J Parasit Dis
https://doi.org/10.1007/s12639-020-01256-y

ORIGINAL ARTICLE

Advanced immunological studies on *Cephalopina titillator* with special references to the epidemiological uses of Dot-ELISA in camel sera

Marwa M. Attia[1] · Heba S. Farag[2] · Hitham Abdel-Saeed[2] · Elshaimaa Ismael[3]

Received: 28 April 2020 / Accepted: 29 July 2020

Abstract *Cephalopina titillator* (*C. titillator*) is a common worldwide nasal bot fly larval infestation of camels, which belongs to the family Oestridae. This study aimed to evaluate two new immunologic diagnostic techniques; indirect-ELISA and Dot-ELISA, for the screening of *C. titillator* infestation in camels. Thirty slaughtered camel heads were examined carefully for the presence of *C. titillator* larvae. One hundred, third-stage larvae (L3), were dissected for the collection of their salivary glands, for the preparation of the salivary gland antigen. Blood samples were obtained for hematological and serological examinations. Results revealed a true prevalence of *C. titillator* in the sampled camels being 80% (24/30). Infested camels showed a significant reduction in leukocytes ($P < 0.0001$) and neutrophils ($P = 0.045$), and a significant increase in eosinophils and monocytes ($P < 0.0001$). The serological examination estimated apparent prevalence as 80% (24/30) and 90% (27/30) by Dot-ELISA and indirect-ELISA, respectively. Dot-ELISA revealed 100% sensitivity, specificity, and accuracy. While, indirect-ELISA displayed 100% sensitivity, 50% specificity, and 90% accuracy. Dot-ELISA exhibited perfect agreement with the gold standard test, so it could be considered an ideal, simple, and accurate immunologic screening technique for the detection of *C. titillator* in camels.

Keywords *Cephalopina titillator* · Indirect-ELISA · Dot-ELISA · Oestridae · Immunological evaluation · Screening

Introduction

Camelus dromedarius is the native camel that is present worldwide, especially in the arid and semi-arid regions of different countries such as Egypt, northern Nigeria, Iran, and Saudi Arabia. Dromedary camels slaughtered for their meat and hides. Camels are exposed to different internal and external parasites and could transfer diseases through different countries in Africa (El-Rahman 2010).

Cephalopina titillator (*C. titillator*) is a common nasal bot fly larva that belongs to the family Oestridae, which infest camel populations within African and Asian countries (Higgins and Kock 1984). *Cephalopina titillator* adult flies roam around their specific host where they deposit their first larval stage in the base of the camels' nostrils. The larvae crawl into the nasal cavities and their sinuses to molt into the second, third larval stages and the full mature larval instars which are sneezed with mucous out the nostrils (Zumpt 1965; Angulo-Valadez et al. 2011). Camel nasal bot fly larvae cause a severe health condition in camels. Clinical signs could vary according to the intensity and the developmental stage of the larvae. Clinical manifestations include difficulty in breathing, plus severe irritation to the mucous membranes and the ethmoid bone, which leads to tissue damage (Otranto 2001). Sever cases in camel myiasis lead to meningitis which could develop

✉ Marwa M. Attia
marwaattia.vetpara@yahoo.com;
marwaattia.vetpara@cu.edu.eg

[1] Department of Parasitology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt

[2] Department of Internal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

[3] Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt

Published online: 05 August 2020

through secondary bacterial or viral infections (Musa et al. 1989).

Several countries recorded different prevalence rates in camels with the *C. titillator* infestation as Egypt (Ashmawy et al. 1985; Zayed et al. 1994), Chad (Graber and Gruvel 1964), Sudan (Steward 1950), Iran (Jalali et al. 2016) and different countries in Asia.

Many studies had reported the prevalence and incidence rates of *C. titillator*, but few reports investigated the diagnostic value of using different serological tests; as indirect-ELISA and Dot-ELISA, for the detection and monitoring of *C. titillator* in camels.

Materials and methods

The examination of slaughtered camels for larval collection

A total of 30 camels were sampled and examined at slaughterhouse in Cairo abattoir. The camels were brought from Sudan and Sinai for slaughtering. The age and sex of each camel were recorded by careful inspection of the slaughtered camels (Abouheif et al. 1990). The heads of the examined camels were separated from the body and sagittally cut to directly inspect the nasal passages and pharynx for the presence of *C. titillator* larvae. The larvae were collected with forceps, placed into vials containing saline, and labeled. Additionally, the two turbinate bones (near the brain) were placed in plastic bags and labeled. The second (L2) and third (L3) larval stages were macroscopically detected by naked eyes.

Samples

Fecal samples

Fecal samples were obtained during clinical examination for parasitological examination, using direct fecal smear and floatation technique, for the exclusion of internal parasitic infections. Detection of parasitic eggs, oocysts, larvae, or adult worms was suggestive for the exclusion from the sera in the present study (Soulsby 1986).

Blood and serum samples

Blood samples (6 ml each) were received from the jugular vein of each camel into plain tube was for serum separation.

Identification of *C. titillator* larvae

All the collected larvae were examined in the parasitology laboratory of the Faculty of Veterinary Medicine, Cairo University. The identification of the collected bot fly larvae was verified using the morphological key described by Zumpt (1965) with the stereoscopic and light microscope.

Ultrmorphological study of the collected *Cephalopina titillator*

The 3rd stage larvae of *C. titillator* were washed several times; labelled with their concentration using buffered phosphate saline (pH 7.2). The larvae were fixed in 2.5% glutaraldehyde for 24 h. The larvae were dehydrated in serial upgraded ethanol degrees. Then, the larvae were dried in CO2 critical point drier (Autosamdri-815, Germany) and glued on stubs; coated with 20 nm gold in a sputter coater (Spi-Module sputter Coater, UK). The larvae were photographed with the electron microscope (JSM 5200, Electron probe Microanalyzer, Jeol, Japan) at the Faculty of Agriculture, Cairo University, Egypt as described by Attia and Salaeh (2020) and AbdElKader et al. (2020).

Serological diagnosis

Preparation of larval antigens

One hundred, third-stage larvae (L3), were dissected for the collection of their salivary glands, for the preparation of the salivary gland antigen. The collected salivary glands (SG) were preserved in 1 ml phosphate-buffered saline (pH 7.2), ground in ice by a homogenizer, then sonicated for 5 min at pulse rate 60–80; according to Attia et al. (2019a). The protein content of the SG was measured using the modified Lowry's methods (Lowry et al. 1951).

Preparation of hyper-immune sera (HIS)

Male rats (*Rattus norvegicus* albino) of 100 g weight, were adopted to produce hyper-immune sera (HIS) against SG protein; as designated by Attia et al. (2019a). Rats were separated into two groups; a negative control group and SG antigen immunized group. The protocol of immunization included three doses of the 1 ml a mixture of 0.5 mg SG antigen and 0.5 ml the mineral oil adjuvant. The first dose was subcutaneously delivered, while the following two doses (each dose 1 ml as first dose) were given intramuscularly. Two weeks post-immunization, rats were subjected to bleeding for sera collection. The collected HIS were stored at − 20 °C until analyzed. This study was approved

by the ethical committee of the Faculty of Veterinary Medicine, Cairo University.

Sera examined

Serum samples used for indirect-ELISA and Dot-ELISA procedures included: HIS of rats against SG antigen; negative sera from control rats; positive sera from naturally *C. titllator* infested camels; three sera from camels infected with another single parasite as Hydatid cyst or *Haemonchus contortus* without *C. titllator* infestation.

Enzyme-linked immunosorbent assay (ELISA)

For the determination of the lowest antigen dilution, a checkerboard titration was applied according to Harlow and Lane (1988), using 96 flat bottomed-wells ELISA plates were used and all the dilution and chemicals were applied according to Attia et al. (2019a, b). Protein A conjugate (IgG) (Sigma, A-5420) and 10 mg Ortho-Phenylene Diamine (OPD) substrate were used. The reaction stopped using the stopping buffer 3 N H2SO4. The Optical density (OD) was recorded from the ELISA reader adjusted at 450 (Bio-Rad, USA). All the sera were considered positive when OD give as or more than the cut-off value.

Dot-enzyme-linked immunosorbent assay (Dot-ELISA)

Two hundred ng of SG antigen was used onto 1 cm nitrocellulose membranes (NC) of 0.22 µm pore diameter (Bio-Rad Laboratories, USA), and incubated at 37 °C for 1 h. The NC was blocked, sera were added, and the conjugate (Anti-rat IgG and Protein A IgG conjugated horseradish peroxidase). The used substrate was (4-chloro-1-naphthol in methanol). The violet color was developed and then stopped by the washing of the NC by distilled water. Positive sera developed violet color, while negative ones exhibited no color; all the procedures followed Attia et al. (2019a).

Statistical analysis

For the indirect-ELISA test, the cut-off point for a positive result was taken as 2× mean of optical density (OD) of negative control sera (Romero et al. 2010). To assess the sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV), and other evaluation points of the screening diagnostics, each screening test was performed through the inspection of the slaughtered camels' heads, indirect-ELISA, and Dot-ELISA. Inspecting heads of the slaughtered camel is regarded as the most sensitive method for the epidemiological detection of *C. titillator* infestation, so it was assigned as the gold standard test.

Kappa statistic (κ) was performed to measure the agreement between different screening tests based on the guidelines of Altman (1999) and adapted from Landis and Koch (1977). The Landis and Koch scale (Landis and Koch 1977) was used to measure the degree of agreement according to the Kappa value, with the scores divided into: < 0 no agreement; 0.0–0.20 slight; 0.21–0.40 fair; 0.41–0.60 moderate; 0.61–0.80 substantial; 0.81–0.99 almost perfect; 1 perfect.

Data were expressed as means and standard errors and were statistically analyzed using independent sample *t* test and analysis of variance (ANOVA) test. Pearson's correlation coefficient (r) and regression analysis (R^2) tests were performed to evaluate the association between the studied variables. All statistical analyses were performed using PASW Statistics, Version 18.0 software (SPSS Inc., Chicago, IL, USA). Statistical significance was considered when P value is less than 0.05.

Results

Prevalence of infestation and gross examination

Examination of heads of 30 camels, in the current study, revealed that 24 of camels (80%) were infected with *C. titillator* instars. The observed minimum and maximum intensities of *C. titillator* larvae per camel were 4 and 20 larvae respectively, with a mean of 11.9 larvae per camel.

The infestation was confined to the nasopharyngeal cavity and turbinates in which numerous larvae were active and freely crawling around. A few larvae were detected deep in the turbinate bones and ethmoid area.

Results of Pearson correlation (Table 1) indicated that there was a weak positive association between camels' age and larval infestation intensity ($r = 0.40$, $P = 0.029$). For each one-year increase in camels' age, we observed a 1.74 ($\pm$ 1.55, $\pm$ 95% CI) increase in the larval count ($F(1, 28) = 5.31$, $P = 0.029$, $R^2 = 0.16$); as demonstrated in Fig. 1. Results in Table 1 indicated that the total mean larval infestation intensity was not significantly different for age classes of camels ($F(3, 20) = 0.85$, $P = 0.484$).

On average, total larval infestation intensity in females (M = 13.30, SE = 1.09) was greater than that in males (M = 10.93, SE = 1.38). This difference, 2.37 larvae (95% CI − 1.52, 6.26), was not significant ($t(22) = 1.27$, $P = 0.219$) (Table 1).

Table 1 Mean intensities (± SE) of *Cephalopina titillator* larvae found according to age groups and sex in the infested camels

	Total larvae mean	L2	L3
Age class (years)			
4	11.67 ± 2.38	2.83 ± 1.08	8.83 ± 2.39
5	10.38 ± 1.74	0.88 ± 0.58	9.50 ± 1.69
6–7	14.33 ± 1.43	2.67 ± 0.33	11.67 ± 1.41
8	11.75 ± 1.55	1.25 ± 0.75	10.50 ± 1.32
P value	*0.484*	*0.151*	*0.720*
F(3, 20)	*0.85*	*1.97*	*0.45*
Sex			
Female	13.30 ± 1.09	2.10 ± 0.41	11.20 ± 0.96
Male	10.93 ± 1.38	1.71 ± 0.61	9.21 ± 1.35
P value	*0.219*	*0.634*	*0.282*
t(22)	*1.27*	*0.483*	*1.10*

SE standard error, *L2* 2nd stage larvae, *L3* 3rd stage larvae

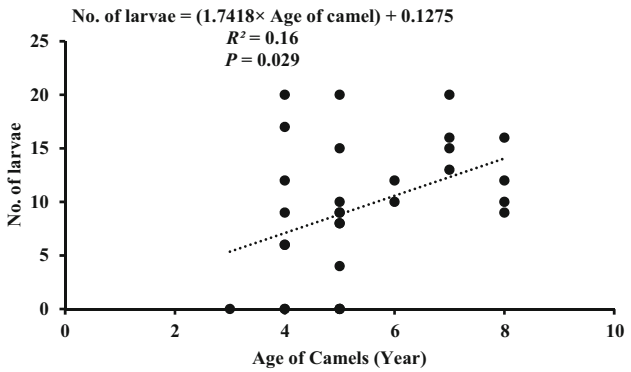

Fig. 1 Relation between age of infested camels and intensity of larvae (no.)

Second stage larvae of *C. titillator* (L2)

The color of the freshly recovered L2 from the turbinate was whitish. The length of the larvae ranged from 0.6 to 1.4 cm (1 cm ± 0.2) while its maximum width was 0.3–0.5 cm (0.4 cm ± 0.1). Each segment was provided with several tubercles. The larvae were provided with posterior spiracles in deep pits, as demonstrated in Fig. 2.

Third stage larvae of *C. titillator* (L3)

The fully mature larvae were whitish to yellowish coloured with a dark brown line on the ventral surface. The larvae varied in length from 1.9 to 2.6 cm (2.2 cm ± 0.1) and its maximum width ranged from 0.5 to 1.1 cm (0.8 cm ± 0.2). It was provided with posterior spiracles located deeply at the posterior end of the 12th segment; Fig. 2. The third stage larvae were photographed by SEM for morphological description of the surface ultrastructure as; the anterior end or pseudocephalon had two long curved maxillae; with absence of mandibles. The two antennary lobes were large and supported by sensory papillae; the 1st and 2nd thoracic segments were supported by small spines ventrally. While the 3rd segments and abdominal segments were supported by large fleshy spines with tapering end and small spines. The last abdominal segment contains two peritremes in deep pit; this last abdominal segment formed from dorsal and ventral lips. The lips contain number of sensory papillae at its surface and small spines (Fig. 3).

Serological analysis

The cut-off value for camels' sera was 0.40 for the indirect-ELISA test. Positive OD values for camels' sera ranged between 0.60 and 2.8 in indirect-ELISA. Examination of the heads of slaughtered camels (Gold standard test) as well as Dot-ELISA testing of camel's sera showed positivity in 24 samples (80%), while 27 (90%) camels were found to be positive for *C. titillator* with indirect-ELISA (Table 2). The indirect-ELISA test of camels' sera showed a sensitivity of 100%, and specificity of 50%, with an accuracy of 90%. While, the Dot-ELISA test showed a sensitivity of 100%, and specificity of 100%, with an accuracy of 100% (Table 2).

Kappa (κ) test was run to measure the agreement between the indirect-ELISA, Dot-ELISA, and GS test results of healthy and *C. titillator* infested camels. Based on the results in Table 3, we could report that there were significant substantial agreements between the results of the indirect-ELISA and GS tests, as well as between the results of the indirect-ELISA and Dot-ELISA ($\kappa = 0.62$, $P < 0.0001$). Additionally, there was a significant perfect

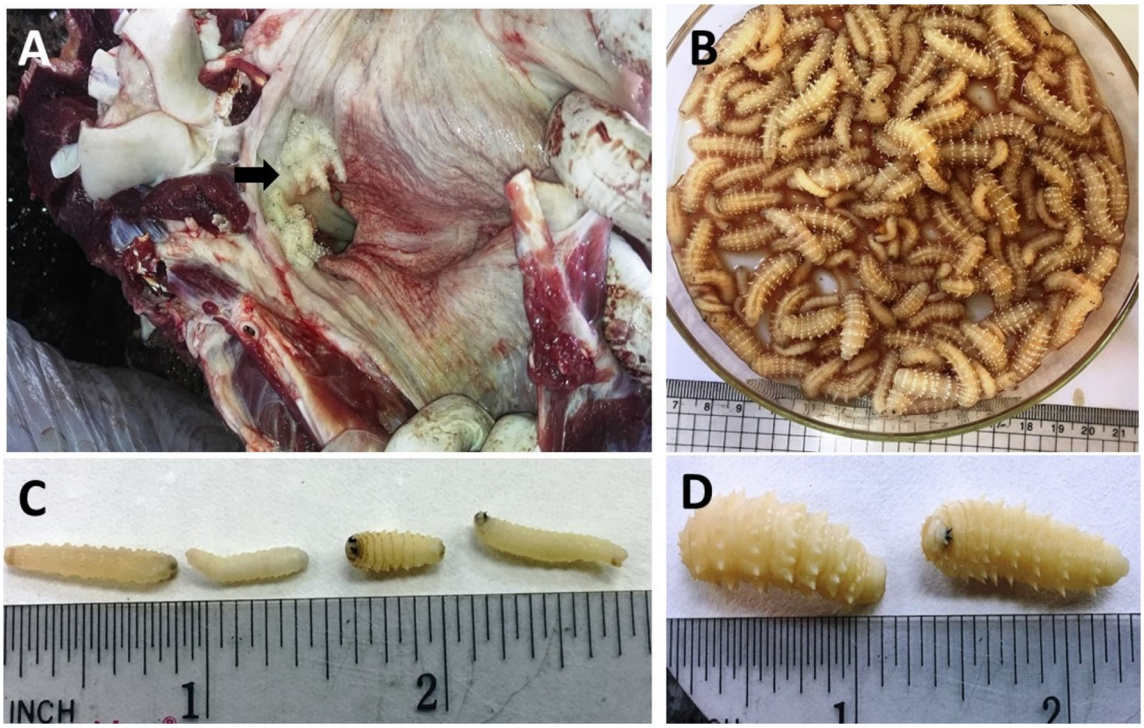

Fig. 2 *Cephalopina titillator* recovered from the camel skull. **A** The heads of slaughtered camel showing the presence of larvae in the pharynx. **B** The collected 2nd and 3rd stage larvae; **C** 2nd stage larvae and **D** showing 3rd stage larvae

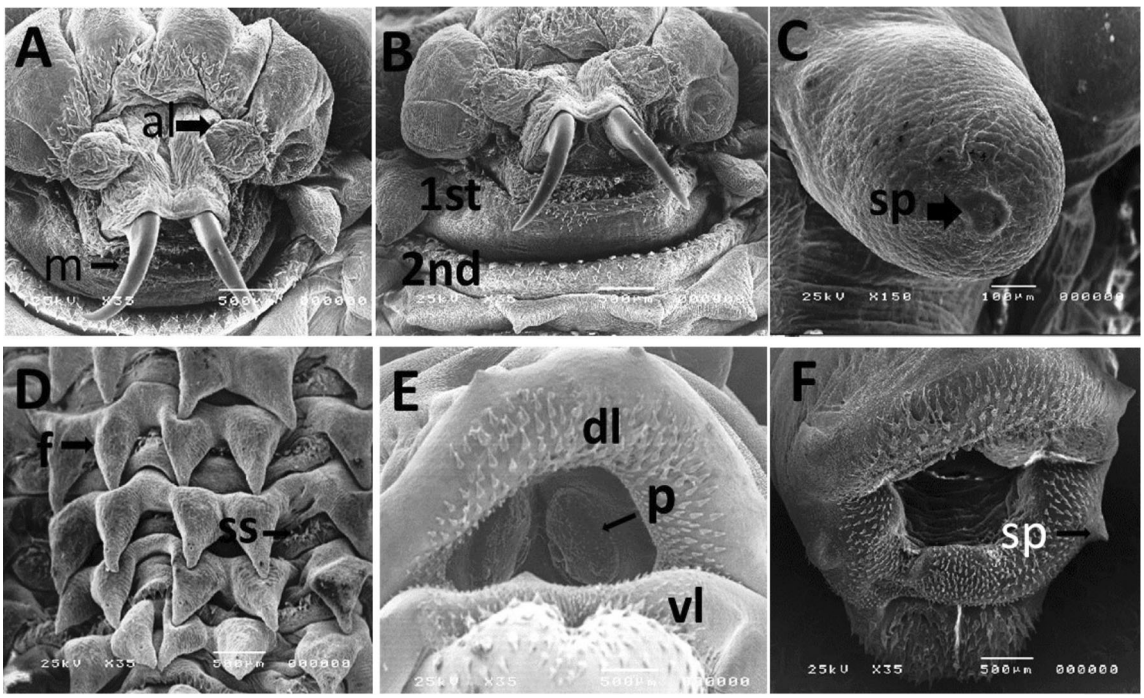

Fig. 3 Scanning electron micrograph of 3rd stage larvae of *C. titillator* showing: **A** the anterior end or pseudocephalon had two long curved maxillae (m) and two antennary lobes (al). **B** Pseudocephalon with the 1st and 2nd thoracic segments were supported by small spines. **C** High magnification of antennary lobe with sensory papillae. **D** Showing the large fleshy spines (f) with tapering end and small spines (ss) behind the fleshy one. **E** Showing the last abdominal segment which contains two peritremes (p) in deep pit; this last segment formed from dorsal (dl) and ventral lips (vl). **F** The lips contain number of sensory papillae (sp) at its surface and small spines

Table 2 Evaluation of the diagnostic values of *C. titillator* larval antigen used in indirect-ELISA and Dot-ELISA compared to GS tests

	Indirect-ELISA	Dot-ELISA
Sensitivity	100%	100%
Specificity	50%	100%
Type 1 error (false positive)	50%	0
Type 2 error (false negative)	0	0
Apparent prevalence	90%	80%
PPV	88.89%	100%
NPV	100%	100%
Accuracy	90%	100%
Likelihood ratio positive	2.00	–
Likelihood ratio negative	0	0

ELISA enzyme-linked immunosorbent assay, *PPV* positive predictive value; *NPV* negative predictive value; true prevalence determined by GS was 80% in camels, *GS* gold standard (examination of the skulls of the slaughtered camels)

Table 3 The agreement between indirect-ELISA, Dot-ELISA and GS test results of camel samples

Diagnostic test	Kappa value (κ)	Agreement	*P* value
Indirect-ELISA versus GS	0.62	Substantial	< 0.0001
Dot-ELISA versus GS	1.00	Perfect	< 0.0001
Indirect-ELISA versus Dot-ELISA	0.62	Substantial	< 0.0001

ELISA indirect enzyme-linked immunosorbent assay, *GS* gold standard (examination the skulls of the slaughtered camels)

agreement between the results of the Dot-ELISA and GS tests ($\kappa = 1.00$, $P < 0.0001$).

Results of Pearson correlation (Table 4) indicated that there was a significant strong positive association between larval infestation intensity and indirect-ELISA titer (OD) ($r = 0.87$, $P < 0.0001$). For each one increase in larval count, we observed a 0.097 ($\pm$ 0.02, $\pm$ 95% CI) increase in the ELISA titer (OD) of the infested camels ($F(1, 28) = 90.28$, $P < 0.0001$, $R^2 = 0.76$); as demonstrated in Fig. 4.

Discussion

The current study revealed that *C. titillator* is a common parasite of the camel population, which points out that the myiasis is widespread in areas where camels are found in Egypt. The overall frequency of infestation among 30 inspected camels' heads was 80%. Previous records from Egypt and other countries proved that the fly is a common and well-known parasite of camel (Al-Ani and Amr 2016; Al-Jindeel et al. 2018). Additionally, the occurrence of this parasite in camels in different countries is usually high. The reported prevalences of infestation in neighbor countries were 79% in Egypt (El-Rahman 2010), 89.02% in Iraq (Al-Jindeel et al. 2018), 87.5% in Jordan (Al-Ani and Amr 2016). Different stages of the larvae could be observed among the infested camels.

The current study noted that the rate and intensity of infestation with *C. titillator* larvae in females are insignificantly higher than males. Some studies estimated that sex is a significant factor that influences the infestation rate with *C. titillator* larvae (Bekele 2001; Atiyah et al. 2011). Furthermore, the current study noted higher rates of

Table 4 Correlation between indirect-ELISA titer, intensity of larvae infestation (no.) and age of infested camels

	Pearson's correlation (*r*)	*P* value
Indirect-ELISA titer versus no. of larvae	0.87	< 0.0001
Age of camel versus no. of larvae	0.40	0.029

ELISA enzyme-linked immunosorbent assay

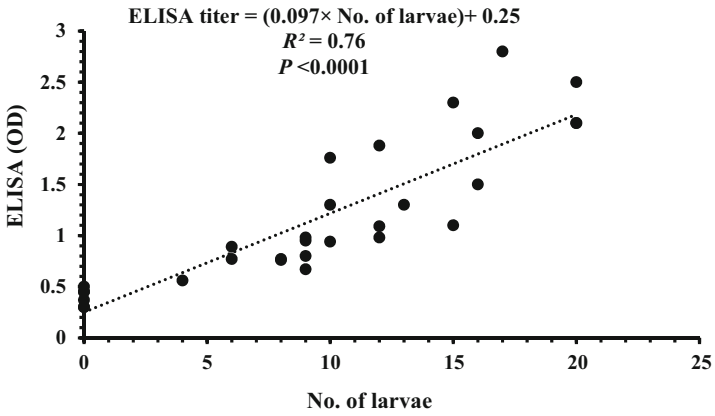

Fig. 4 Relation between intensity of larvae (no.) and ELISA OD values of Camels' sera

infestation and larval intensities of *C. titillator* larvae in the age group 4–8 years than younger ages, which agreed with several earlier studies as recorded by Bekele (2001) and Oryan et al. (2008). This difference may be associated with the continuous exposure of the old camels to the adult fly or due to immunosuppression caused by increasing age (Atiyah et al. 2011).

Infestation with *C. titllator* is very hard to detect in live animals and could not be differentiated from other nervous and or respiratory diseases, which cause similar clinical signs. Besides, *C. titllator* larvae can't be seen by excretory or secretory body fluid, as blood or fecal samples.

Until now, there are no available commercial laboratory kits for diagnosis of nasal bot fly larvae in live camels. In the current study, the gold standard test (inspection of the heads of slaughtered camels) was used in evaluating serological techniques used for screening camels infested with *C. titillator*. The main objective of most screening tests is to decrease the morbidity rate in the tested animal groups through early detection, so efficient treatment and control measures could be applied. The ideal screening test should differentiate correctly between those who have or do not have the infestation and be cheap and not invasive. Prevalence, sensitivity, and specificity are key determinants of the efficacy of the screening test (Maxim et al. 2014).

Many ELISA techniques could be prepared to estimate the humoral antibodies to *C. titellator* in camels' sera. A wide variety of antigens were previously developed that yielded variable results according to the protein structure from which the antigen was prepared, methodological modifications, and variable immune responses (Hendawy et al. 2013).

Cephalopina titellator infestation seems to stimulate cellular and humoral immunity due to the extended period of infestation and interaction with the host's immune system (Stevens et al. 2006; Oryan et al. 2008). Our study demonstrated the association between the intensity of larval infestation and the detected ELISA OD value. Yousef et al. (2016) recorded that the immune response in camels was stimulated by the intensity of infestation and the lesions induced by L2 and L3 larvae of *C. titillator*. In our study, we used the salivary gland (SG) antigen, which was recommended by Hendawy et al. (2013), as the most immunogenic element of *C. titillator* that could elicit IgG response against *C. titillator* larvae.

Our results revealed that 27 (90%) samples defined as positive for *C. titillator* by indirect-ELISA, while 24 (80%) positive samples were defined by Dot-ELISA. The apparent prevalence of infestation among camels differed according to the adopted ELISA technique, where Dot-ELISA demonstrated a perfect agreement in results with the GS test.

Indirect-ELISA is a laboratory diagnostic technique that requires a special apparatus to read the data. However, Dot-ELISA is a potential field test, as it is a simple, quite easy to apply test, and does not require a special apparatus for reading the results, but demands a few hours to analyze the camel sera (Attia et al. 2019a), so it is used for diagnosing different helminths and protozoa as recorded by Gupta et al. (2008) and Attia et al. (2019a). Results indicated that they could be used as immunological screening tests in the laboratory (indirect-ELISA) or the field (Dot-ELISA), as the results of the two tests were nearly similar to the surveying study recorded by Zayed (1998) and Jalali et al. (2016).

Our study recorded high sensitivity and specificity, especially in Dot-ELISA, but less specificity for indirect-ELISA. However, Dot-ELISA needs a few hours to analyze the camel sera. Accordingly, this study is the first to use and evaluate Dot-ELISA for the diagnosis of *C. titillator* in camels and presented acceptable results compared to indirect-ELISA.

Conclusion

In this study, the sensitivity and specificity of two different tests was evaluated. The Dot-ELISA could be applying in the diagnosis of camel diseases and/or in the laboratory. the Dot-ELISA is the rapid and the newest one in diagnosis of *C. titllator*.

Author's contribution All authors sharing in the aim of works; HSF and EI collection of the samples; MMA Identify the parasites and applying the serological analysis; HA-S applying and analyze the haematological parameters. All authors sharing in writing this manuscript and revise it.

Funding All authors declare that they are no funding supporting this work.

Compliance with ethical standards

Conflict of interest All authors declare that there are no competing of interest.

References

AbdElKader NA, Sheta E, AbuBakr HO et al (2020) Effects of chitosan nanoparticles, ivermectin and their combination in the treatment of *Gasterophilus intestinalis* (Diptera: Gasterophilidae) larvae in donkeys (*Equus asinus*). Int J Trop Insect Sci. https://doi.org/10.1007/s42690-020-00171-2

Abouheif M, Basmaeil S, Bakkar M (1990) A standard method for jointing camel carcasses with reference to the effect of slaughter age on carcass characteristics in Najdi camels. I. Wholesale cut weight Asian-Australas. J Anim Sci 3(2):97–102

Al-Ani F, Amr Z (2016) Seasonal prevalence of the larvae of the nasal fly (*Cephalopina titillator*) in camels in Jordan. Revue d'élevage et de médecine vétérinaire des pays tropicaux 69(3):125–127

Al-Jindeel TJ, Jasim HJ, Alsalih NJ, Al-Yasari AMR (2018) Clinical, immunological and epidemiological studies of nasopharyngeal myiasis in camels slaughtered in Al-Muthanna province. Adv Anim Vet Sci 6(7):299–305

Altman DG (1999) Practical statistics for medical research. Chapman & Hall/CRC Press, New York

Angulo-Valadez CE, Ascencio F, Jacquiet P, Dorchies P, Cepeda-Palacios R (2011) Sheep and goat immune responses to nose bot infestation: a review. Med Vet Entomol 25(2):117–125

Ashmawy KI, Fahmy MM, Hilali M (1985) Incidence and seasonal variations of the larvae of *Cephalopina titillator* infesting camels (*Camelus dromedarius*) in Egypt. In: Proceedings of the 12th symposium. Scandinavian Society of Parasitology, Tromsy, Norway, 17–19 June, Abo Akademi, pp 43–44

Atiyah W, Dawood K, Dagher A (2011) Prevalence of infestation by camel nasal myiasis fly larvae in Al-Diwania city abattoir. Al-Qadisia J Vet Med Sci 2:20–26

Attia MM, Salaeh NMK (2020) Ultrastructure of adult *Gasterophilus intestinalis* (Diptera: Gasterophilidae) and its puparium. Int J Trop Insect Sci 40:327–335. https://doi.org/10.1007/s42690-019-00084-9

Attia MM, Soliman SM, Saleh NMK (2019a) Advanced and rapid serodiagnosis of oestrosis (*Oestrus ovis*; Diptera: Oestridae) in sheep using indirect and Dot-ELISA. Jordan J Biol Sci 12(3):275–281

Attia MM, Ismael E, Saleh NMK (2019b) A sensitive serodiagnostic tool for the detection of active infection of zoonotic visceral and nasopharyngeal linguatulosis. Vet World 12(6):883–889

Bekele T (2001) Studies on *Cephalopina titillator*, the cause of 'Sengale' in Camels (*Camelus dromedarius*) in semi-arid areas of Somali State, Ethiopia. Trop Anim Health Prod 33(6):489–500

El-Rahman SSA (2010) Prevalence and pathology of nasal myiasis in camels slaughtered in El-Zawia Province-Western Libya: with a reference to thyroid alteration and renal lipidosis. Glob Vet 4(2):190–197

Graber M, Gruvel J (1964) Etudes des agents des myiases des animaux domestiques et sauvages d'Afrique equatoriale. Rev Elev Med Vet Pays Trop 17(3):535–554

Gupta A, Dixit AK, Dixit P (2008) Dot-ELISA in the diagnosis of parasitic diseases of animals. J Parasit Dis 32(1):10–14

Harlow ED, Lane D (1988) A laboratory manual. Cold Spring Harbor Laboratory, New York, p 579

Hendawy SHM, Allam NAT, Kandil OM, Zayed AA, Des-ouky AAY, El-Seify MA (2013) Immunological evaluation of the diagnostic values of *Cephalopina titillator* different larval antigens in camels from Egypt. Glob Veterinaria 10(2):158–164. https://doi.org/10.5829/idosi.gv.2013.10.2.71161

Higgins A, Kock R (1984) The camel in health and disease. I. A guide to the clinical examination, chemical restraint and medication of the camel. Br Vet J 140:485–504

Jalali MHR, Dehghan S, Haji A, Ebrahimi M (2016) Myiasis caused by *Cephalopina titillator* (Diptera: Oestridae) in camels (*Camelus dromedarius*) of semi-arid areas in Iran: distribution and associated risk factors. Comp Clin Pathol 25(4):677–680

Landis JR, Koch GG (1977) The measurement of observer agreement for categorical data. Biometrics 33(1):159–174

Lowry OH, Rosbrough NJ, Farr AL, Randal RJ (1951) Protein measurement with Folin phenol reagent. J Biol Chem 193:265–275

Maxim LD, Niebo R, Utell MJ (2014) Screening tests: a review with examples. Inhal Toxicol 26(13):811–828

Musa MT, Harrison M, Ibrahim AM, Taha TO (1989) Observation on Sudanese camel nasal myiasis caused by the larvae of *Cephalopina titillator*. Rev Elev Med Vet Pays Trop 42(1):27–31

Oryan A, Valinezhad A, Moraveji M (2008) Prevalence and pathology of camel nasal myiasis in eastern areas of Iran. Trop Biomed 25(1):30–36

Otranto D (2001) The immunology of myiasis: parasite survival and host defense strategies. Trends Parasitol 17(4):176–182

Romero JA, Arias MS, Suárez JL, Paz-Silva A, Francisco I, Alonso F, Cortiñas FJ, Dacal V, Romasanta A, Morrondo P et al (2010) Application of the analysis of serum antibodies (IgM and IgG) to estimate the seroprevalence of ovine oestrosis and to evaluate the effect of chemotherapy. J Med Entomol 47:477–481

Soulsby EJL (1986) Helminths, arthropods and protozoa of domesticated animals, 6th edn. Bailliere & Tindall, London

Stevens JR, Wallman JF, Otranto D, Wall R, Pape T (2006) The evolution of myiasis in humans and other animals in the old and new world (part II): biological and life-history studies. Trends Parasitol 22:181–188

Steward JS (1950) Notes on some parasites of camels (*C. dromedarius*) in the Sudan. Vet Record 62:835–837

Yousef HA, Abdel Meguid A, Afify A et al (2016) Analysis of larval antigens of *Cephalopina titillator* in the camel mucus for diagnosis of infestation. Biologia 71:438–443. https://doi.org/10.1515/biolog-2016-0051

Zayed AA (1998) Localization and migration route of *Cephalopina titillator* (Diptera: Oestridae) larvae in the head of infested camels (*Camelus dromedarius*). Vet Parasitol 80(1):65–70

Zayed AA, Abdel-Maguid A, Madbouly MH, El-Moursy AA, El-Khateeb RM (1994) Incidence and monthly prevalence of *Cephalopina titillator* infesting dromedary camels slaughtered in Egyptian abattoir, Egypt. Vet Med Assoc 54:13–22

Zumpt F (1965) Myiasis in man and animals in the Old World. Butterworth, London, pp 159–164