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First Record of an Outbreak of *Dictyocaulus viviparus* Infection in Cattle and Buffalo Farms in Assiut, Upper Egypt with Special Reference to the Role of Filth Flies in the Disease Transmission

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Abstract

Bovine dictycaulosis is a serious disease in ruminants caused by lungworm *Dictyocaulus viviparus* with a wide spectrum of clinical manifestations. The infection is closely related to pasture contamination with the infective larvae. A cross-sectional study was conducted from July 2020 to June 2021 to detect the prevalence of bovine dictyocaulosis in two animal-rearing stations in Assiut, Upper Egypt with risk factor analysis. Also, the role of filth flies in the transmission of infective larvae was investigated. A total of 251 animals (143 cattle and 108 buffaloes) were examined using the modified Baermann technique and postmortem examination. Multiple fly pools were also collected and examined for the detection of *Dictyocaulus* larvae in different seasons microscopically and by PCR techniques. The overall prevalence of *D. viviparus* infection in cattle and buffaloes was 20.98% and 52.78%, respectively. The infection was significantly higher in the young aged and symptomatic animals (96.7%). Most lungworm infection was recorded during autumn, spring, and winter seasons, respectively. Coprological examination coincides with microscopic examination of fly wash regarding the prevalence and the seasonal variation. *Musca domestica*, *Borborillus vitripennis*, *Fannia canicularis*, and *Sepsis punctum* were the most abundant fly species and showed the highest rates of infection. To the authors' knowledge, this is the first report of an outbreak of bovine dictycauolosis in Upper Egypt. The obtained results proved the role of filth flies in the transmission of *D. viviparus* larvae during the outbreak. Control programs for bovine dictyocaulosis should include the control of filth flies around the rearing places.

KEYWORDS
Bovine lungworms, *Dictyocaulus viviparus*, Filth flies, Risk factors, Upper Egypt.

INTRODUCTION

Lungworms are considered one of the most prevalent parasitic nematodes causing bronchitis and pneumonia in ruminants worldwide (Panciera and Confer, 2010). They are widely distributed throughout the world with a high prevalence rate in temperate zones and the highlands of tropical and subtropical countries (Silva *et al*., 2005). The most important species affecting large and small ruminants belong to two different families: the Dictyocaulidae and the *Metastrongylidae* (Mandal, 2006). The *Dictyocaulidae* family includes *Dictyocaulus viviparus* in bovines and *Dictyocaulus* filaria in ovines (Adem, 2016; Fesseha and Mathewos, 2021). The lungworm *D. viviparus* causes a severe lung disease which is called dictyocaulosis or parasitic bronchitis, also known as ''husk'' (Taylor *et al*., 2007). These species are responsible for economic losses due to poor productivity, failure to thrive, and deaths especially in first-grazing pasture calves (May *et al*., 2018). In the last decades, the incidence of the disease is on a rise affecting different age groups of cattle. There is a considerable increase in the morbidity between calves in the second year of grazing or adult cows whether dairy or beef cattle (Matthews, 2008; Motus *et al*., 2018). Several outbreaks in dairy cattle herds have been recorded causing significant economic losses (Schunn *et al*., 2013). In addition, the recorded expenses regarding laboratory diagnosis and treatment of infected cows ranged between 159 to 300 € per cow in a moderate outbreak in dairy herds (Holzhauer *et al*., 2011; Wapenaar *et al*., 2007a).

The clinical picture of dictyocaulosis varied widely from mild respiratory symptoms such as loss of appetite, increase respiratory rate, and coughing to emphysema and pneumonia. The infection could be lethal in severely affected animals (Cantacessi *et al*., 2011). The life cycle of *D. viviparus* is direct where the adult nematodes live in the bronchi and bronchioles of infected animals laying eggs containing first-stage larvae that rapidly hatch and passed in the feces. Then under favorable environmental conditions, they develop into third-stage larvae which are ingested by grazing buffalo and cattle. Finally, the larvae are transported to the lungs, where they penetrate the alveoli and develop into adults (within 3–4 weeks) (Adamson and Anderson, 1993; von Samson-Himmelstjerna and Schnieder, 1999). Healthy animals acquire infection through ingestion of grass contaminated with the infective larvae. The prevalence and morbidity of the disease are mostly related to the degree of pasture contamination (Cantacessi *et al*., 2011). Generally, the infection usually induces acquired immunity that could last for 6–12 months in the absence of reinfection (Michel and MacKenzie, 1956).

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Synanthropic flies are considered significant annoying pests of animals and humans where they are mostly associated with human dwellings and domestic animals in both rural and urban areas (Olsen, 1998; Ommi *et al*., 2015). Filth flies are either coprophagic flies that use animal manure and human excrement, or saprophagous flies that use garbage, animal bedding, and decaying organic matter for nutrition, oviposition, and breeding (Graczyk *et al*., 2005; Robinson, 2020; Stafford, 2008). Therefore, different adult fly species, like houseflies, are natural carriers of more than 100 species of pathogenic microorganisms, such as viruses, fungi, bacteria, and parasites in different regions of the world (Banjo *et al*., 2005). They are of responsible for disease transmission of more than 65 human and animal diseases worldwide, especially in tropical and subtropical countries (Graczyk *et al*., 2005).

Several species of filth flies belonging to families Muscidae, Sarcophagidae, and Calliphoridae are implicated in disease transmission (Graczyk *et al*., 2005; Stafford, 2008). The mechanical transmission of pathogens by filth files has been reported via their mouthparts, vomit drops, feces, and/or via body hairs and sticky pads of their feet (Graczyk *et al*., 2005). Several studies have been conducted worldwide to detect the role of filth flies in the transmission of parasites such as helminths eggs and/or larvae like eggs of *Enterobius vermicularis*, *Strongyloides stercoralis, T. trichiura* and *Toxocara canis*, *Diphyllobothrium*, *Hymenolepis*, *Taenia*, and *Dipylidium* species. They may also transmit protozoal trophozoites and cysts such as *Trichomonas, Entameoba histolytica, Cryptosporidium*, and *Giardia lamblia* (Adenusi and Adewoga, 2013). As a result, control measures to reduce the fly abundance are a serious challenge for most livestock production which is influenced by ideal breeding and feeding conditions for fly populations around animal farms.

Routine diagnostic techniques developed to date are still less sensitive in the diagnosis of parasitic nematode infection in animals (Roeber *et al*., 2013). The routine diagnostic techniques for the examination of parasitic infections in livestock usually rely on relatively insensitive tools, like coprological microscopic examination and fecal egg counts. Traditional diagnostics are hampered by the overlap between morphologically similar species and parasitic stages (Chilton, 2004). Although microscopic identification of nematode larvae from cultured fecal samples is still the commonly used diagnostic technique for specific nematode diagnosis in most laboratories, it poses some challenges, including time and labor intensive protocols and the need for expertise and skilled laboratory personnel (Borkowski *et al*., 2020; Wyk and Cabaret, 2004). Therefore, with the advances in molecular tools of diagnosis, molecular identification of *Dictyocaulus* infection has the advantages of simplicity, non-invasiveness, and rapid identification of *Dictyocaulus* spp. larvae isolated from fecal samples (Pyziel *et al*., 2015).

In Egypt, there is no information is available about the naturally infected cases of *D. viviparus* infection in cattle and buffaloes, up to the authors' knowledge. Therefore, the present study aims to describe an outbreak of lungworm infection in dairy cows and buffaloes in Upper Egypt clarifying possible risk factors for infection with special reference to the role of the synanthropic fly population in disease transmission.

MATERIALS AND METHODS

Study area

A cross-sectional study was performed during the period July 2020 to June 2021 to detect the prevalence of Dictyocaulosis infection in two animal rearing stations in the municipality of Assiut governorate, Upper Egypt that showed an outbreak of verminous pneumonia. The first animal station (A) located at El-Hammam, Abnoub district at about 9.01 Km northeast of Assiut city (27°15'26.7"N 31°09'56.3"E). The area of the station is about 14 acres containing 14 barns of Dutch cows (Holstein Friesian) of different ages are raised at about 15-17 animals/barn. The second animal station (B) is in Bani Murr village, El-Fath district at about 4.37 Km northeast of Assiut city (27°13'03.0"N 31°11'23.4"E). The area of this station is about 5.5 acres with 9 barns including Egyptian buffaloes at different ages (each 20-27 animals/barn). A total of 251 animals in two animal stations were included in the study. Animal station (A) included 143 cattle and animal station (B) included 108 buffaloes.

Animal conditions

Different age groups of animals were kept in separate barns, fed on green fodders and grass which decrease in the summer season because of the low rainfall, therefore hay is used beside green fodders, especially in these months. Concentrates are also given to animals twice a week as a supplement.

Clinical examination of animals

A detailed history with farm records including animal information regarding age, sex, feeding, etc. was provided by veterinarians. Other data including the clinical symptoms, morbidities, and mortalities were recorded. All animals were clinically evaluated for the presence of any respiratory signs. The death rate of severely infected animals was recorded.

Post-mortem (P.M) examination of dead animals

Thorough gross inspection of lungs in all dead animals, for congestion, consolidation, and gross lesions. The trachea was opened and examined for the presence of adult nematodes or exudates.

Diagnosis of *Dictyocaulus* infection

Collection of stool samples

About 25 g of fresh fecal sample was collected from the rectum of each animal in a separate sterile stool cup. The collected samples were transported to the Zoology laboratory in the Faculty of Science, Assiut University, and stored at room temperature. Larvae and eggs of lungworms were recovered using Baermann's technique and examined under Stereomicroscope (Olympus) according to (Hernández-Chavarría and Avendaño, 2001).

Collection of fly samples

The collection of adult flies from the recorded farms of the outbreak was done using a handmade insect sweep net that had been well-cleaned and sterilized before each sampling (Clark, 1992). It was made in the Zoology and Entomology Department at the Faculty of Science, Assiut University. The collected flies were exposed to low temperatures (-20°C) in a freezer in jars to be immobilized, examined using 4x magnification binocular microscope, and classified to the family and species levels using the taxonomic keys (Ebrahim and Salem, 2010; Sawaby *et al*., 2018). Fly samples were washed with phosphate-buffered saline (PBS) pH 7.4 and dissected into the external parts of flies and guts.

They were batched into pools each containing 10 flies that were placed into a sterilized 1.5 ml epindruff tube filled with 1 ml PBS, vigorously washed using vortex then centrifuged at 3000 rpm for 5 minutes. The sediment was examined for parasites using light microscopy by direct smear and concentration methods (Sedimentation and flotation techniques), the collected larvae were identified according to Wyk and Cabaret (2004).

Molecular diagnosis of the collected larvae from fly samples

Genomic DNA from the collected larvae from fly wash was extracted using the QIAamp® DNA Mini Kit (Cat. no. 51304) for isolation of DNA of nematode larvae according to the manufacturer's protocol. DNA quality was detected using 1.5% agarose gel electrophoresis stained with ethidium bromide (10µg/ml) (Sambrook *et al*., 1989) and DNA yield was determined by Nanodrop at optical density 240- 260.

PCR amplification

Conventional PCR technique was done to confirm the diagnosis of Dictycaulous infection targeting mitochondrial Cox1 gene using universal primers LCO1490 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HCO_2198 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ that were previously described (Ács *et al*., 2016). The reaction volume was 25 µl containing 5 µl of DNA template (100 ng), 12.5 µl Emerald Amp GT PCR master mix, 1 µl of each of forward and reverse primers (10 pmoles), and 5.5 µl nuclease-free water. The PCR cycling conditions were as follows: an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing temp. at 50°C for 30 s and extension at 72°C for 40 s, with a terminal extension at 72°C for 10 min. PCR was performed in Biometra thermocycler. The amplified PCR was visualized by 1.5% agarose gel electrophoresis stained with ethidium bromide. DNA ladder 100 bp was used (Jena Bioscince, GmbH, Germany) and photographed under UV light with a gel documentation system.

Statistical analysis

The data collected in the present study were analyzed by IBM-SPSS version 20.0. Descriptive data were presented as percentages to label the prevalence of lungworm infection. The detection of possible risk factors for infection was evaluated using Chi-square test and Fischer exact test. The 95% confidence intervals of proportion and odds ratios were determined. A P value of < 0.05 was considered statistically significant.

RESULTS

Clinical investigations and post-mortem examination

Cattle (Animal station A)

Calves less than 3 months had the highest infection rate, 66.67% of animals were symptomatic, and symptoms ranged from moderate (sporadic cough and slight increase in respiratory rate), to severe (fever 39ºC, rapid respiratory rate and productive cough with purulent nasal discharge). One calf died after a short period of complete anorexia and P.M examination of lungs revealed severe congestion with pleural effusion. While 33.33% of growing calves were symptomatic with slight to moderate signs, sporadic dry coughing was also reported with no deaths. Adult cattle were asymptomatic but two cases were positive in stool examination, their milk yield was reduced, with no other signs of disease such as normal temperature, respiratory rate, or heart rate, and with good body condition. The intensity of infection in relation to different animal age groups was presented in Table 1.

Buffaloes (Animal station B)

This animal station included 108 buffaloes that showed a serious outbreak as most animals were positive and the total infection rate reached 52.77%, however, the number of positive cases and the severity of symptoms increased in younger animals. All of the young neonates (100%) got infected, and were standing with their neck extended and the head held lowered, cachectic, and off food. In addition, the most obvious clinical signs were the high increase in the respiratory rate (reaching 40 per min.), fever (39.5 to 40ºC), tachycardia (95.8±5), dry coughing, nasal discharge, dyspnea and mouth breathing (in 50% of calves) and high death rate (10.5%) out of all infected buffaloe calves. The P.M. examination of the lungs revealed frothy exudates filling the trachea and bronchi in all inspected lungs, severe congestion, and two of them had consolidation. 90.48% of young weaned calves showed positive larvae in stool examination. There were 2 calves apparently healthy. The main symptoms include cachexia, nasal discharge, increased respiratory rate, and coughing. Eight calves showed more severe symptoms (high respiratory rate exceeding 40, fever 40ºC and completely stop feeding). Seven out of 21 young-weaned buffaloes died. P.M. examination of the lungs revealed severe congestion and frothy purulent exudate in the trachea and bronchi (Fig. 1H). Growing calves were positive (63.6%) in stool examinations. They showed symptoms that ranged from moderate to severe, two of them died after a short period of stop feeding, high respiratory rate, and coughing with nasal discharge.

Table 1. Prevalence of lungworm infection in fecal samples of cattle and buffaloes animal farms in Assiut governorate, Upper Egypt.

Species	Age	No. examined	No. of positive cases	%	Dead animals (No./%)
Cattle (Animal station A)	Calves <3months	24	16	66.67%	1 (3.33%)
	Heifers (4-15months)	36	12	33.33%	0
	Adults (15months-4years)	83	2	2.40%	0
	Total	143	30	20.98%	1 (3.33%)
Buffalo (Animal station B)	Young neonates (<month)	14	14	100%	6 (10.5%)
	Young weaned (1-3 months)	21	19	90.48%	7 (12.28%)
	Growing (3 months-year)	33	21	63.60%	2 (3.5%)
	Adults (1-3 years)	40	3	7.50%	1 (1.75%)
	Total	108	57	52.78%	16(28.1%)

Post-mortem examination of the lungs revealed congestion and exudation in both cases. Adult buffaloes were apparently healthy, they were grazing normally recumbent and ruminating. However, lower milk yield was recorded. Three cases were depressed, anorexic, and had repetitive coughing and purulent nasal discharge, one of them was extremely cachectic and died.

Table 1, shows the infection rate in each animal station, animal farm (B) had a serious outbreak of dictyocaulosis, especially between the young neonates, weaned, and growing calves, while the disease was less severe in the animal station (A), and more distributed between calves less than 3 months.

Diagnosis of D. viviparus infection

The stool examination and fly wash released hundreds of the *D. viviparus* larvae in different developmental stages. The retrieved larvae presented a mean length of 350 µm (± 20.15 µm) and a mean width of 15 µm (±0.9 µm). In addition, morphological identification of these larvae suggested that they were similar to *Dictyocaulus* sp., with intestinal cells containing numerous granules. The larvae have rounded heads, and bluntly pointed tails and were filled with intestinal food granules that were typical of the family Dictyocaulidae. In addition, a few eggs were also detected, the eggs containing a fully grown first-stage larva as shown in Fig. 1.

The Role of filth flies in the transmission of Dictyocaulus larvae

Ten fly species (Fig. 2) belonging to six dipteran families; Muscidae, Sphaeroceridae, Fannidae, Sepsidae, Ulidiidae, and Calliphoridae were collected from animal stations (A) and (B) during the study period in four seasons. Four fly species were the most abundant fly species including *Musca domestica* followed by *Borborillus vitripennis*, *Fannia canicularis*, and *Sepsis punctum*, respectively. However, six species presented in low numbers with sporadic cases of infections such as *Physiphora alceae, Meroplius minutus, Stomoxys calcitrans, Musca sorbens, Calliphora vicina*, and *Lucilia sericata*. These fly species contributed to Dictyocaulosis disease transmission through the dispersal of *D. viviparus* larvae and eggs in the animal rearing sites as they were isolated from the fly wash as shown in Fig. 1, demonstrating the morphological criteria of *D. viviparus* larvae and their embryonated eggs.

The abundance of fly species and the prevalence of *D. viviparus* larvae in relation to seasons in each animal station were presented in Table 2. The infection intensity was more abundant in fly species in Autumn followed by the Spring season while Winter season showed low infection in all fly species. However, in Summer, there were no Dictycaulous larvae detected except a single infection was detected on Stomoxys calcitrans and Musca sorbens.

Molecular diagnosis by conventional PCR technique

To confirm the role of filth flies in dictyocauliasis disease transmission, molecular diagnosis by conventional PCR targeting Cox1 gene specific for *Dictyocaulus* mitochondrial DNA was performed. DNA was extracted from the retrieved larvae that were collected from the fly wash. PCR amplification of the larval DNA showed PCR products with positive bands at 657 bp (Fig. 3).

Seasonal variation and risk factors analysis associated with D. viviparus infection in cattle and buffalo farms

Various risk factors such as species, sex, age groups, respiratory symptoms, and seasonal variation have been shown in Table 3. The data revealed that females were significantly more affected than males. Also, buffaloes exhibited a more serious outbreak than cattle with a significant increase in infection in younger animals than adult calves. Further, lungworm infection was significantly related to the autumn season with high infection rates followed by the spring and winter seasons. Most symptomatic animals (96.7%) showed positive larvae in stool since they were

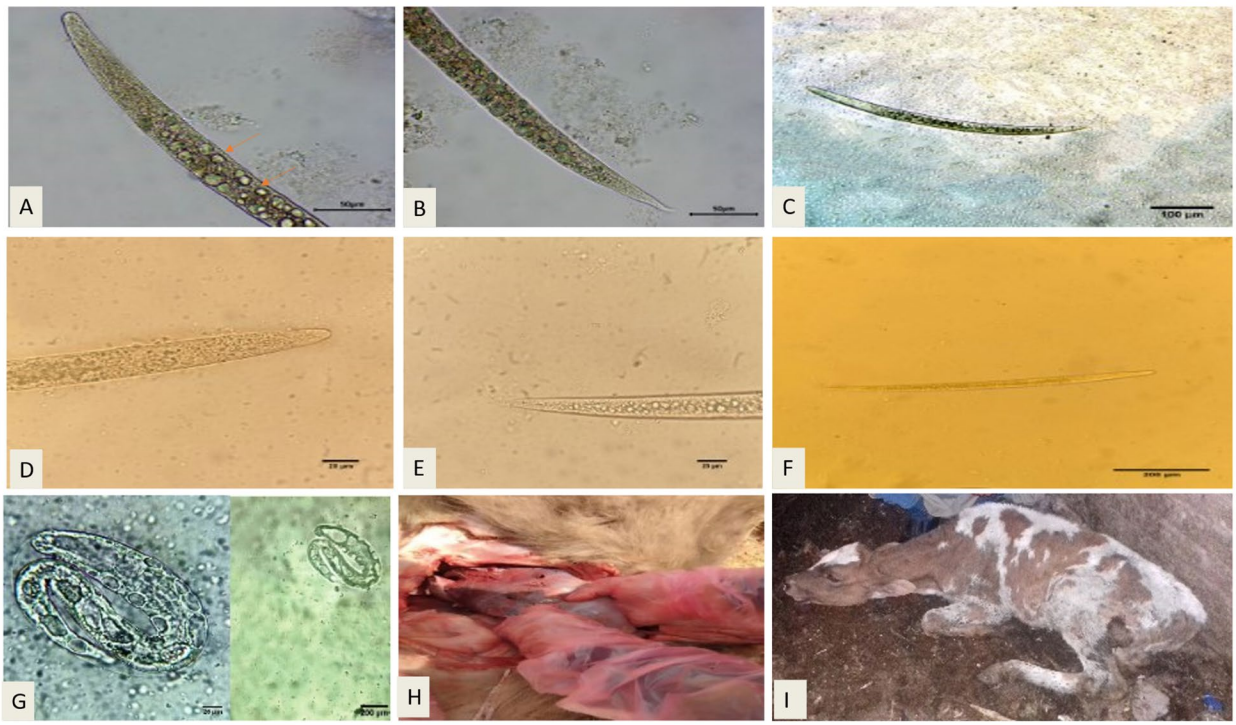

Fig. 1. (A-C): Microphotographs showing *Dictyocaulus viviparus* larvae released from filth fly wash: note the rounded anterior end and intestinal food granules (arrows), (B): the short tail with bluntly pointed posterior end. (C): first stage *D. viviparus* larva released from the fly wash 40x and 10x. (D-F): photographs showing the rounded anterior end of *D. viviparus*, the posterior end, and first stage larva in stool and released from Baermann's technique. (G): the *D. viviparus* eggs contain fully developed larva 40x and 10x (H): Post-mortem examination shows lung congestion. (I): A cow presented with dyspnea, orthopnea, and cachexia.

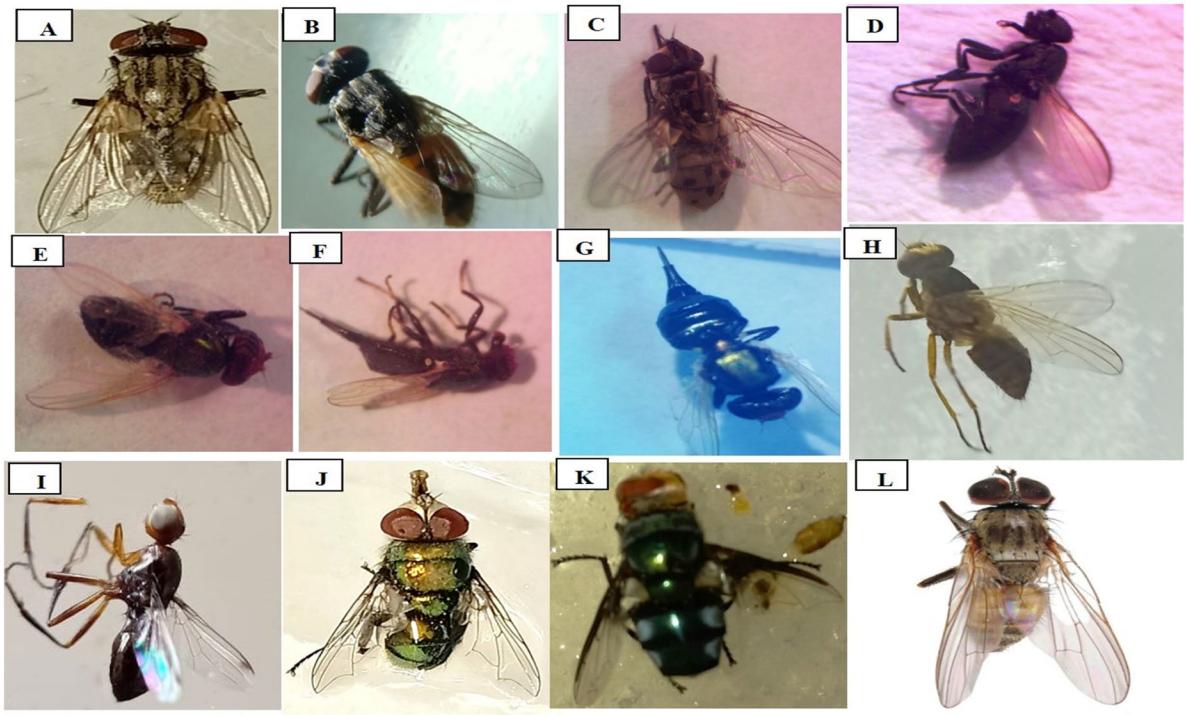

Fig. 2. The collected fly specimens in the present study: (A – C): Family Muscidae (*M. domestica*, M. sorbens and Stomoxys calcitrans, respectively. (D): Family Sphaeroceridae (*Borborillus vitripennis*), (E - G): Family Ulidiidae (*Physiphora alceae* male and female, (H, I): Family Sepsidae (*Meroplius minutus* and *Sepsis punctum* respectively), (J, K): Family Calliphoridae (*Lucilia sericata* and *Calliphora vicina* respectively), (L): Family Fanniidae (*Fannia canicularis*).

found significantly associated with infection ($p < 0.001$).

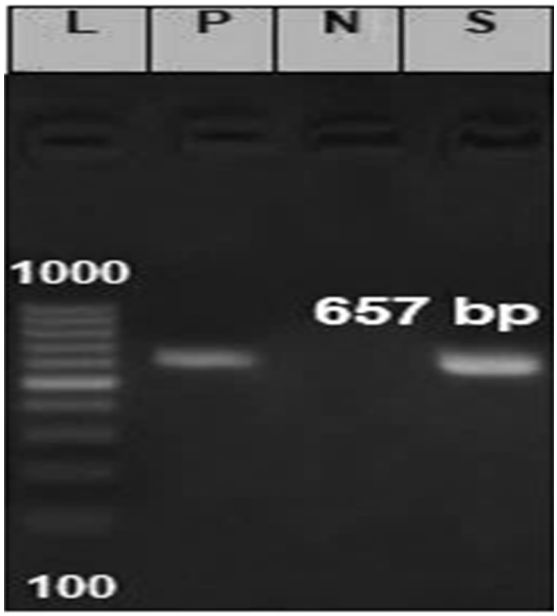

Fig. 3. DNA amplification of Nematode Cox1 gene of larvae isolated from fly wash: PCR products resolved in 1.5% agarose gel electrophoresis and stained with ethidium bromide. The gel image showed positive PCR products at 657 bp in lanes (S, P). (N)= negative control, (P)= positive control, (M) marker = 100 bp DNA ladder.

DISCUSSION

Genus *Dictyocaulus* (Lungworms) are parasitic nematodes with a significant veterinary impact producing severe economic losses in the animal industry. *Dictyocaulus* filaria affects small ruminants while *D. viviparus* is a bovine lungworm affecting cattle and buffalos and causing parasitic bronchitis (Dictyocauliasis) with a wide range of various symptoms (Adem, 2016; Hailu, 2019). This disease is frequently documented in many tropical, subtropical countries and temperate zones (Lat-Lat *et al*., 2007). However, there is insufficient information about the distribution of *D. viviparus* in Egypt which could be due to the absence of systematic surveys, inexperienced veterinary care, and deficient parasitological tools for diagnosis. Therefore, in the present study, infection with *D. viviparus* was recorded in two animal-rearing stations in the Assiut governorate, Upper Egypt. To the authors' knowledge, this is the first report of this outbreak in the studied area.

The prevalence of lungworm infection in cattle and buffaloes was 20.98% and 52.8%, respectively with a significant difference between the two species. The high incidence of infection in the present study may be attributed to that the cross-sectional study targeted the animal farms that experienced an outbreak of parasitic bronchitis in the studied area. Higher prevalence rates of *D. viviparus* have been reported in dairy calves and sheep in many tropical, subtropical countries like Brazil (Henker *et al*., 2017), India (Zafari *et al*., 2022), Malaysia (Lat-Lat *et al*., 2007), and Pakistan (Mahmood *et al*., 2014). In addition, temperate countries are not immune to this parasite and showed several outbreaks such as in Ireland (Murphy *et al*., 2006), Netherlands (Ploeger, 2002), and Sweden (Höglund *et al*., 2004). It is also found in the highlands of some African countries such as Congo, Tanzania, and Ethiopia (Chanie and Ayana, 2013; Thamsborg *et al*., 1998). In Egypt, previous studies of lungworm infection in sheep and goats have been conducted. The lungworm D. filaria was detected in sheep in Qena governorate (Mohamed *et al*., 2008) and among goats in the same locality in southern Egypt where the modified Baermann method showed D. filaria larvae in 32.84% of fecal samples of tested goats (Fereig *et al*., 2018). In Nile Delta, ovine lungworm infection was detected in low numbers (4.5%) in local breed sheep.

Risk factor analysis for infection was done and revealed that the prevalence of Dictyocaulosis was higher in young animals in both animal species. The infection rate in neonates was the highest (100%). Also, in young weaned and calves aged <3 months (90.48% and 66.67%), the infection was recorded to be more than those of mid- aged (growing) and adults, this is consistent with a previous study in Faisalabad, Pakistan (Mahmood *et al*., 2014). Further, many studies recorded a high prevalence in young aged animals (Jamshidi *et al*., 2020; Terefe *et al*., 2013; Tewodros, 2015), while others documented that lungworm infection rates increase

with increasing animal age (Berrag and Urquhart, 1996; Henker *et al*., 2017; Regassa *et al*., 2010). This could be attributed to the strong immune system in old aged animals causing an absence of larval shedding in the stool (Holzhauer *et al*., 2011; Strube, 2012).

Results from this study showed that female calves were found to have a higher prevalence (46.09 %) than male calves in both livestock farms. This is consistent with other studies that observed high infection rates in females (Regassa *et al*., 2010; Terefe *et al*., 2013). This could be ascribed to female immune suppression during parturition and early lactation (Craig, 1998). This finding contradicts recent research from Malaysia which found that male calves are more susceptible to lungworm illness than females (Lat-Lat *et al*., 2010). Previous research claimed that because of testicular hormones, bulls, and male sheep were more susceptible to worm infections than females (Barger, 1993).

Concerning the seasonal distribution of *D. viviparus* infection, the present study revealed that the high infection prevalence of lungworms was in autumn (16.7%) followed by spring (11.3%) and winter (6.7%). This result agreed with a previous study that was conducted to estimate the prevalence and describe the contributing risk factors of lungworm infection in sheep and cattle in Ethiopia, which is considered an important region for importing ruminants to Egypt. This study revealed that most of the sheep and cattle were heavily infected with lungworms and that the peak lungworm infection was recorded during autumn and

Table 2. Frequency of Dictycaulous larvae in contaminated fly pools collected from animal rearing stations at different seasons

Fly species	Station	Infested fly pools with *Dictyocaulus* larvae No. (%)				*P*. value
		Summer	Autumn	Winter	Spring	
Musca domestica	A	0(0%)	25(25%)	0(0%)	12(19%)	=0.032*
	B	0(0%)	75(75%)	29(100%)	51(81%)	
	Total	0(0%)	100/197 (50.76%)	29/106 (27.36%)	63/226 (27.88%)	
	P. value	-	<0.001**	-	<0.001**	
Musca sorbens	A	1(100%)	1(50%)	0(0%)	0(0%)	-
	B	0(0%)	1(50%)	0(0%)	0(0%)	
	Total	1/6 (16.67%)	2/2(100%)	0(0%)	0(0%)	
	P. value	-	0.62	-	-	
Stomoxys calcitrans	A	0(0%)	1(50%)	0(0%)	0(0%)	-
	B	1(100%)	1(50%)	1(100%)	1(100%)	
	Total	1/8 (12.50%)	2/5(40%)	1/3(33.3%)	1/6(16.67%)	
	P. value	-	0.62	-	-	
Borborillus vitripennis	A	0(0%)	11(28.9%)	0(0%)	9(19.1%)	0.39
	B	0(0%)	27(71%)	8(100%)	38(80.9%)	
	Total	0(0%)	38/55(69.09%)	8/45(17.78%)	47/94(50%)	
	P. value	-	<0.001**	-	<0.001**	
Fannia canicularis	A	0(0%)	6(33.3%)	0(0%)	3(33.3%)	0.89
	B	0(0%)	12(66.7%)	0(0%)	6(66.7%)	
	Total	0(0%)	18/23(78.26%)	0(0%)	9/35(25.7%)	
	P. value	-	0.001**	-	0.001**	
Sepsis punctum	A	0(0%)	3(27.3%)	0(0%)	1(20%)	0.75
	B	0(0%)	8(72.7%)	0(0%)	4(80%)	
	Total	0(0%)	11/11(100%)	0(0%)	5/16(31.25%)	
	P. value	-	<0.001**	-	0.001**	
Meroplius minutus	A	0(0%)	1(50%)	0(0%)	1(50%)	-
	B	0(0%)	1(50%)	1(100%)	1(50%)	
	Total	0(0%)	2/3(66.67%)	1/3(33.3%)	2/5(40%)	
	P. value	-	0.48	-	0.45	
Physiphora alceae	A	0(0%)	1(50%)	1(100%)	1(50%)	-
	B	0(0%)	1(50%)	0(0%)	1(50%)	
	Total	0(0%)	2/3(66.67%)	1/4(25%)	2/7(28.57%)	
	P. value	-	0.48	-	1	
Calliphora vicina	A	0(0%)	0(0%)	0(0%)	0(0%)	-
	B	0(0%)	1(100%)	0(0%)	0(0%)	
	Total	0(0%)	1/2(50%)	0(0%)	0(0%)	
	P. value	-	-	-	-	
Lucilia sericata	A	0(0%)	0(0%)	0(0%)	1(100%)	-
	B	0(0%)	0(0%)	0(0%)	0(0%)	
	Total	0(0%)	0(0%)	0(0%)	1/3(33.3%)	
	P. value	-	-	-	-	

*= significant P. value <0.05

Table 3. Risk factor analysis of lungworm infection in cattle and buffaloes in Assiut governorate.

Sex/Species/Age	No. of animals	Positive		Prevalence (%)	OR	95% CI	*P*-value
		No.	%				
Sex							
Male	136	34	25	25	0.39	0.229-0.665	0.001**
Female	115	53	46.1	46.09	1	-	-
Species							
Cattle (Rearing- station A)	143	30	20.98	20.98	1	-	-
Buffalo (Rearing- station B)	108	57	52.8	52.78	0.24	0.137-0.413	<0.001**
Total	251	87	34.7				
Age groups							
Neonate calves (<month)	20	18	90	90	1	-	-
Weaned calves (1-3months)	39	31	79.5	79.49	0.43	0.082-2.253	0.32
Heifers and Growing (3months-1year)	69	33	47.8	47.83	0.10	0.022-0.473	0.004**
Adults (Above 1 year)	123	5	4.1	4.07	0.01	0.001-0.026	<0.001**
Season							
Winter	240	16	6.7	6.67	1	-	-
Spring	239	27	11.3	11.3	1.78	0.934-3.403	0.08
Summer	251	2	0.8	0.8	0.11	0.026-0.494	0.004**
Autumn	251	42	16.7	16.73	2.81	1.535-5.157	0.001**
Respiratory clinical signs							
Showing respiratory clinical signs	61	59	96.7	96.72	1	-	-
Apparently healthy	190	28	17.1	14.74	0.01	0.001-0.025	<0.001**

CI = Confidence interval, OR = Odd ratio, Reference group=1. **= significant P-value.

spring (Fesseha and Mathewos, 2021). Another study that is consistent with the obtained results was performed in Upper Egypt in Qena governorate on D. filaria in goats, the higher infection rate was observed during winter and autumn (50% and 44%, respectively) (Fereig *et al*., 2018). Other researchers disagreed with this finding and reported a higher prevalence during hot dry seasons such as summer (Alemu *et al*., 2006; Borji *et al*., 2012; Regassa *et al*., 2010).

Several factors influence the epidemiology of lungworm infection in animals. Typically, pasture contamination with lungworm larvae gradually increases during the spring and early summer, while the disease manifestations become more evident in the cold seasons where a damp and cool environment is ideal for the growth of *Dictyocaulus* larvae (Frewengel, 1995; Wapenaar *et al*., 2007b).

It was observed that lungworm larvae are relatively inactive and are incapable of traveling more than a few inches away from the dung pats (Shite *et al*., 2015). Hence, the dispersal of infective lungworm larvae over pasture was thought to be influenced by many factors including Pilobolus fungus sporangia, dung beetles, earthworms, rain, wheeled vehicles, and animal movements (Borji *et al*., 2012; Eysker, 1991; Radostits *et al*., 2007).

Synanthropic flies are ecologically adapted to live in close association with human and animal habitations (Gabre and Abou-Zied, 2003). They are important vectors of animal and human diseases transmitting a wide variety of infectious agents either mechanically or biologically including parasites, bacteria, and viruses (Graczyk *et al*., 2001). Therefore, this study aimed to declare the possible role of filth flies in the transmission of lungworm infection during the ongoing outbreak in cattle and buffalo rearing places in Assiut, Upper Egypt.

The present study showed that filth flies play a significant role in the transmission and distribution of Dictyocaulosis. The most prevalent fly species carrying the *Dictyocaulus* larvae were *Musca domestica* followed by *Borborillus vitripennis*, *Fannia canicularis*, and *Sepsis punctum*, respectively. The number of carried larvae increased significantly in the autumn and spring seasons, which was closely related to the time when the morbidities increased between the animal flocks. Hence, these flies contributed to the increased burden of the disease which in turn led to increased morbidities and mortalities and heavy economic losses. Also, there was a high incidence of *Dictyocaulus* larvae in the fly pools collected from the animal farm (B) including buffaloes than from cattle farm (A) and that coincides with the observed significant increase in *Dictyocaulus* infection in buffaloes ($p<0.001$).

Many previous studies focused on the role of flies in parasite transmission. A previous study conducted in Egypt found that *Musca domestica* had a significant role in the transmission of nematode parasites like hookworm, *T. trichura*, and *Ascaris* eggs (El-Sherbini and El-Sherbini, 2011). Another Sudanese study investigated flies that were collected from slaughterhouses and proved their role in the transmission of many protozoans and helminths such as *Entamoeba histolytica/* dispar, *E. coli*, *Giardia lamblia, Hymenolepis nana*, and *Taenia* species (Ibrahim *et al*., 2018). In North Eastern Nigeria, the role of housefly in the mechanical transmission of parasites was studied, and detected four parasitic species in the wash of exoskeletons of the collected housefly including *Ascaris lumbricoides*, *Trichuris trichiura*, Hookworm, and *Hymenolepis nana* (Balla HJ *et al*., 2014). In Iraq, several parasites were found on the external body surface and digestive tract of house flies whether protozoal cysts or helminth eggs and larvae (Al-Aredhi, 2013).

In fact, diagnosis of lungworm infection was mainly dependent on traditional parasitological diagnostic means besides the presence of clinical signs as well as gross and histopathological findings. Clinically, lungworm infections must be differentiated from other viral or bacterial causes of respiratory diseases affecting calves (Henker *et al*., 2017). Therefore, the detection of *D. viviparus* larvae by the Baermann technique is sensitive for diagnosis (Holzhauer *et al*., 2003). However, false negative results are common that are believed to be due to prepatent infections (Matthews, 2008). Therefore, molecular-based diagnostic techniques are valuable in disease diagnosis to avoid delayed diagnosis and missing reporting of an outbreak with subsequent eco-

nomic losses in the animal livestock.

CONCLUSION

The present study highlights a serious outbreak of Dictyocaulosis causing serious morbidities and mortalities affecting cattle and buffaloes in Assiut governorate, Upper Egypt. The study declared that calves in the early grazing years are highly susceptible. However, pasture contamination could occur with low numbers of lungworm larvae. In addition, prevalence of lungworm infection increases during autumn and spring. Thus, further studies are needed to clarify the effect of seasonal variations on the epidemiology of bovine Dictyocaulosis in Egypt. Moreover, Upper Egypt should be considered as an area at risk of lungworm infections. Proper management and efficient diagnosis is encouraged to avoid unnecessary economic losses. The control programs of Dictyocaulosis in buffalo and cattle farms should include control of filth flies around the rearing places.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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