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Identification of nematode infective larvae in black bucks (*Antilope cervicapra*)

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Abstract
Strongyle nematode egg positive faecal samples of blackbucks from Tal-Chhapar Sanctuary of Rajasthan were pooled, cultured for their development to the infective third stage larvae. Infective strongyle nematode larvae recovered from faecal cultures in the present investigation, were identified on the basis of measurements of their total length, extension of tail sheath beyond the tip of the larvae (µm), intestinal cell number, shape and other morphological characters. The coproculture analysis revealed four nematode genera *viz Bunostomum* sp., *Haemonchus* sp., *Trichostrongylus* sp. and *Strongyloides* sp.

Keywords: *Antilope cervicapra*, Strongyle larvae, Rajasthan, Tal Chhapar sanctuary, wildlife

Introduction
In nature, wild animals in nature live on large areas and have consequently a low genetic resistance against parasitic infections because of low exposure and suffer from a variety of parasitic infections [1]. However, there is a dearth of information on parasitic infections of wild animals due to lack of systematic investigations [2]. Diagnosis of parasitic nematode infections of ruminants, both qualitative and quantitative, is largely still dependent on relatively inaccurate methods such as faecal worm egg counts [3], which give no indication of the identities of most of the common worm genera.

Larva identification is imperative to find out strongyle genera causing the infection, except for those genera with morphologically distinct ova *viz. Strongyloides* sp., *Nematodirus* sp. Moreover, studies regarding identification infective strongyle larvae in wild ruminants particularly blackbucks are scarce with only a single study done by Fatima *et al.* (2019) [4]. Keeping in view these facts, the present study has been aimed to identify strongyle worm genera prevalent in blackbucks of Tal-Chappar sanctuary which will be immensely helpful in efficient anthelmintic treatment and control in near future.

Materials and Methods
The study area comprises of Tal-Chhapar sanctuary, which is located in Churu district of north-western Rajasthan in India and is spread over 7.19 Sq. Km area. Tal-Chhapar sanctuary comes under principal arid zone of the country and is characterized by large variation in temperature which reaches up to 48°C in June and minimum temperature falls below 4°C in December – January. The area is characterized by stormy southwest winds and frequent dust storms with an average rainfall of 300 mm [5]. The faecal samples from blackbucks of Tal-Chhapar sanctuary were placed in sterile polythene bags and labelled carefully indicating the host's detail, location and month of collection, kept in a cool transport box and brought to the Laboratory for further examination. Faecal samples were qualitatively examined by faecal floatation and sedimentation techniques as described by Soulsby [6] for strongyle eggs. Copro-culture was set up to identify the various strongyle larvae on the basis of gut cells number and morphological peculiarities of third stage infective larvae of strongyles. Faecal cultures provide an environment suitable for the hatching of strongylid eggs and their development to the infective third stage larvae. Different eggs require different conditions but the general method given below was suitable for the culture of strongylid eggs in faeces.

For this purpose, strongyle nematode egg positive faecal samples of blackbucks were pooled, cultured following the method of Roberts and Sullivan (1950) [7]. About 40 grams of faeces were broken up finely, using a large pestle and mortar along with spatula. If the lump remained harder a small quantity of water was added to make it desirably soft and if the faeces were too

soft in consistency animal charcoal was added to get the required consistency. A large lump of faeces of desirable consistency was taken in a small Petri dish and spread evenly and this dish was placed in another larger Petri dish having a small quantity of water in it. The larger Petri dish was then covered with another Petri dish to minimize evaporative losses and incubated at 27 ºC in BOD incubator for 7 days. The water in larger Petri dish contained larvae which migrated from the faecal mass in small Petri dish after hatching out from the eggs. Many of the larvae reached the infective third stage by that time facilitating specific diagnosis.

The infective larvae migrating to water in outer Petri dish were pipetted out and centrifuged at 1500 rpm for 3 minutes. The supernatant was discarded and the sediment was warmed over the spirit lamp for few seconds to kill and stretch the infective larvae. A drop of sediment was taken on the slide, and mixed with a drop of lugol's iodine and the stained larvae were examined under microscope.

The third stage infective strongyle nematode larvae recovered from faecal cultures in the present investigation, were identified on the basis of measurements of their total length, extension of tail sheath beyond the tip of the larvae (µm), intestinal cell number, shape and other morphological characters. The larvae were identified on the basis of key provided by Wyk *et al.* (2004) [7], Wyk and Mayhew (2013) [3], Bowman (2014) [9] and Fathima *et al.* (2019) [4]. The measurements were expressed in microns (µm) and composition in percentage (%).

Results and Discussion:

Micrometry analysis of various infective third-stage nematode larvae from black bucks were in compliance with previous analyses by Wyk and Mayhew (2013) [3] and Fathima *et al*. (2019) [4] in small & large ruminants and blackbucks, respectively.

The micrometry analysis revealed the total length of larvae and tail sheath length for *Hemonchus* sp. ranging from 648-741 µm with (697.33±8.59 µm) and (65-78 µm 70.67±1.10 µm), respectively. The tail sheath was sharply pointed and tip of tail kinked and intestinal cells numbering 16 were triangular in shape as shown in Table 1 & Figure 1. The results are consistent to the findings of Jeyathilakan and Sathianesan (2012) [10] and Fathima *et al*. (2019) [4].

Trichostrongylus sp. was observed to have shortest tail sheath length with a conical and blunt tip ranging from 24.5-36.75 µm with an average of 33.09±1.58 µm as shown in Table 1 and Figure 2, which is in accordance to the findings of Jeyathilakan and Sathianesan (2012) [10] and Fathima *et al*. (2019) [4].

Total length of *Bunostomum* sp**.** larvae ranged from 425-543 µm (497.5±7.48 µm) with tail sheath ranging from 54-95 µm (77.66±2.85 µm) with wide body and long thin tail as shown in Figure 3. Micrometry results are in close agreement to the findings of Jeyathilakan and Sathianesan (2012) [10] and Wyk and Mayhew (2013) [3] who observed similar results in small ruminants and cattle. In contrast, a shorter tail sheath length was observed in blackbucks by Fathima *et al*. (2019) [4].

Strongyloides sp. larvae recorded with total length ranging from 553-637 µm with an average of 595.37±10.36 µm and oesophagus comprising more than one third of the total body length as shown in Figure 4, which is correlated to the findings of Bowman (2014) [9].

Table 1: Mean measurements (µm) of 3rd stage infective strongyle larvae of black bucks (Mean±S.E.)

Nematodes	Total length (Range)	Oesophagus Length	Extension of tail sheath beyond tail (Range)	Intestinal cell no. and shape	Salient features
Haemonchus sp.	697.33±8.59 (648-741)	134.86±2.25 105.45-154	70.67±1.10 65-78	16 Triangular	Narrow bullet shaped head, the pointed tail of larva and tail sheath is usually 'kinked'.
Bunostomum sp.	497.5±7.48 (425-543)	101.58±2.09 82.18-129.50	79.66±2.85 54-95	16 Triangular	Short straight larvae with wide body sudden tapering to long thin tail.
Trichostrongylus sp.	680.18±7.11 (638-706)	163.66±1.34 154-182	33.09±1.58 24.5-36.75	16 Triangular	The tail sheath is conical and blunt at the tip.
Strongyloides sp.	595.37±10.36 (553-637)	40% of total length	-	-	No sheath and slender body with long oesophagus 1/3 to ½ of the total length of larvae.

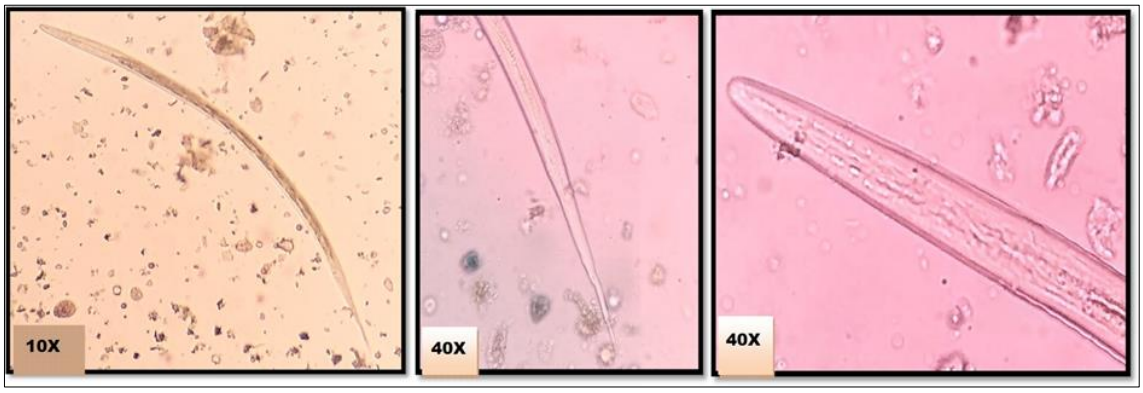

Fig 1: Larvae of *Haemonchus* sp.

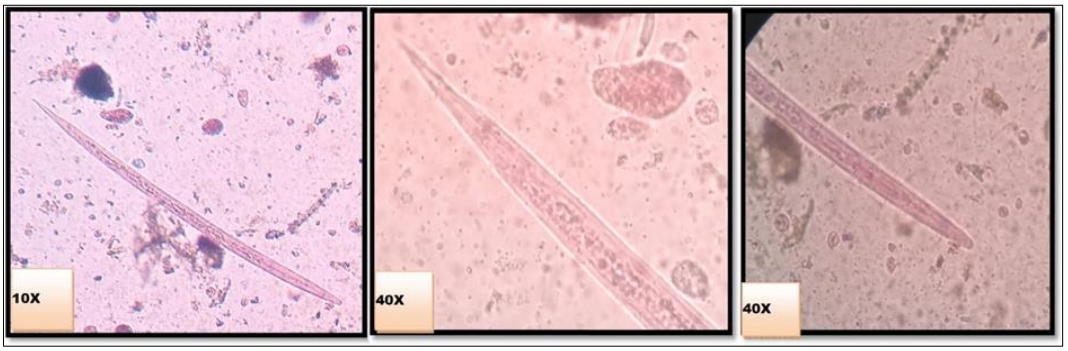

Fig 2: Larvae of *Trichostrongylus* sp.

Fig 3: Larvae of *Bunostomum* sp.

Fig 4: Larvae of *Stongyloides* sp.

Conclusion

Present study represents a comprehensive report on strongyle genera prevalent in blackbucks and their identification of gastrointestinal nematode genera in black bucks of Tal Chhapar Sanctuary, Churu, Rajasthan.

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Conflict of interest

We declare that we have no conflict of interest.

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