

STUDIES ON *STRONGYLUS ASINI*. I. EXPERIMENTAL INFESTATION OF EQUINES

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ABSTRACT

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Infective larvae were harvested from a culture of eggs collected from adult *Strongylus asini* recovered from a free-ranging Burchell's zebra, *Equus burchelli*, in the Kruger National Park. Worm-free zebra, horse and donkey foals were successfully infested, but infestation failed in a mule foal. At slaughter, 117-125 days post-infestation, *S. asini* in their 4th moult were recovered from the liver and portal veins. This is the first report of successful experimental infestation of these hosts with *S. asini*.

INTRODUCTION

Fully mature *Strongylus asini* were recovered by Jaszkowski & Colglazier (1956) from cysts in the liver of a Grevyi zebra, *Equus grevyi* that had died in the Chicago Zoo USA. These worms were the cause of extensive liver damage and peritonitis which was responsible for the zebra's death. In this paper we report on the recovery of *S. asini* (Fig. 1) from free ranging Burchell's zebra, *Equus burchelli*, in the Kruger National Park in the Republic of South Africa.

McCully, Kruger, Basson, Ebedes & Van Niekerk (1969) state that the extensive liver pathology and other lesions in the Burchell's zebra, both in the Kruger National Park and Etosha Park in South West Africa/Namibia, were due to *Strongylus vulgaris*. In the present report we will show that the cause of these liver lesions in the Kruger National Park was *S. asini* and compare the host susceptibility of various equines to *S. asini*.

MATERIALS AND METHODS

Infective larvae

Female *S. asini* were collected from the caecum and ventral colon of 2 zebras slaughtered during a parasite survey in the Kruger National Park in April 1981. Additional *S. asini* were collected from 2 zebras slaughtered in May 1981. The worms were placed in normal saline in a Petri dish and stored overnight in an incubator at 28 °C. A large number of eggs were laid. After 24 hours the worms were cut into small pieces and placed in a tea sieve. Saline was poured over these worm pieces while they were being lightly ground with a glass rod, and the eggs were washed through the sieve.

All the eggs collected were mixed with horse faeces which had been obtained from a worm-free horse, i.e. an animal that had repeatedly been dewormed beforehand. Vermiculite was added to the dung for aeration and cultures were made in 1ℓ wide-mouthed glass jars, which were then incubated at 28 °C.

The infective larvae were harvested 11 and 12 days later, concentrated in a measuring cylinder and stored at 4 °C in a refrigerator.

Experimental animals

A young zebra stallion approximately 6 months old was dartsed with a combination of M99* and Flupyrindol** and transported to the Hoechst Research Farm at Malelane.

A horse filly about 1 year old was purchased at Carolina, Transvaal, and transported to the farm.

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A donkey and a mule foal were bought when they were about 1 month old and reared on a concrete floor at the farm. At the time of infestation they were about 3 months old.

All the animals were kept in pens with concrete floors. The animals were fed worm-free hay *ad libitum* and a pellet concentrate 3 times a day. Clean fresh water was always available.

All 4 trial animals were dosed with Albendazole*** at 50 mg/kg with a stomach tube twice a day for 2 days.

The mass of each animal at the time of infestation was:

Zebra	: 130 kg
Horse	: 150 kg
Mule	: 55 kg
Donkey	: 55 kg

Infective larvae of *S. asini* were dosed by means of a stomach tube and 300 larvae were given to each animal on 21 May 1981 (Day 0) and a further 1 500 larvae to each animal 6 days later on 27 May 1981 (Day +6). All the foals were killed from 21-23 September 1981. The age of the worms in days at slaughter in the different hosts was:

Zebra	: 117 and 123,
Horse	: 118 and 124,
Mule	: 119 and 125,
Donkey	: 119 and 125.

Post-mortem technique

The autopsies were conducted according to the techniques described by Malan, Reinecke & Scialdo (1981 a, b). In addition, special attention was given to the following:

- The portal venous system was extensively opened for observing the lesions and recovery of all the worms.
- The peritoneum, abdominal muscular layers, mesentery and kidney fat were dissected and examined for worms.
- The lungs were cut into small pieces and placed in a 2ℓ wide-mouthed jar containing normal saline, and the jar incubated in a water-bath at 40 °C for 4 hours. Any worms that might have migrated from the lungs into the saline solution were recovered by washing the contents on a sieve with apertures of 150 μm.

The helminths recovered were left in saline for 12 h and then killed in glycerine-alcohol mixture heated to 60 °C. The worms were stored in plastic specimen jars in the glycerine-alcohol mixture (60% absolute alcohol, 5% glycerine, 35% H₂O).

RESULTS

Zebra

The general condition of the zebra was poor, with little subcutaneous, mesenteric or perirenal fat.

*** Smith Kline

Liver: Numerous nodules measuring 2–3 cm in diameter could be seen elevating the capsular surface (Fig. 2). More nodules could be palpated deeper in the parenchyma. They were oval to round and were quite hard. Cutting through the nodules revealed in each a hard fibrous capsule surrounding a cystic cavity which contained *S. asini* and a thick red-brown fluid. Most contained a single worm, but some were larger and multi-lobular and contained up to 4 worms.

The nodules were associated with the portal blood supply to the liver. When opening the portal vein at the portal fissure and following the vein and its branches into the liver substance, numerous nodules containing 1 or more helminths were seen protruding into the lumens of the branches of the portal vein.

A large thrombus, roughly 14 cm long and having a diameter of 15 mm, was found in the portal vein.

The periportal and hepatic lymph nodes were markedly enlarged.

A. mesenterica cranialis: No worms or lesions were found.

Lungs: A caseous nodule was found in the lung tissue, but no worms were found.

Ascites: Four hundred ml of a straw-coloured fluid was measured.

Hydropericardium: Forty-five ml of the same coloured fluid was present.

Horse

The carcass was in a healthy condition.

Liver: No macroscopic changes were seen in the initial examination *in situ* of the liver. One nodule was identified by palpation, but when the portal vein was opened at the portal fissure, 15 large (2 × 2 cm) nodules were observed (Fig. 3). In addition, 2 thrombi were found in the intrahepatic branches of the portal vein.

An additional 30 worms were recovered from nodules adjacent to the distal branches of the portal vein.

A. mesenterica cranialis: No lesions or worms were seen.

Ascites: Two hundred ml of a straw-coloured fluid was measured.

Hydropericardium: Eighteen ml of straw-coloured fluid was measured.

Mule

No worms or lesions were seen. The mule was in very good condition.

A. mesenterica cranialis: No worms or lesions were seen.

Ascites: Two hundred and fifty ml of fluid was recovered.

Hydrothorax: Fourteen ml of fluid was present.

Donkey

The general condition of this animal was very poor.

Liver: The liver had the same external appearance as that of the zebra (Fig. 2), with numerous nodules throughout. The major difference was noted, however, when the portal vein was opened. Instead of numerous parasitic nodules, a severe thrombophlebitis was observed. Many *S. asini* were present in the thrombotic material which adhered to the roughened intimal surface of the vein (Fig. 3). The proximal intrahepatic branches of the portal vein were those most prominently involved.

Numerous nodules were found in the smaller branches of the veins, the majority being associated with the right lobe of the liver.

A. mesenterica cranialis: No worms were recovered and no lesions were seen.

Ascites: Fourteen ml of a straw-coloured fluid was measured.

Hydropericardium: Eighteen ml of a straw-coloured fluid was measured.

DISCUSSION

Boulenger (1920), using specimens of *S. asini* sent to him from donkeys in Zanzibar and Nairobi, described *S. asini* as a large (18–42 mm long × 2.5 mm wide) strongyle, dark-red in colour, its head divided from the rest of the body, with a fairly large mouth (320 × 470 μm) and a well-marked-off mouth collar. The dorsal gutter is well-developed and terminates behind the anterior margin. There is a single tooth at the base of the dorsal gutter which, like that of *S. vulgaris*, is divided into lateral projections which are much shorter than those of *S. vulgaris* (See the illustration of *S. asini* in Fig. 1).

Nairobi, situated ± 1°S and Skukuza in the Kruger National Park ± 25°S, being separated by about 2 600 km, represent different ecosystems. It is postulated that the normal hosts of *S. asini* should be sought among the wild zebra populations of Africa and that they as the true hosts possibly overflowed into domestic equines on communal grazing grounds, such as occurred in the donkeys in which Boulenger (1920) found the original specimens of *S. asini*.

To test this theory 3 domestic equines (horse, donkey, mule) and a Burchell's zebra were artificially infested with infective larvae harvested from eggs of *S. asini* collected from zebras in the Kruger National Park. The donkey and zebra were the most susceptible species of the hosts tested and both of them showed debility and emaciation in the terminal phases of this study prior to slaughter for autopsy.

The worm nodules in the *Vena portae* of the zebra were more prominent than those in the donkey, while more worms were found associated with the thrombophlebitis present in this vein in the donkey. This may indicate that part of the larval development occurs in the lumen of the *V. portae* in the donkey. If so, it is reminiscent of the parasitic endarteritis produced by larval stages of *S. vulgaris* in equines.

Neither the horse nor the mule exhibited obvious clinical signs, and, while the horse showed only a few nodules in the *V. portae*, the mule showed none at all. At slaughter 117–125 days post-infestation, *S. asini* in the 4th moult were recovered from the liver and portal vein of the zebra, donkey and horse, but none in the mule. We surmised that *S. asini* infestation is capable of causing mortality in the zebra if the severe pathology in an experimental infestation is typical of a field case.

The worms recovered were all in the 4th moult. The mean length of the females was 32.1 mm, ranging from 25–38.5 mm. The mean length of the males was 29.9 mm, ranging from 22.5–34 mm. Theiler (1923) reports that the mean length of female *S. vulgaris* is 20 mm, while that of the male *S. asini* ranged from 18–32 mm and females from 30–42 mm.

From these trials it seems that the parasitic life cycle can be summarized as follows:

Mouth → gut → penetration → portal system → liver tissue → portal system → caecum and colon.

The aim of the further trials will be to prove this postulate. *S. asini* were never recovered from the *A. mesenterica cranialis* or its branches and this is additional evidence that this is not a strain of *S. vulgaris* which normally migrates to this artery and not to the *V. portae*.

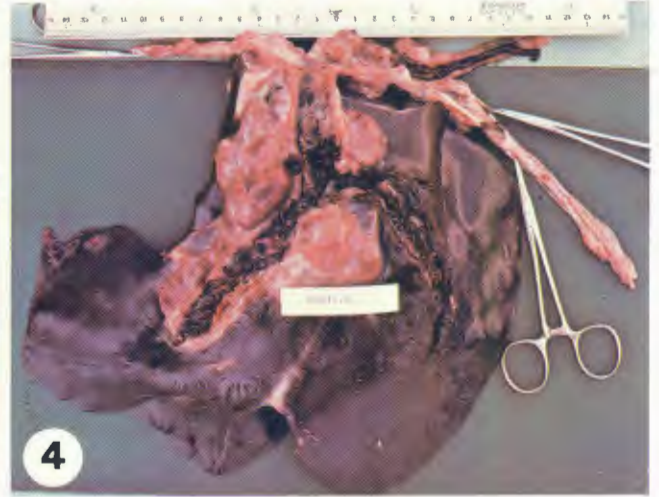
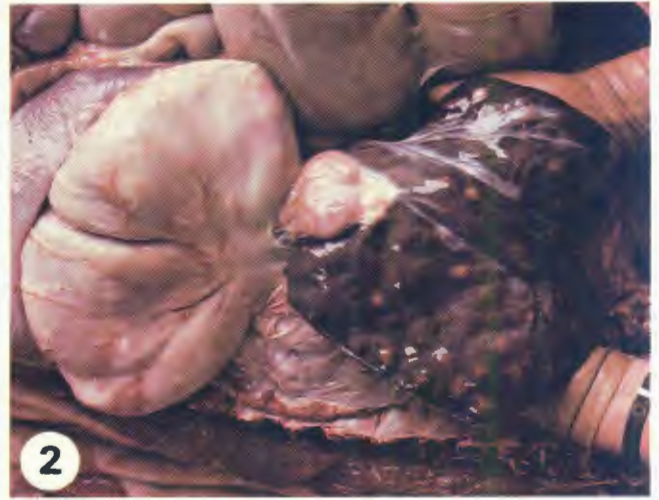


FIG. 1 Anterior end, lateral view of *S. asini*. Although the mouth capsule resembles that of *S. vulgaris*, the dorsal gutter is shorter and does not reach the anterior margin, and the teeth are much shorter than those of *S. vulgaris*

FIG. 2 Liver of an experimentally-infested zebra *in situ*. Note the numerous nodules 2–3 cm in diameter, elevating the surface of the capsule. At least 1 but as many as 4 *S. asini* were recovered from each nodule

FIG. 3 The *Vena portae* of a horse experimentally infested with *S. asini*. Note thrombi in the lumen and nodules in walls of the portal vein

FIG. 4 *S. asini* in the portal vein of an experimentally-infested donkey. Note the severe thrombophlebitis and the numerous *S. asini* in the lumen

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