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The prevalence of *Cysticercus bovis* in slaughtered cattle in El-Bassatine abattoir, Cairo, Egypt.

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ABSTRACT

In humans who eat raw or undercooked meat, *Cysticercus bovis*, a larval stage of *Taenia saginata*, one of the most prevalent foodborne cestodes worldwide, causes cysticercosis in cattle. Current techniques for detecting *C. bovis* in cattle rely on a visual assessment of the meat. This study aimed to validate the molecular diagnosis of *C. bovis* obtained through visual inspection at the El Bassatine abattoir in Cairo, Egypt. From January 2022 to December 2022, a cross-sectional investigation was carried out to ascertain the prevalence of *C. bovis* in cattle slaughtered at the El Bassatine abattoir in Cairo, Egypt. A total of 65264 cow carcasses (64884 males and 380 females) were inspected for obvious signs of *C. bovis*. Positive samples were subsequently confirmed by polymerase chain reaction (PCR) amplification for HDP2 gene sequencing. Out of the 65264, 561 cattle tested positive for *C. bovis*, resulting in a frequency of 0.86%. According to seasonality and animal sex, *C. bovis* was not found in female cattle throughout the year, while in male cattle, the prevalence was 173 (1.01%), 200 (1.18%), 114 (0.75%), and 74 (0.47%) in winter, spring, summer, and fall, respectively. The most infested organ was the heart (0.76%), then the masseter muscle (0.07%), skeletal muscle (0.03%), and tongue (0.02%). Through molecular analysis, a diagnostic band was identified that targeted genes for *C. bovis* was the HDP2 gene with an amplicon size of 599 bp. Because of this, although meat inspection can be used as a main screening method for *C. bovis*, more precise molecular testing is required for a correct diagnosis.

1. INTRODUCTION

Cattle Animal farms are impacted by the major public health concern of cysticercosis. The most common human tapeworm in the world is *Taenia saginata*, a foodborne cestode (FAO/WHO 2014). Cattle are intermediate hosts; humans are the ultimate hosts. An adult worm generates 3–7 proglottids every day, each containing 30,000–50,000 eggs, as it grows in the small intestine (Lesh and Bardy, 2020). When humans eat raw or undercooked meat, they become infected with *Cysticercus bovis*, the larval stage of *T. saginata* that causes cysticercosis in cattle.

Grazing on pasture polluted with human feces carrying Taenia eggs might cause cattle to become directly or indirectly infested (Marshall et al., 2016). When raw beef with live *T. saginata* cysts is consumed by humans, infection results (Braae et al., 2018). Eight to twenty persons could become infected from a bovine carcass (Sato et al., 2018).

The visual inspection of meat is used to regularly detect *C. bovis* in cattle (FAO/WHO 2014). The main method of detecting bovine cysticercosis in Egyptian abattoirs is visual inspection of a carcass using a knife and eye technique at specific predilection sites and organs such as the heart, tongue, masseter muscles, esophagus, and diaphragm (El-Sayad et al., 2021). This method needs to be modified, though, as lesions from *C. bovis* can be mistaken for those from other species, such Sarcocystis and Actinobacillus, or ~~for~~ other local alterations (Ogunremi et al., 2004). Both morphological and histological methods can be used to diagnose cysticercosis in cattle. It was demonstrated that molecular analysis—specifically, unique PCR techniques derived from the HDP2 gene sequence—is more precise, expedient, and sensitive than morphological features (Gonza'lez et al., 2000).

Consequently, the purpose of this study was to ascertain the prevalence of *C. bovis* in cattle that are killed at the El Bassatine abattoir in Cairo, Egypt, during the year 2022, and to validate the molecular diagnosis of *C. bovis* that is identified through visual inspection.

2. MATERIAL AND METHODS

2.1. Study area and period

This study was conducted from January 2022 until the end of December 2022 during different seasons in the El Bassatine abattoir, Cairo, Egypt. This study was approved by the Scientific Research Ethics Committee, Faculty of Veterinary Medicine, Benha University, Egypt and the Ethical approval number is BUFVM-21-12-2021.

2.2. Sample collection

A total of 65264 cattle carcasses (64884 males and 380 females) were examined for gross lesions of *C. bovis* by Trained veterinarians under close supervision of local authorities. Veterinarians individually inspect each slaughtered animal during their routine duties. The samples were represented by 17203 during winter (17092 males and 111 females), 17074 during spring (16981 males and 93 females), 15285 during summer (15170 males and 115 females), and 15702 during autumn (15641 males and 61 females). All samples were collected and transferred without

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delay to the laboratory in the ice box for further investigation.

2.3. Visual inspection for Cysticercosis in slaughtered cattle

The slaughtered cattle were evaluated using standard and detailed visual inspection methods. During this investigation, the routine postmortem inspection consisted of a visual inspection of carcass musculature revealed during dressing and an assessment of muscular surface exposed by incisions in masseter muscles, heart, tongue, diaphragm, and esophagus muscles. If any locations tested positive for *C. bovis*, the infested carcasses were inspected further for shoulders, thighs, and skeletal muscles (Gracey et al., 1999).

2.4. Molecular confirmation of Cysticercosis in slaughtered cattle

For molecular identification, viable cysts were maintained in at -80 °C. DNA was extracted from the cysts using the DNeasy Tissue Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Primers were used in a traditional polymerase chain reaction (PCR) to identify the *C. bovis* HDP2gene as reported by González et al. (2002).

Centrifugation (16,000 × g, 10 min at room temperature) was performed after the pellet was resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) in the presence of 0.3 M sodium acetate, pH 4.8. The pellet was then precipitated twice with isopropanol for 5 min. The DNA pellet was re-suspended in TE buffer after being cleaned with 100% and 70% ethanol. Until it was used, the extracted DNA was kept at -20°C.

A Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany) was used to execute the amplification. A total of 25 µl of PCR mix using 2X Master Mix and 5 µl of DNA template (10 pmol of a forward primer (5′-CAGTGGCATAGCAGAGGAGGAA-3′) and 10 pmol of a reverse primer (5′-GGACGAAGAATGGAGTTGAAGGT-3′)) were applied. The following technique was used to amp up the DNA: first denaturation at 94°C for 2 minutes, then 35 cycles of denaturation, annealing, and extension at 94°C, 56°C, and 72°C for 1 minute each, with a single final extension lasting 7 minutes at 72°C. The amplified DNA fragments were photographed, seen using an ultraviolet transilluminator, and separated by gel electrophoresis using 1.5% agarose gel stained with ethidium bromide solution (0.5 µg/ml). The size of the fragment was established using a 100 bp DNA ladder.

2.5. Statistical analysis

Descriptive statistics including frequency, percentage, and proportion were used in the statistical analysis, which was carried out using STATA 17 (STATA Inc., USA).

3. RESULTS

Following a postmortem analysis, it was determined that 561 of the 65264 male cattle (64884 males and 380 females), or 0.86 % of the total cattle were likely infected with *C. bovis* cysts. We looked at the sex-specific infection rate in cattle (Fig. 1). In contrast to the year-round absence of *C. bovis* detection in females (0%, 0/380), the frequencies of infestation in males varied seasonally. In winter, spring, summer, and autumn, the prevalence of male cattle was 173 (1.01%), 200 (1.18%), 114 (0.75%), and 74 (0.47%), respectively.

The anatomical distribution of the visually identified cysts in different seasons and in different killed cow organs was also studied (Fig. 2). The most affected muscle was the heart (0.76%), then the masseter muscle (0.07%), skeletal muscle (0.03%), and tongue (0.02%).

Eleven (73.3%) of the fifteen probable cysts that were removed from cattle had PCR confirmation (Fig. 3).

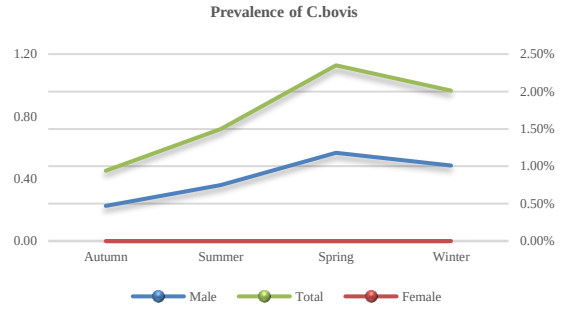

Fig. 1 Prevalence of *C. bovis* in slaughtered cattle at El- Bassatine abattoir, Cairo during different seasons in 2022

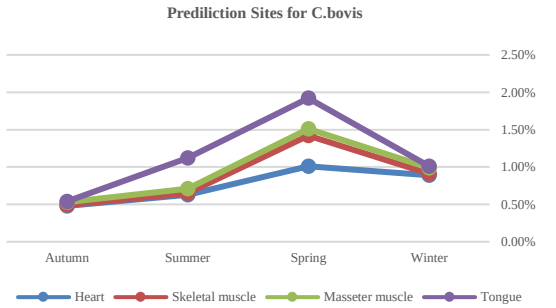

Figure 2 Prevalence (%) of *C. bovis C. bovis* in predilection sites in slaughtered cattle during different seasons in 2022.

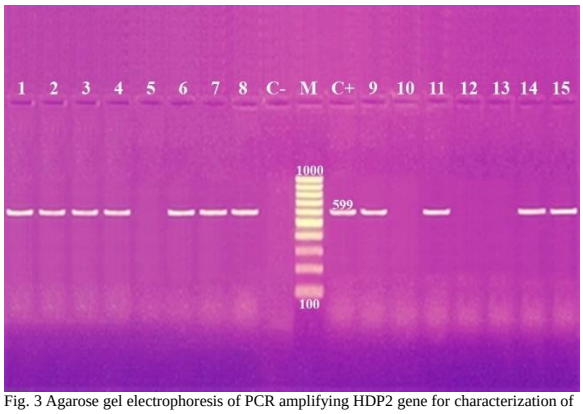

Fig. 3 Agarose gel electrophoresis of PCR amplifying HDP2 gene for characterization of C. bovis. Lane M: 100 bp ladder as molecular size DNA marker. Control positive C. bovis for HDP2 gene.. Lanes 1, 2, 3, 4, 6, 7, 8, 9, 11, 14 & 15: Positive samples for C. bovis. gene. Lanes: 5, 10, 12 & 13: Negative C. bovis.

4. DISUCCUSION

The meat business suffers financial losses as a result of cysticercosis; a carcasses with a serious infection are condemned. Fig. (1) showed that in 2022, the incidence of *C. bovis* in cattle varied according to the season and sex.

In this investigation, the incidence of *C. bovis* varied by season in bovine males, but it was not found in female cattle throughout the year. During the winter, spring, summer, and autumn, the incidence of *C. bovis* was 173 (1.01%), 200 (1.18%), 114 (0.75%), and 74 (0.47%), respectively. On the other hand, out of 65264 samples, 561 (0.85%) tested positive for C. bovis. These findings conflict with those of Zakaria et al. (2023), who concluded that females were more susceptible than males while also agreeing somewhat with the prevalence rate of *C. bovis* (0.69%) in cattle. They also differ significantly from Gebreab's (1995) findings, which

showed that the disease was present in Sudan at 0.8% and in Aswan at 7.5%, as well as from Dyab et al. (2017) findings. The discrepancy in results may be explained by the rate of slaughtered females and the stringent measures taken to investigate and detect *C. bovis* in abattoirs with strict hygienic measures for drainage systems in such abattoirs (Dorny et al., 2010). Seasons can impact an egg's ability to grow, survive, and reach grazing cattle. Accordingly, Usip et al. (2011) suggest that temperature and humidity may have an impact on the epidemiology of cysticercosis.

According to Fig. (2), the masseter muscle, skeletal muscle, and tongue had the highest percentages of impacted predilection sites for *C. bovis* 494 (0.76%), 44 (0.07%), 19 (0.03%), and 14 (0.02%), respectively. Additionally, the prevalence of *C. bovis* for the same organ is impacted by seasonal change. Heart revealed 171 (10.01%), 96 (0.63%), 75 (0.48%), and 152 (0.89%). In winter, spring, summer, and autumn, respectively, the masseter muscle measured 14 (0.08%), 15 (0.09%), 9 (0.06%), and 6 (0.04%), while the skeletal muscle measured 3 (0.02%), 7 (0.41%), 7 (0.41%), and 2 (0.02%). The tongue measured 4 (0.02%), 7 (0.41%), 2 (0.02%), and 1 (0.01), respectively. These findings corroborated those of Costa et al. (2012), who discovered that the heart had the highest prevalence of *C. bovis* (1.90%). Furthermore, Mekonnen (2017) discovered that the heart muscle had the greatest infestation rate (1.69%), followed by the tongue (1.04%), masseter muscle (1.04%), liver (0.78%), and diaphragmatic muscle (0.39%). However, Garedaghi et al. (2011) found that the cysts favored the masseter, heart, triceps, tongue, and thigh muscles. *C. bovis* was shown to be present in 67.74% of the tongue, 52% of the shoulder, 60% of the heart, and 75% of the masseter muscle by Belachew and Ibrahim (2012). In contrast, it was previously mentioned that the parasite has no preference for a particular predilection location, and Hailu et al. (2019) found that animal behavior and blood dynamics were the primary causes. Oncosphere dispersal is influenced by topographical and ecological elements that alter the animal's blood dynamics. During the meat inspection, the preferred sites changed as a result. Also, it can be attributed to the experience of meat inspectors in identifying the infestation.

As shown in Fig. (1), HDP2 gene characterization for *C. bovis* was done for confirmation. Eleven of the fifteen samples that were analyzed had the HDP2 gene detected, which was consistent with the findings reported by Gonzalez et al. (2006), who discovered that the multiplex HDP2-PCR procedure produced two amplification products, 600 and 170 bp, containing *T. sagina* tag DNA. Consequently, the *T. saginata* cysts from infected cow muscle, heart, masseter, diaphragm, tongue, and liver were conclusively identified by this PCR. The findings raised serious concerns about the use of conventional PCR as a backup method for *C. bovis* diagnosis to prevent needless meat loss in cases when an infestation is suspected.

5. CONCULOSION

The study's findings, in summary, showed that the prevalence of cysticercosis in calves killed at the El Bassatine abattoir was overstated based only on visual assessment of the flesh. Even while visual screening is crucial for many animals that are killed, more precise and sophisticated techniques are needed to identify cysticercosis and prevent needless carcass losses. The authors contend that, despite the low prevalence, every positive case of cysticercosis warrants a unique epidemiologic inquiry to further lower the risk.

CONFLICT OF INTEREST

No conflict of interests.

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