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Parasitic and bacterial assessment of edible offal of slaughtered animals at Sharkia abattoirs, Egypt

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ABSTRACT

Foodborne illness as a result of microbiological contamination (parasitic infestations and bacterial contamination) of slaughtered carcasses and edible offal have a significant impact on human health and causes economic losses particularly in developing countries. A total of 4,723 of cattle slaughtered at Sharkia governorate abattoirs, Egypt from January, 2018 to December 2018 were investigated for the presence of *Cysticercus bovis*. Furthermore, a total of 80 samples of edible kidney and liver (40 of each) have been collected randomly from different animal species (Cattle, buffalo, camel and sheep, 10 of each) to detect *S. aureus, E. coli and Salmonella spp*. The results revealed that the prevalence of *C. bovis* was 0.78% and the most infected site was heart (86.49%) followed by masster muscles (13.51%). In addition, the present results showed that the examined edible offal were contaminated with *S. aureus* and *E. coli* while, the examined samples were free from *Salmonella spp*.

Key words: Abattoirs, Edible offal, *C. bovis*, *S. aureus*, *E. coli*, *Salmonella*

INTRODUCTION

Meat and offal in healthy slaughtered animals are mainly free from parasites or pathogens which are fit for human consumption **(Anderson et al., 1992)**. In Egypt, with the high rates of human population growth meat, edible offal consider the common animal protein for human consumption **(Dyab et al., 2017)**.

Edible offal represent about 20-30% of live weight of cattle, buffalo, sheep, and goats **(Umaraw et al., 2015)**. Hence, the bacterial contamination of meat carcasses and edible parts in abattoirs originated from handling, storage, personal hygiene, clothes, improper clean knives, hide, and gut fecal contents on feet or from the environment **(Adzitey et al., 2011; Lavilla Lerma et al., 2013; Tanganyika et al., 2017)**.

Cysticercus bovis is one of the most serious parasitic meat infestations can be detected during meat inspection. Cattle acquire the infection with cysticercosis by accidentally drinking water or ingesting pasture contaminated with *T. saginata* eggs that voided in human stool **(Hashemnia et al., 2015)**. The eggs hatch and released oncospheres which penetrate the intestinal mucosa and travel through the bloodstream which reach mainly to muscles tissues and internal organs where develop into *C. bovis* **(Laranjo-Gonzalez et al., 2018)**. Human infected with adult stage *Taenia saginata* in small intestine through consumption of raw or undercooked beef meat containing larval stage (*C. bovis*) which causes gastrointestinal syndromes as abdominal pain, diarrhea and weight loss **(Abuseir et al., 2007; Beyene and Hiko, 2019; Dorny and Praet, 2007)**. However, *C. bovis* in cattle is asymptomatic, it causes significant economic losses due to condemnation, decrease infected carcass and organs quality **(Boone et al., 2007; Figueiredo et al., 2019; Mirzaei et al., 2016)**. In Egypt, bovine cysticercosis causes great economic losses which the annual financial losses in slaughtered carcasses was 87032 Egyptian Pounds **(Elkhtam et al., 2016)**. Moreover, additional treatment and handling of the infected carcasses and viscera during food inspection such as cooling, transport also causes great economic losses to cattle industry **(Laranjo-Gonzalez et al., 2016; WHO, 1983)**.

Based on routine meat inspection, several reports were recorded about the comparative high prevalence of *C. bovis* worldwide particularly in African countries **(Alemneh et al., 2017)**. In Egypt, **Abdo et al. (2009)** found that the prevalence of *C. bovis* among examined cattle was 1.6 %; while, 6.09% of slaughtered cattle recorded by **Elkhtam et al. (2016)**. However, a high prevalence of *C. bovis* was reported in Ethiopia which ranged from 8.6% **(Beyene and Hiko, 2019)** to 26.25% **(Abunna et al., 2008)**.

Therefore, meat spoilage mainly occurred due to microbial contamination of carcass and internal organs which reduce shelf-life and causing many health hazards and intoxication between consumers as a result of harmful bacterial contamination **(Nychas et al., 2008; Phillips et al., 2006)**.

In Egypt, some abattoirs have poor infrastructure facilities where animals are still slaughtered, handling, eviscerated, processed under poor hygienic conditions that minimize microbiological quality,

safety of the meat carcass and increase threat to consumers' health **(Bello et al., 2015; Khalafalla et al., 2016)**.

It is widely recognized that the most food-borne hazards associated with fresh meat are pathogenic bacteria that can cause food poisoning, illness and gastroenteritis in humans, such as *Salmonellae spp.*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter spp.*, and *E.coli* O157:H7 **(Bersisa et al., 2019; Farzaneh et al., 2016; Gansheroff and O'Brien, 2000; Schlegelova et al., 2004; Zweifel et al., 2014)**. Furthermore, isolation and identification of *Staphylococcus aureus*, *E. coli* and *Salmonella spp.* is much important as it considers a principle indicator for microbiological quality of raw meat **(Jay, 1995)**.

Several previous studies were conducted about prevalence of *S. aureus* and *E. coli* in beef carcass as **(Brichta-Harhay et al., 2008)** in US who recorded that *E. coli* O157:H7 was prevalent on beef carcasses hides and preevisceration of 46.9% and 16.7% respectively. So, to evaluate the bacteriological quality of slaughtered animals and their offal, there is a need to isolate and to identify pathogenic microorganisms to develop a strategy to reduce food illness. As well, to improve the control of bovine cysticercosis, epidemiological investigations based on a reliable, complete update regional data with assessing epidemiological risk factors are necessary **(Laranjo-Gonzalez et al., 2016)**. Therefore, the goal of this work was to study the prevalence of *C. bovis* in cattle at Sharkia governorate, abattoirs. Besides, risk factors such as age and seasons were assessed. Moreover, the prevalence of *S. aureus, E. coli and Salmonella spp*. in edible offal (kidney and liver) of cattle, buffaloes, camel and sheep were thus determined.

MATERIALS AND METHODS

Parasitological Examination

A total of 4,723 of slaughtered cattle were randomly examined from slaughterhouses at Sharkia governorate during the period extended from January, 2018 till December 2018. During normal routine meat visual inspection regulation, masster muscles, liver, heart and kidney were examined for presence of *C. bovis* **(Gracey and Collins, 1992)**. *C. bovis* was collected in a sterile phosphate buffer saline (PBS) and were transferred to laboratory of Parasitology, faculty of veterinary medicine, Zagazig University, Egypt for further examinations.

Examination of *Cysticercus bovis* in cattle

Cysts were examined between two slides pressing and it divided into viable or degenerating as viable cysts were determined as translucent, fluid filled bladder and mature as it has protoscolex while, immature cysts has no protoscolex. Degenerating cysts were calcified when it was solid, and it contains cheesy when smooth, or dull when they contained nothing and were apparently neither viable nor degenerating for criteria of viable cyst **(Fahmy et al., 2015)**.

Bacteriological examination of organs

A total of 80 samples of kidney and liver (40 of each) were randomly collected from different animal species (Cattle, buffalo, camel and sheep, 10 of each) from Sharkia governorate abattoirs in the period extended between January, 2018 and December 2018. The samples were transferred to laboratory of meat hygiene, food control department, faculty of veterinary medicine, Zagazig University, Egypt.

Preparation of samples

Samples have been prepared by techniques recommended by **APHA (1976)**. Twenty five grams from each tissue sample were transferred under aseptic condition to a sterile polyethylene bag containing 225ml of 0.1% sterile buffered peptone water (Oxoid CM9). The content of the bag was then homogenized using stomacher to have a dilution of 10^{-1} and then were allowed to stand for 5 minutes. From the original dilution, 1ml was transferred aseptically to a test tube containing 9ml sterile 0.1% buffered peptone water to prepare a dilution of 10^{-2}, then from which further tenfold decimal serial dilution up to 10^{-7} were prepared. Additionally, the swabs were suspended in 10 ml sterile peptone water 0.1% (Oxoid CM9), then from which further tenfold decimal serial dilution up to 10^{-7} were prepared.

Isolation and identification of *S. aureus*

According to **ISO (1999)**, 0.1 ml of the prepared dilution was spread into plates contained Baird Parker media (Biolife, Italy) with Egg yolk-Tellurite emulsion (Himedia, India) incubated at 37 ºC, and observed after 48 hours. Characteristic black colonies (1-1.5 mm in diameter, black, shiny convex colonies, with narrow white margin) surrounded by a narrow white margin with a zone of clearing were counted to obtain the total *S. aureus* counts per gm. Each suspected colony was collected and cultured on slope agar for additional biochemical and

microscopical identification. The collected isolates were morphologically identified **(Cruickshank et al., 1975)**. Furthermore, biochemical identification of *S. aureus* were carried out by Catalase activity test, Oxidase test, Detection of Arginine decarboxylase (ADH), Bile esculin test, Mannitol test, Coagulase test, Thermostable nuclease test "D-Nase activity" **(MacFaddin, 2000)**

Detection and typing of enterotoxin (Shingaki et al., 1981)

The clear culture supernatant fluid was tested serologically by Reverse Passive Latex Agglutination technique "RPLA" using kits for the detection of staphylococcal enterotoxins A, B, C and D (**SET-RPLA**, **Denka Sekeu LTD, Japan)**.

Detection of *S. aureus* enterotoxin genes by PCR

The genomic DNA extraction was performed using GeneJET Genomic DNA Purification Kit (Thermo Scientific, #K0721). One ml of an overnight incubated broth was centrifuged at 13000 rpm for 2 minutes at 4 ^{o}C and the supernatant was discarded. The pellet resuspended in 180 ul of Gram positive bacteria lysis buffer which consists of 20mM Tris-Hcl, 2mM EDTA, 1.2% Triton X-100 and lysozyme 20mg/ml. and then incubated for 30 min at 37^{o}C. All preparations of subsequently DNA extraction were performed using supplier's instructions. Furthermore, template DNA extracted from the standard strains *S. aureus* ATCC 13565 (SEA), ATCC 14458 (SEB), ATCC19095 (SEC), FRI 361 (SED) as a positive controls for PCRs reactions. Accurately, PCR primers as specific for detection of enterotoxin genes as virulence factors of *S. aureus* were listed in (Table 1).

DNA Amplification reaction of *S. aureus*

PCR was performed in 25 µl reaction mixture as previously described by **Rall et al. (2008)**. DNA was amplified in a thermal cycler Master cycler, Eppendorf, Hamburg, Germany. The reaction mix (25 µl) consisted of 5 µl of the bacterial lysate, 5 µl of 10x assay buffer for Taq polymerase containing 1.5 mM MgCl2, 2 µl of 10mM dNTP mix 1 µl each of forward and reverse primer (10 pmol) and 1.25 U of Taq DNA polymerase made up to 25 µl using sterile distilled water. The following conditions: initial denaturation for 5 min at 94°C followed by 30 cycles of denaturation (94°C for 2 min), annealing (50°C for 1 min), and extension (72°C for 1 min). Then, a final extension step (72 °C for 5 min) was performed. DNA amplicons were analyzed by 1% of agarose gel electrophoresis.

Table 1: Primer sequences of *S. aureus*

Target gene	Primer	Oligonucleotide sequence (5′ → 3′)	Product size (Base pair)	References
Sea	SEA-1	ttggaaacggttaaaacgaa	120	**(Johnson et al., 1991; Rall et al., 2008)**
	SEA-2	gaaccttcccatcaaaaaca		
Seb	SEB-1	tcgcatcaaactgacaaacg	478	
	SEB-2	gcaggtactctataagtgcc		
Sec	SEC-1	gacataaaagctaggaattt	257	
	SEC-2	aaatcggattaacattatcc		
Sed	SED-1	ctagtttggtaatatctcct	317	
	SED-2	taatgctatatcttataggg		

Determination of total coliform count (MPN)

Three tubes most probable number (MPN) method recommended by **ICMSF (1978)** was adopted as follows; one ml of decimal dilution was inoculated separately into each of three MacConkey broth tubes (HiMedia, Mumbai) with inverted Durham's tubes. The inoculated tubes were incubated at 37º C, and then examined after 24 hours and 48 hours. Positive tubes with acid and gas productions were recorded. The most probable number of coliforms was calculated.

Isolation and Identification of *E. coli*

A loopful of each positive MacConkey broth tubes (acid and gas) was streaked into eosine methylene blue (EMB) agar (Oxoid, CM 69). The inoculated plates were incubated at 35°C for 24 h. Colonies with a metallic sheen were isolated for further identification. Suspected isolates of *E. coli* were identified based on morphological characters either microscopical examination **(Cruickshank et al., 1975)** or motility test **(MacFaddin, 2000)**. Moreover, biochemical tests were performed to identify *E. Coli* as Indole test, Methyl Red Test, Voges – Praskauer test, Urease test, Hydrogen sulphide production test, Gelatin hydrolysis test, Oxidation–Fermentation test, Nitrate reduction test, Oxidation–Fermentation test, Nitrate reduction test, Detection of Ornithine decarboxylase (ODC), Detection of L-lysine decarboxylase (LDC), Detection of Arginine decarboxylase (ADH), Detection

of β- galactosidase (ONPG), Fermentation of sugars **(Krieg and Holt, 1984)**.

Serological identification of *E. coli*:

Serologically, the isolates were identified as **(Kok et al., 1996; Simmon, 1926)** by using rapid diagnostic polyvalent and monovalent *E. coli* antisera sets **(DENKA SEIKEN Co., Japan)** for diagnosis of the Enteropathogenic types.

Isolation and identification of *Salmonella spp.* (ISO, 2002)

Twenty five grams of the sample was pre-enriched in 225ml of buffered peptone water 1%, and then incubated at 37^{o}C for 18 h ±2h. After that, 1 ml of pre enrichment broth was transferred into a tube containing 9 ml of Rappaport Vassiliadis with soya (RVS broth) and incubated at 43^{o}C for 24hr ±3hrs.

Selective plating and identification

A loopful from the culture obtained in the previous step was streaked on the plates of Xylose Lysine Desoxycholate ager (XLD agar), incubated at 37^{o}C ±1^{o}C and examined after 24hr±3hrs, for presence of suspected colonies of *Salmonella* which have a black center and lightly transparent zone of reddish color due to color change of the indicator. The suspected colonies were picked up and purified on Trypticase Soya agar for further identification. Moreover, *Salmonellae* were identified either morphologically (Gram's stain) or biochemical tests using triple sugar iron agar (TSI) slant TSI incubated at 37^{o}c for 24h, Lysine Iron agar LIA incubated at 37^{o}C for 18-24hrs, citrate utilization, indole production, methyl red, motility and voges – proskauer test **(Krieg and Holt, 1984)**.

Statistical analysis

Regarding to *C. bovis*, data were analyzed with Chi square ($\chi 2$) tests using IBM SPSS Statistics for windows software version 21. P-values <0.05 were considered statistically significant. Results of microbiological analysis were converted to $\log_{10}$ CFU/g values and reported as mean values ± standard error (S.E). Statistical analysis of data was done by using the statistical package for social sciences **(SPSS-16.; Chicago, IL, USA)** software. One way analysis of variance (ANOVA), differences among individual means were compared by Tukey–Kramer honestly test, at 95% level of confidence, P<0.05 was considered as significant.

RESULTS

Morphological identification and prevalence of *C. bovis*

Based on visual and palpation examinations, *C. bovis* was small cysts which their size approximately ranged from 1 – 2 cm. The present results showed that 21 (56.75 %) cysts are mature, fluid filled cyst, viable as it has single invaginated scolex, while 16 (43.24 %) of 37 cysts were degenerated. Regarding the prevalence of *C. bovis*, the present study revealed that the prevalence rate among cattle carcasses was 0.78% (Table 2). On the other hand, the infection rate of heart was 86.49% followed by masster muscles which reached up to 13.51%. The difference between prevalence rates among different organs is statistically significant (P <0.05).

Concerning seasonal dynamics, our results as shown in (Table 2) revealed that the prevalence of *C. bovis* in summer and autumn was higher than winter and spring but no statistically significant difference between the prevalence rate of any four different seasons (P=0.08).

Regarding to age, our study showed that animals more than three years were likely to be infected with *C. bovis* than young ones (Table 3). Moreover, the difference between two groups of age was statistically significant (P<0.05).

Table 2: Prevalence of *C. bovis* in slaughtered cattle with infected organs regarding to seasonal variation

Season	No. of examined cattle	No. of infected cattle	%	Organs infected							
				Heart	%	Masster Muscles	%	Liver	%	Kidney	%
Winter	1533	3	0.20	2	100	0	0	1	33.33	0	0
Spring	1496	9	0.60	8	88.89	1	11.11	0	0	0	0
Summer	660	13	1.97	10	76.92	3	23.08	0	0	0	0
Autumn	1043	12	1.15	11	91.67	1	8.33	0	0	0	0
Total	4732	37	0.78	31	86.49	5	13.51	1	2.70	0	0

Table 3: Age variation of infected carcasses with bovine cyticercosis

Age	No. of examined carcasses	No. of infected carcasses	%
Under three years	3049	9	0.30
Above three years	1683	28	1.66
Total	4732	37	0.78

Bacteriological examination of kidney and liver from different animal species slaughtered at Sharkia governorate abattoirs:

Concerning to the prevalence of *S. aureus* in kidney and liver, our findings showed that overall prevalence of *S. aureus* in the examined kidney and liver was 17 (21.25%). It was isolated from 3(30%), 2(20%), 2(20%) and 3(30%) of the examined cattle, buffalo, camels and sheep kidney respectively. Regarding to liver samples, it was isolated from 2(20%), 3(30%) and 2(20%) of the examined cattle, buffalo and camels respectively (Figure 1). However, there was no *S. aureus* isolated from sheep' liver. The mean count of *S. aureus* was 4.8 ± 0.15, 4.5 ± 0.06, 4.2 ± 0.24 and 4.4 ± 0.12 log_{10} cfu/g in the examined cattle, buffalo, camel and sheep kidney samples respectively with minimum counts of 3.9, 4.4, 4 and 4.2 log_{10} cfu/g respectively and maximum counts of 5.4, 4.6, 4.5 and 4.9 log_{10} cfu/g respectively. On the other hand, the examined liver samples of cattle, buffalo and camel, *S. aureus* counts ranged from 4.3 to 4.7, 4 to 4.6 and 4 to 4.3 log_{10} cfu/g respectively with mean counts of 4.5 ± 0.19, 4.3 ± 0.17 and 4.1 ± 0.15 log_{10} cfu/g respectively (Table 4).

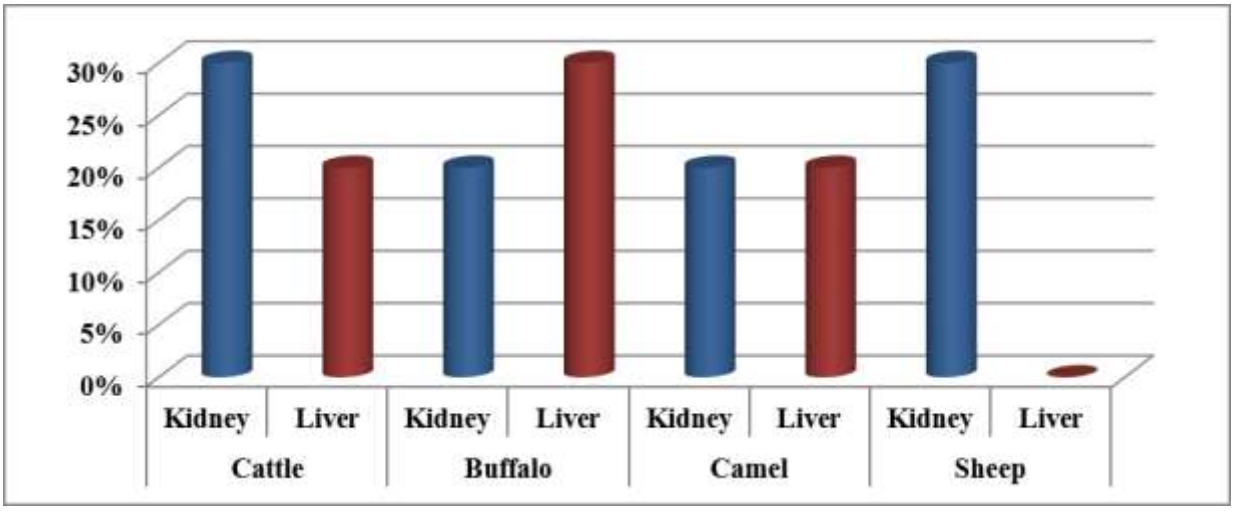

Figure 1: Prevalence of *S. aureus* in kidney and liver

Table 4: Statistical analytical results of *S. aureus* in kidney and liver (N= 10 of each organ) log_{10} cfu/g).

S. aureus	Cattle		Buffalo		Camel		Sheep	
	Kidney	**Liver**	**Kidney**	**Liver**	**Kidney**	**Liver**	**Kidney**	**Liver**
Minimum	3.9	4.3	4.4	4	4	4	4.2	ND
Maximum	5.4	4.7	4.6	4.6	4.5	4.3	4.9	ND
Mean ± S.E	4.8 ± 0.15	4.5 ± 0.19	4.5 ± 0.06	4.3 ± 0.17	4.2 ± 0.24	4.1 ± 0.15	4.4 ± 0.12	ND

N: Number of examined organs (10 of each) cfu: colony forming unit
S.E: Standard error of mean ND: Not detected
Means are not significantly different (p> 0.05)

Detection of *S. aureus* enterotoxin genes in examined samples

Based on multiplex PCR technique, 13 identified strains of *S. aureus* were screened for presence of enterotoxins. Using agarose gel electrophoresis patterns, the sizes of amplified products (amplicons) were identical to those predicted from the design of the primers (Table 1). It was found that SEA, SED and SEA & SEB was detected in 2**(**15.4%), 1(7.7%) and 1(7.7%) of the tested *S. aureus* strains respectively, while, 9(69.2%) of the tested *S. aureus* strains were negative for enterotoxin genes (Table 5). In the present study, SEA & SED genes were amplified in only one *S. aureus* isolate. As well, we didn't detect SEC gene in any isolate.

Table 5: Prevalence of enterototoxin genes of *S. aureus* strains isolated from the examined samples (n= 13 strains).

Target gene	No.	%
SEA	2	15.4
SEB	1	7.7
SEA & SED	1	7.7
Negative strains	9	69.2

SEA: *S. aureus* enterotoxin A, SEB: *S. aureus* enterotoxin B, SED: *S. aureus* enterotoxin D

Most probable number (MPN) of coliform:

Our results showed that the value of MPN of coliform ranged from 2.5 to 3.6, 3.3 to 5, 2.5 to 5 and 3 to 5 log_{10} cfu/g, with mean values of 2.9 ± 0.11, 4.4 ± 0.23, 3.6 ± 0.29 and 3.6 ± 0.22 log_{10} cfu/g in the examined cattle, buffalo, camel and sheep kidney samples respectively. However, the mean values of MPN of coliform were 4.5± 0.19, 4.1± 0.24, 3.1± 0.20 and 3± 0.12 log_{10} cfu/g, with minimum values of 3.5, 3, 2.5 and 2.5 log_{10} cfu/g, and maximum values of 5, 5, 4, 3.8 log_{10} cfu/g in the examined cattle, buffalo, camel and sheep liver samples respectively (Table 6).

Table 6: Statistical analytical results of MPN of coliform in kidney and liver (N= 10 of each organ) log_{10} cfu/g).

MPN of coliform	Cattle		Buffalo		Camel		Sheep	
	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver
Minimum	2.5	3.5	3.3	3	2.5	2.5	3	2.5
Maximum	3.6	5	5	5	5	4	5	3.8
Mean ± S.E	2.9^{C} ± 0.11	4.5^{a}± 0.19	4.4^{A} ± 0.23	4.1^{a} ± 0.24	3.6^{B} ± 0.29	3.1^{b} ± 0.20	3.6^{B} ± 0.22	3^{b} ± 0.12

N: Number of examined organs (10 of each)
cfu: colony forming unit
S.E: Standard error of mean
Means carrying different superscript letters are significantly different ($p < 0.05$)

Prevalence of *E. coli*

The obtained results in (Table 7) showed that the prevalence of total *E. coli* in kidney and liver samples from cattle, buffaloe, camel and sheep was 9 (11.25%). In addition, the prevalence of *E. coli* was 20% in both buffaloe, camel kidneys and livers. Meanwhile, *E. coli* failed to be detected in the examined cattle liver and kidney as well as sheep liver.

Serological identification of the isolated *E. coli* strains

In the current study, sero-diagnosis of the isolated *E. coli* strains revealed 6 serologically different strains as follow: O111:H2 3(27.3%), EHEC; O26: H11 2(18.2%), EHEC**;** O128: H2 2(18.2%) ETEC; O146:H21 2(18.2%), EPEC; O111: H2 1(9.1%), EHEC; and O75 1(9.1%), EPEC (Table 8).

Table 7: Prevalence of *E. coli* in kidney and liver (N= 10 of each organ).

Prevalence (positive samples)	Cattle		Buffalo		Camel		Sheep	
	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver
Number	0	0	2	2	2	2	1	0
%	0	0	20%	20%	20%	20%	10%	0
Total	9 (11.25%)							

Table 8: Serological identification of *E. coli* (N=11).

Serodiagnosis	Prevalence	Strain characterization
O111 : H2	3 (27.3%)	EHEC
O26 : H11	2 (18.2%)	EHEC
O128 : H2	2 (18.2%)	ETEC
O146 : H21	2 (18.2%)	EPEC
O111 : H2	1 (9.1%)	EHEC
O75	1 (9.1%)	EPEC

N: Number of examined strains.
EHEC: Enterohemorrhagic E. coli, ETEC: Enterotoxigenic, EPEC: Enteropathogenic.

Regarding *Salmonella*

Our findings revealed that *Salmonella* wasn't isolated from the examined kidney and liver samples of cattle, buffalo, camel and sheep.

DISCUSSION

Bovine cysticercosis is a zoonotic disease that is associated with a major economic significance and concerns with public health **(Asaava et al., 2009)**.

Regarding the prevalence of *C. bovis*, the present study revealed that the prevalence rate among cattle carcasses was 0.78%. This finding approximately agreed with **El-Alfy et al. (2017)** who found the prevalence of *Cysticercus bovis* cysts in cattle were 0.51% in Dakahlia province and a little lower than a study by **Abdo et al. (2009)** who found that the prevalence was 1.6% in Assiut governorate, Egypt. Also, low contamination rate was reported in Belgium in bovine carcasses of 0.12% - 0.16%

(EFSA, 2013). However, the obtained results were lower than those recorded in several previous reports in Egypt as **(Dyab et al., 2017)** and **(Elkhtam et al., 2016)** who found that the prevalence of *C. bovis* among slaughtered cattle was 7.5% and 6.09% in Aswan and Menofia governorates respectively. Furthermore, a much higher prevalence (20%) was recorded by **Abdel-Hafeez et al. (2015)**. The difference in the prevalence rates is possibility due to many factors as differences in production systems, climatic variations among different areas and sample size, monitoring measures as well as eradication programs between countries **(Abdo et al., 2009; Allepuz et al., 2009)**. This interpretation supported by **El Shazly et al. (2006)** who reported approximately low infection rate with *Taenia saginata* (1.1%) in patients at Mansoura University Hospitals as eggs can pass in stool and represent a source of infection for cattle. On the other hand, the heart was the most infected organ with *C. bovis* than other organs. Our results agreed with **(Abunna et al., 2008)** who reported that the distribution of cysticerci were high in heart (29.2%) and masseter (26.7%). In addition, **Abdel-Hafeez et al., (2015)** and **Maeda et al. (1996)** found that the heart muscles were the most infected organ. On the other hand, **Oryan et al. (1995)** stated that the most common infected parts were the muscles of the shoulder followed by masseter muscle. The distribution of *T. saginata* cysts in different tissues of naturally infected cattle may depend on geographical area, breed and age of the animal, as well as activity of the muscle group **(Maeda et al., 1996; Pawlowski and Schultz, 1972)**.

Concerning seasonal dynamics, our results revealed that the prevalence of *C. bovis* in summer and autumn was higher than winter and spring but no statistically significant difference between the prevalence rates of any four different seasons. These findings agreed with results obtained by **Hashemnia et al. (2015)** and **Mirzaei et al. (2016)**. In contrast, **Oryan et al. (1995)** recorded that the highest incidence during spring and autumn and lowest in summer and winter. Regarding to age, our study showed that the infection rate was higher in old animals than young ones. The results obtained were approximately similar to **Abdo et al. (2009)** and **Mirzaei et al. (2016)** who found that the infection rate was higher in older cattle than young age. This might attributed to old age mainly above 2 years exposed to accumulative of the different sources of infection **(Abdo et al., 2009)**. However,

Oryan et al. (1995) found no variation in the infection rate in animals according to the age as the animals gained life long immunity to superinfection.

Food safety have received a big attention for food service organizations due to mishandling which lead to serious illness for consumers, and great economic losses **(Rani et al., 2017)**.

Concerning to the prevalence of *S. aureus* in kidney and liver, our findings showed that overall prevalence of *S. aureus* in the examined kidney and liver was 17 (21.25%). However, there was no *S. aureus* isolated from sheep' liver. Similar results were obtained by **Ibrahim et al. (2013)**. Meanwhile, lower count was reported by **Khalil et al. (2018)** who reported that the mean values of Staphylococcal count / g of beef liver was $1.1\times10^2 \pm 1.4\times10$ cfu/g. Such variations may be attributed to the method of isolation and enumeration, the habitats from which samples were collected as well as the sanitary measures adopted in an abattoir. Furthermore, the presence of Staphylococci in edible offal may be due to contamination during dressing and evisceration in slaughterhouse, contaminated equipment, handling and processing **(Datta et al., 2012)**.

S. aureus is represented the most serious cause of food-borne illnesses in the world as enterotoxins are the main cause of gastrointestinal symptoms **(Gucukoglu et al., 2012; Ibrahim et al., 2013)**. The present study revealed that SEA, SED and SEA & SEB enterotoxins genes were detected in some of tested *S. aureus* strains. For occurrence of SEA in *S. aureus* isolates, our findings were approximately agreed with **Kitai et al. (2005)** who reported that the occurrence SEA in *S. aureus* isolates in raw chicken meat was 17.9%. However, our results were higher than obtained by **Pu et al. (2011)** (1%), **Hwang et al. (2007)** (7%). For SEB gene**,** our results (7.7%) were lower than reported by **Kitai et al. (2005)** 64.1% and **Mathenge et al., (2015)** (13.9%). However, **(Khalil et al., 2018)** didn't detect any SEB gene in the examined *S. aureus* isolates. Also, we didn't detect SEC gene in any isolate. Similar results were reported by **El Bayomi et al. (2016), Hwang et al. (2007)** and **Pelisser et al. (2009)**. SEA are the most common enterotoxins and a main cause of gastroenteritis **(Khalil et al., 2018)**, therefore, a highly percent was detected in our study. The higher incidence of the microbial contamination of the offal might

be attributed to unsanitary conditions during slaughtering at slaughterhouses.

Coliforms are indicator organisms that indicate the potential fecal contamination. Moreover, isolate such microorganisms from food in large quantity indicates the probability of culturing the organism in unhygienic condition or the usage of polluted water during processing **(National Research Council, 1985)**.

Our results showed the value of most probable number of coliform in the examined cattle, buffalo, camel and sheep kidney and liver samples. Nearly similar results was reported by **Ibrahim et al. (2013)**. Furthermore, **Gomes and Furlanetto, (1987)** who recorded that MPN was 2.4×10^5 cfu/g in beef liver. However, higher results obtained by **El-Shamy (2011)** which reached up to $9.7\times10^5\pm3.3\times10^5$ in liver samples, while, $7.44\times10^3\pm 1.86\times10^3$ and $4.27\times10^3\pm 0.89\times10^3$ cfu/g were recorded for liver samples of cattle and camel respectively **(Faten et al., 2013)**. The source of coliform contamination in edible offal began during skinning from the hide and hair of animal by knives and workers also during evisceration due to puncture of internal organs or from air, worker utensils or clothes, water used for carcass and offal wash **(Abdalla et al., 2009; Abdelsadig, 2006)**. High coliform count of edible offal may be related to the unhygienic conditions of offal collection after evisceration and contamination with fecal matters, this implies that these offal are viable source of various diseases **(Ukut et al., 2010)**. Regarding to the prevalence of *E. coli*, Our results were lower than obtained by **Salem (2001)** who found that the prevalence of *E. coli* was 40% and 60% in liver samples from butcher's shop and street cars respectively. However, *E. coli* strains isolated from cattle liver samples were 3 (15 %) and 2 (10%) in camel liver samples **(Faten et al., 2013)**. Moreover, **Surkiweicz et al. (1977)** found that the prevalence of *E. coli* in chopped liver was 1%. Wherefore, presence of pathogenic microorganisms such as *E. coli* in abattoirs due to contamination of offal during the slaughter and processing of animals and it should pay attention for the possible public health hazards as well as the liability for the occurrence of foodborne intoxication **(Bintsis, 2017; Garcia et al., 2010)**.

Regarding to serological identification of the isolated *E. coli* strains, the present work revealed 6 serologically different strains. Nearly the same *E. coli* serotypes were identified by **Khalafalla et al. (1989)** who

found O111, O128 and O26 from cattle livers. On the other hand, our results were higher than **Edris et al. (2013)** who indicated that isolates *E. coli* from bovine liver was serotyped as O26 which reached 8% (EHEC) and O128 (4%) (ETEC); while, in kidneys O26 (4%) (EHEC) and O119:H6 (4%) (EPEC). Furthermore, **Faten et al. (2013)** found that the serotyping of *E. coli* isolated from cattle livers were O26:K60(B6) EPEC, O127: K63(B8) EPEC, untypable with prevalence of 10%, 5 % and 5% of examined samples respectively and added that the serotyping of *E. coli* isolated from the examined camel liver samples was O111: K58 (B9) EHEC and O26 : K60(B6) EHEC which the infection rate reached up to 5% of examined samples in both strains. Regarding to *Salmonella***,** our findings revealed that *Salmonella* wasn't detected in the examined kidney and liver samples. This result agreed with findings obtained by **Surkiewicz et al. (1977)** who didn't able to isolate *Salmonella* from the examined offal samples. Meanwhile, our study disagreed with **Atabay et al. (2012)** who isolated *Salmonella* from 8.57%, and 5.71% from bovine liver and kidney respectively. Besides, **Faten et al. (2013)** who found *Salmonella* in 3 (15%) of liver samples. Furthermore, **Samuel et al. (1980)** showed that isolation rate of *Salmonella* was 32% at evisceration and increased to be 82% at inspection which explained due to contamination of gastrointestinal tract. In this study, we assumed that the internal organs weren't exposed to high surface of *Salmonella* contamination.

CONCLUSION

The present study confirmed that bovine cysticercosis is prevalent at Sharkia governorate, Egypt. These obtained data highlighted the need of detailed meat inspection to provide useful information for control of the disease, to reduce economic losses and improve food safety. Furthermore, in the present work the examined edible offal revealed high level of contamination with *S. aureus* and *E. coli* of public health significance which the possible sources of contamination might from soil, water, equipment and utensils and improper handling. So, the hygienic measures to improve meat quality were therefore addressed and control measures were implemented from animal slaughtering till human consumption to decrease public safety risks.

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الملخص العربي

التقييم الطفيلى والبكتيرى للأحشاء الداخلية فى الحيوانات المذبوحة بمجازر محافظة الشرقية، مصر

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***قسم الطفيليات – كلية الطب البيطرى – جامعة الزقازيق**

****قسم مراقبة الأغذية – كلية الطب البيطرى – جامعة الزقازيق**

*****وحدة مكافحة العدوى – مستشفيات جامعة الزقازيق - الزقازيق**

تؤثر الأمراض المنقولة بالغذاء سواء الطفيلية أو البكتيرية الناتجة عن تلوث ذبائح الحيوانات وأحشائها الداخلية تأثيراً ضاراً على صحة الإنسان بالإضافة إلى الخسائر الإقتصادية الناجمه عنها. أجريت هذه الدراسة على ٤٧٢٣ من ذبائح الأبقار مختلفة الأعمار بمجازر محافظة الشرقية خلال الفترة من يناير ٢٠١٨ إلى ديسمبر ٢٠١٨ للكشف عن مدى إصابتها بالكيسات المُذَنَّبَة (اليرقة المثانية البقرية). كما تم جمع ٨٠ عينة من الأكباد والكلاوي الصالحة للأكل (٤٠ من كل منها) بشكل عشوائي من ذبائح الحيوانات المختلفة (الأبقار والجاموس والجمال والأغنام ، ١٠ من كل منها) للكشف عن المكورات العنقودية الذهبية وميكروب الإيشيريشيا كولاي والسالمونيلا.

أوضحت النتائج أن معدل انتشار اليرقة المثانية البقرية كان ٠.٧٨٪ وكان أكثر الأماكن إصابة هى عضلات القلب (٨٦.٤٩٪) يليها العضلات الماضغة (١٣.٥١٪)

من ناحية أخرى ، تم عزل ميكروب المكور العنقودي الذهبي من عينات الكلاوي المأخوذة من ذبائح الأبقار والجاموس و الجمال والأغنام وكذلك تم عزله من عينات الأكباد لنفس الحيوانات، بينما لم يتم عزل المكور العنقودي الذهبي من عينات الأكباد المجمعة من ذبائح الأغنام. على الجانب الأخر ، تم عزل ميكروب الإيشيريشيا كولاي من عينات كلاوي ذبائح الجاموس و الجمال والأغنام وكذلك أكباد الجاموس والجمال ، بينما لم يتم عزل ميكروب الإيشيريشيا كولاي من كلاوي وأكباد الأبقار وأيضا لم يتم عزله من أكباد الأغنام. على النقيض، أظهرت النتائج خلو جميع عينات الكلاوي والأكباد المأخوذة من ذبائح الأبقار والجاموس والجمال والأغنام من ميكروب السالمونيلا. خلصت الدراسة أن تلوث أحشاء ذبائح الحيوانات بمجازر الشرقية ببعض ميكروبات التسمم الغذائي يمكن أن يُعزى إلى المعالجة غير الصحية وغير السليمة للحيوانات أثناء الذبح.

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