare between proportions. The probability of less than 0.05 was used as a cut off point for all significant tests.

Results

Out of 300 animals examined for *Sarcocystis spp.* Sarcocysts could not be seen by macroscopic examination in all animals' muscles, but by homogenization method, 240 (80%) were infected. The highest preva-

lence rate was in goat (92%), cattle (80%) and then pigs (68%). The esophagus was the most infected organ in cattle (68%) and pigs (56%), while tongue was the most frequent infected organ in goats (72%).

Table 1: Infection rate and organ distribution of *Sarcocystis* spp. in different slaughtered animals.

Animals	examined	Infected	Esophagus		Tongue		Heart		P Value
			D^+	H^+	D^+	H^+	D^+	H^+	
Cattle	100	80 (80%)	49 (49%)	68 (68%)	37 (37%)	53 (53%)	14 (14%)	35 (35%)	P≤0.05
Goat	100	92 (92%)	38 (38%)	67 (67%)	42 (42%)	72 (72%)	28 (28%)	37 (37%)	P≤0.05
Pigs	100	68 (68%)	36 (36%)	56 (56%)	24 (24%)	50 (50%)	20 (20%)	42 (42%)	P≤0.05
Total	300	240 (80%)	123 (41%)	191 (63.6%)	103 (34.3)	175(58 .3%)	62 (20.6%)	114 (38%)	

D^+: Direct microscopy H^+: Homogenization

Histopathologically, in cattle thin walled cysts of *Sarcocystis cruzi* was seen (Fig. 2b), which measured less than 1µm. The cyst wall showed palisade-like villar protrusions of *S. hirsuta* or *S. hominis* (Fig. 2c). *T. gondii* infection was neither diagnosed either by direct microscopy nor homogenization of liver or brain samples. The IHA test showed that 50.9% (300 samples) were positive, with significant difference (P≤0.004). The prevalence (Fig. 3) of anti-*Toxoplasma* IgG & IgM were negative in cattle and pigs for IgG but 48% were positive for IgM in cattle and 40% in pigs. In goats, 64% were positive for IgG and 28% for IgM, with significant difference (P≤0.006). *C. bovis* (Tab. 2) was in 20% of cattle, without significant difference (P≤ 0.5). Heart was the commonest predicted site for *C. bovis* (16%) followed by tongue (6%). Also, 12% pigs were infected with *C. celluosae,* but without significant difference (P≤0.5). Also, heart was the commonest predicted site for *C. cellulosae* (12%) followed by tongue (4%).

Table 2: Infection rate, organ distribution of *C. bovis* / *C. celluosae* cysts recovered from slaughtered cattle/pigs.

Animals	No. examined	Infected animals	Esophagus	Tongue	Heart	P Value
Cattle	100	20 (20%)	2(2%)	6 (6%)	16 (16%)	P≤0.05
Pigs	100	12 (12%)	1 (1%)	4 (4%)	12 (12%)	P≤0.05

All examined pigs were negative for *T. spiralis* by all used techniques.

Discussion

In the present study, 80% of animals were positive for *Sarcocystis* species, with significant difference (P≤0.001). The data agreed with (Latif *et al*, 1999; Fukuyo *et al*, 2002).

In the present study, 80% of cattle were infected with *Sarcocystis* species, with significant difference (P≤0.005). This result agreed with other studies, in Egypt (Khalifa *et al*, 2008; Sayed *et al*, 2008), Iran (Hamidinejat *et al*, 2010) and in Argentina (Moré *et al*, 2011). On the other hand, it was higher than that reported by Badawy *et al*. (2012) and Ghoneim *et al*. (2014) in Egypt, Latif *et al*. (2013) in Malaysia and Obijiaku *et al*. (2013) in Nigeria. The esophageal muscles were the most preferred for *Sarcocystis* species infection. This agreed with previous studies (Sayed *et al*, 2008; Obijiaku *et al*, 2013; Ghoneim *et al*, 2014). But, Grikenene and Senutaite, (1993) and Ruas *et al*, (2001) found that *Sarcocystis* in all cardiac muscle (100%). In the present study, sarcocysts was not seen by macroscopic examination in all animals' muscles. This agreed with others (Nourani *et al*, 2010, Badawy *et al*, 2012).

In cattle, there were *Sarcocystis*: *S. cruzi*, *S. hominis* and *S. hirsuta*. The thin walled cysts (*S. cruzi*) were more frequently than the thick walled cysts (*S. hirsuta* or *S. hominis*) in all muscles samples of the infected animals. *S. hirsuta* transmitted by felids was less frequently than that of *S. cruzi,* because of low sporocysts production in felids (Fayer, 2004) and the felids tend to

bury their feces. The species of *Sarcocystis* involved in the present work identified microscopically as *S. cruzi*, which depended on the morphological characters of cysts and sporozoites (Calero-Bernal *et al*, 2015). Also, sarcocyst in cattle histologically possessed thick and radially striated cyst wall, which might be *S. hirsuta* or *S. hominis* (Le-vine, 1977). Cysts of *S. hirsuta* or *S. hominis* were morphologically identical by light microscope and could be differentiated only by ultrastructure (Dubey *et al*, 1989). Moreover, out of 100 goats were examined, 92% were infected with *Sarcocystis* species. This data was statistically significant (P≤0.006). This result was in line with other studies (Woldemeskel and Gebreab, 1996) in Ethiopia, (Latif *et al*, 1999) in Iraq, (Al-Quraishy *et al*, 2004) in Saudi Arabia and Morsy *et al*. (2011) in Egypt. Conversely, others reported low prevalence rate as Al-Hoot *et al*. (2005) in Saudi Arabia, Mahran (2009) in Egypt, Kargar *et al*. (2012) in Iran and Kutty *et al*. (2015) in Malaysia.

In the present study, in goats there were *S. capracanis*, *S. hircicanis* identified by micro-scopic cysts and *S. caprafelis* identified by macroscopic cysts (Dafedar *et al*, 2008). But, no macroscopic cysts were detected, which agreed with others (Kudi *et al*, 1991; Singh *et al*, 1992; Morsy *et al*, 2011). The low prevalence of *S. caprafelis* might be due to the less or non- contamination of pastures by dog feces. Kudi *et al*, (1991), Singh *et al*. (1992) and Mahran (2009) reported that the esophagus was the predilection site for microscopic cysts however, in the present study, the tongue was the most infected organ followed by heart.

Generally, pork or pig-meat products are consumed worldwide (Balić *et al*, 2015), but in Islamic countries it is prohibited and there was little studies on parasites transmitted by Egyptian pigs. However, pig husbandry is being practiced as a meat source for some Egyptian families in form of backyard farming. Out of 100 pigs were examined, 68% were infected with *Sarcocystis* species, with the esophagus as the highly infected organ. These data were matched with (Mowafy, 1998) in Egypt, (Gupta and Gautam, 1984; Saleque and Bhatia, 1991) in India, (Obijiaku *et al*, 2013) in Nigeria.

This high prevalence of *Sarcocystis* spp. Infection in different animals species may be due to usage of multiple techniques to detect the *Sarcocystis*, the common usage of dogs as guards by animals owners or presence of other definitive hosts in grazing areas. All of the previous mentioned reasons ensure shedding of infective oocysts into the grazing areas. Also, the differences in organ distribution may be due to oocyst contamination and differences in nutritional status of the hosts that may lead to variations in the immunity against infection and parasites as well (Abdel-Ghaffar *et al*, 2009).

Diagnosis of toxoplasmosis by detecting cysts in tissue is very difficult. So, the detection of *Toxoplasma*-antibody response by particularly by IHAT for man and animals was accepted as a dependable tool for diagnosis (Saleh *et al*, 2014). The present results went with the fact that *T. gondii* infection neither by direct microscopy nor by meat homogenization. However, by IHAT, of 300 samples 50.9% were positive.

In the present study, out of 100 cattle sera slaughtered, 48 samples were seropositive for *T. gondii* infection with an overall prevalence of 48%. This was matched with (Ibrahim *et al*, 1997) in Egypt, (Santos *et al*, 2009) in Brazil and (Holec-Gąsior *et al*, 2013) in Poland. However, it was higher than reported by El Ridi *et al*. (1990) in Egypt, El Fahal *et al*, (2013) in Sudan, Gharekhani (2014) in Iran, Chikweto *et al*. (2011) and Kalita and Sarmah (2015) in India. In goats, the prevalence rate was 64%. This was similar to that recorded by (Fahmy *et al*, 1979; Hefnawy *et al*, 2000) in Egypt, (Prelezov *et al*, 2008) in Bulgaria. On the other hand, it was higher than that reported in Egypt (El-Manyawe *et al*, 2001, 2010; Abou-Zeid *et al*, 2010), in Saudi Arabia (Amin and Morsy**,** 1997; Sanad and Al-Gha

bban, 2007), in Uganda, (Bisson *et al*, 2000), in India (Chikweto *et al*, 2011) and in Somalia (Kadle, 2014).

In pigs, the prevalence rate was 40% that was comparable with others (El Moghazy *et al*, 2011) in Egypt, (Gebrem- edhin *et al*, 2015) in Ethiopia, (Wu *et al*, 2012) in China. But, it was higher than reported by Botros *et al*. (1973) in Egypt, and Chikweto *et al*. (2011) in India.

The differences in prevalence in different regions and animals species might be due to several factors as samples size, techniques used, cats shedding oocysts and environmental pollution (Bisson *et al*, 2000) as well as pet cats (Must *et al*, 2015). Also, the IgM positive antibodies in some samples exhibited a recent or active infection, which might be a zoonotic source due to tachyzoites shedding in all body fluids, including milk (Dubey, 1994).

In the present study, 20/100 (20%) of cattle were infected with *T. saginata* by macroscopic detection of *C. bovis*. This result agreed with Opara *et al*. (2006) in Nigeria, Abunna *et al*. (2008) in Southern Ethiopia. But, the present finding was higher than that reported by Egyptian authors (Haridy *et al*, 1999; Abdo *et al*, 2009; Kandil *et al*, 2012; Elmonir *et al*, 2015), or Ethiopian (Ibrahim and Zerihun, 2012).

Besides, the heart muscles were the most preferred predilection site for *C. bovis* followed by tongue. This agreed with others (Abdo *et al,* 2009; Cueto González *et al*, 2015), but Birhanu and Abda (2014) found that tongue was the most preferred predilection site. Pawlowski and Schultz (1992) and Maeda *et al.* (1996) reported that, the *C. bovis* distribution in tissues varied according to many factors such as breed, age and country origin of the cattle. The prevalence of *C. cellulosa*e in pigs was 12/100(12%). This finding went with Pouedet *et al*. (2002) in Cameroon and Sikasunge *et al*. (2008) in Zambia. However, it was higher than reported by Haridy *et al*. (1999) in Egypt and Kozłowska-Łój and Łój-Maczulska (2014) in Poland.The heart was the most preferred predilection site for *C. cellulosae* followed by tongue. But, Haridy *et al*. (1999) reported that in pigs, the highly infected parts were the whole body skeletal muscles followed by the heart and lastly the head parts including tongue. Willingham *et al.* (2003) reported that infection was common in the tongue than in the heart.

The higher prevalence of cysticercosis in developing countries is associated with The main risk and behavioral factors contributing to the high prevalence include poor knowledge of cysticercosis and lack of knowledge on the proper pork preparation methods (Edia-Asuke *et al*, 2015).

In the present study, no pig was infected with *T. spiralis* by using the different techniques. Morsy et al. (2000) using trichinoscope only available in Cairo abattoirs demonstrated trichinosis in slaughtered pig. Chastel (2004) by PCR reported that the most prevalent diseases of the ancient Egyptian were trichinellosis as well as other helminthic and protozoa parasites and microbial diseases. Seef and Jeppsson (2013) reported that the new influenza virus that was first detected in people in April 2009, was initially referred to colloquially as "swine flu", since it contained genes from swine, avian and human influenza viruses. It can, however, not be transmitted by eating pork or dealing with pigs. In Egypt, several hundred thousand pigs were killed in May, in spite of advice from global health authorities that such an action was unnecessary. Since then rearing pigs was in better hygienic environment. Bruschi (2012) reported that trichinellosis declined significantly as a zoonotic disease, particularly in the developed countries where a reduction of the domestic cycle was observed in the last decades

Conclusion

In the present study, in all animals, homogenization of host tissues was the most sensitive method to detect light *Sarcocystis* infection This may be because of mincing and osmotic lysis of samples enhance the detec-

tion of cysts. Sarcocystosis is the most prevalent infection in El- Minia city and could not be detected by visual means. For *T. gondii*, inspection must include indirect serological tests. Cysticercosis is prevalent in the study area in cattle and pigs. This pointed out to the usefulness of meat inspection procedure for detecting cysticercosis. Finally, as a preventive measure, animal meat should be properly cooked before consumption. Attempts to reduce prevalence of these infections may have some impact on the economics of meat production industries.

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Explanation of figures

Fig. 1: Organ distribution of *Sarcocystis* spp. Fig.2: Micrographs of oesophageal muscles from cattle showing sarcocyst: a- Micrograph of fresh preparation of oesophageal muscles from cattle showing sarcocyst (black arrow) 40x. Cyst situated in a muscle cell, surrounded by cyst wall and compact with cystozoites (blue arrow), b- Micrograph of oesophageal muscles of cattle stained with H&E showing sarcocyst (black arrow) 100x. Thin walled cyst of *S. cruzi* (red arrow), and c- Micrograph of oesophageal muscles of cattle stained with H&E showing sarcocyst (black arrow) 100x;Thick walled cyst of *S. hominis* or *S. hirsuta* (red arrow). Fig. 3: Seroprevalence of IgM and IgG against *T. gondii* in different animal.