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Preliminary and advanced studies on fasciolosis in ruminants

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BL	Body length
BW	Body width,
CL	Cone length,
CW	Cone width
OS max	Oral sucker maximum diameter
OS min	Oral sucker minimum diameter
VS max	Ventral sucker maximum diameter
VS min	Ventral sucker minimum diameter
OS-VS	Distance between suckers
A-VS	Distance between anterior end of body and VS
(PhL)	Pharynx length
(PhW)	Pharynx width
BA	Body area
(BL/BW)	BL/BW ratio
OSA/VSA	Sucker ratio
OSA	O.S area
VSA	V.S area
VS-Vit	Distance between vs and vitelline glands
Vit-pos	Distance between Vit and posterior end
Vs-pos	Distance between vs and posterior end

6. Summary

A retrospective study was done to estimate economic losses caused by livers condemnation, due to fasciolosis, of slaughtered cattle and buffaloes in Egypt during the period of 2016-2020, Moreover, livers of naturally infected carcasses were obtained from slaughtered animals in Beni-Suef, Cairo and Tanta provinces, Egypt during 2019-2020 for phenotypic characterization of recovered *Fasciola* species and molecular identification of collected worms using PCR targeting the ITS-1 region. Findings of the retrospective study revealed that percentages of livers condemnation of cattle and buffaloes ranged from 0.66% - 0.79% during the period from 2016-2020. The highest percentages were detected in the south Egypt (2.5%-6.0%) with the highest economic losses (261850–616300 USD annually). Morphometrically, collected flukes categorized into *Fasciola hepatica* and *Fasciola gigantica*. No intermediate forms (*Fasciola* sp.-like) were detected. Sequencing analysis of ITS-1 PCR products showed that only *Fasciola hepatica* (26/34) and *F. gigantica* (8/34) isolates were found, with no intermediate forms, *Fasciola* sp.-like, could be identified. Currently, *Fasciola hepatica* was 100% identical with the Egyptian species (LC076196 and JF294998), French species (JF294999), and Iranian species (MF969009 and MK377150). Moreover, the obtained *F. gigantica* species showed 100% identity with Egyptian ones (LC076125, LC076108 and KX198619), Iranian (KF982047 and MF372919), and other GenBank specimens from Vietnam, Cameroon and India.

Investigating the *in vitro* effect of commercially available fasciolicides; albendazole (40 and 400µg/ml), triclabendazole, rafoxanide and nitroxynil (50 and 100 µg/ml, each) against *Fasciola gigantica* adult worms. For all, worms were incubated for 3 hours. Worms's motility was macroscopically and microscopically detected. Reduction of egg

deposition was estimated. Alterations of worm's cuticle post treatments were recorded using scanning electron microscopy (SEM). Nitroxynil had the most flukicidal effect with mild movement quickly disappeared within 15 min post treatment. It showed the highest egg reduction (88.3% and 95% at concentrations of 50 and 100 µg/ml, respectively). Findings of SEM showed severe furrowing and destruction of spines. In rafoxanide-treated group, the motility disappeared 75 min post treatment, and the egg deposition was significantly ($P \leq 0.05$) reduced to 70% and 85% at the same concentrations. Teguments showed thickening, moderate furrowing and destruction of the spines. Albendazole showed the lowest effect: the motility of the worms was observed till 160 min post treatment and the egg reduction was 43% and 75% at the same concentrations. Interestingly, in albendazole-treated flukes, the tegument had severe furrowing and spines were completely sloughed. Similarly, in triclabendazole-treated flukes, worm's motility was observed till 160 min post treatment and the egg reduction was 76.6% and 88.3%. The tegument showed swelling and mild furrowing with moderately-damaged spines.

In conclusion, South Egypt showed the highest economic losses due to fasciolosis, especially Aswan province. *Fasciola hepatica* was more common than *F. gigantica*, while the hybrid form was not detected. Nitroxynil was the most potent flukicide inducing evidential cuticular changes. Although albendazole induced the most potent cuticular damage, it showed the lowest flukicidal effect. Further *in vivo* studies to investigate resistance/susceptibility of *Fasciola* species in cattle and buffaloes must be carried out.