

## RECOVERY OF HELMINTHS POSTMORTEM FROM EQUINES. II. HELMINTHS AND LARVAE OF *GASTEROPHILUS* IN THE GASTRO-INTESTINAL TRACT AND OESTRIDS FROM THE SINUSES

F. S. MALAN, R. K. REINECKE\* and ROSINA C. SCIALDO\*, Hoechst Research Farm, P.O. Box 124,  
Malelane 1320, Republic of South Africa

### ABSTRACT

MALAN, F. S., REINECKE, R. K. & SCIALDO, ROSINA C., 1981. The recovery of helminths postmortem from equines. II. Helminths and larvae of *Gasterophilus* from the gastro-intestinal tract and oestrids from the sinuses. *Onderstepoort Journal of Veterinary Research*, 48, 145-147 (1981).

The tongue, pharynx, oesophagus and gums are examined for larvae of *Gasterophilus* spp., and the nose and sinuses for oestrid larvae. The gastro-intestinal tract is divided into separate specimens—stomach, small intestine, caecum, ventral colon, dorsal colon, descending colon and rectum—and each is examined separately. Aliquots of ingesta of  $\frac{1}{4}$  by mass of the stomach and  $\frac{1}{10}$  by mass of the small intestine, caecum, ventral colon and dorsal colon are collected for microscopic examination. Each part of the wall of the caecum, ventral and dorsal colon is washed and specimens are collected for subsequent examination. The gut wall of the caecum and colon is examined macroscopically for larval stages. Ingesta in the descending colon are examined macroscopically for *Gasterophilus* larvae.

### Résumé

RÉCUPÉRATION POST-MORTEM D'HELMINTHES ÉQUINS. II. HELMINTHES ET LARVES DE *GASTEROPHILUS* DANS L'APPAREIL GASTRO-INTESTINAL ET OESTRIDES DES SINUS

La langue, le pharynx, l'oesophage et les gencives sont examinés pour la présence de larves de *Gasterophilus* spp., et le nez et les sinuses pour les larves oestrides. L'appareil gastro-intestinal est divisé en échantillons séparés—estomac, intestin grêle, caecum, colon ventral, colon dorsal, colon descendant et rectum, et chacun de ces échantillons est examiné séparément. Des aliquots d'ingesta de  $\frac{1}{4}$  de masse de l'estomac et de  $\frac{1}{10}$  de masse pour l'intestin grêle, le caecum, le colon ventral et le colon dorsal sont pris pour l'examen microscopique. Chaque part de la paroi du caecum, du colon ventral et dorsal est lavée et les échantillons sont récoltés pour un examen ultérieur. La paroi du caecum et du colon est examinée macroscopiquement pour les stades larvaires. Les ingesta dans le colon descendant sont examinés macroscopiquement pour les larves *Gasterophilus*.

### INTRODUCTION

Malan, Reinecke & Scialdo (1981) described the postmortem techniques they used for locating arterial lesions caused by *Strongylus vulgaris* and also for examining the abdominal wall for larvae of *Strongylus edentatus*, the liver for lesions caused by larvae of nematodes and those of *Echinococcus* spp., the lungs for larvae of *Echinococcus* spp., and *Dictyocaulus arnfieldi*. This paper describes the recovery of helminths and larvae of *Gasterophilus* spp. from the gastro-intestinal tract and of oestrid larvae from the sinuses.

### MATERIALS AND METHODS

The exposure and removal of the viscera from the carcass, the removal of the arteries supplying the gastro-intestinal tract, the liver and lungs and their examination for lesions and recovery of nematode and cestode larvae have been described in detail (Malan *et al.*, 1981).

#### *The skull and mouth*

The skull is severed from the cervical vertebrae and one of the mandibles placed in a vice. The skull is sawn in half along its length with a butcher's saw. The jaw is halved medially between the incisor teeth, and then both jaws are removed at their articulation with the skull. The nasal septum is removed with sharp-pointed pruning shears, the frontal and maxillary sinuses are opened and larvae of *Rhinoestrus* spp. removed. Mature 3rd stage larvae ( $L_3$ ) are

transferred to vermiculite in jars and allowed to pupate. Adult flies which may possibly emerge are identified.

The turbinate bones are removed, opened along their length, cut into 4 cm<sup>2</sup> pieces, placed in physiological saline in 2l jars and incubated for 3 h at 37 °C in a water-bath. Thereafter, the filtrate is sieved (apertures 150 µm) and larvae in the residue transferred to jars containing 70% alcohol (I. G. Horak, personal communication, 1980).

The gums are pushed away from the teeth with fine forceps and 1st stage larvae ( $L_1$ ) of *Gasterophilus* removed and placed in 70% alcohol. Most  $L_1$  are found between the premolar or molar teeth. The tongue is severed at the entrance to the pharynx and cut across the width into slices 3-5 mm wide, placed in large-mouthed glass jars containing physiological saline and incubated in a water-bath for 2-3 hours at 37 °C. The filtrate is then poured on to sieves (150 µm apertures) and the residue transferred to jars containing 70% alcohol.

#### *Pharynx and oesophagus*

These organs are opened with bowel scissors and any 2nd stage larvae ( $L_2$ ) and  $L_3$  of *Gasterophilus* are removed and preserved in alcohol.

#### *The gastro-intestinal tract*

Two ligatures are tied around the colon at the pelvic flexure and the mesocolon incised to separate the ventral and dorsal parts of the colon. The caecocolic fold is incised and 2 ligatures tied around the left ventral colon at its junction with the caecum and 2 around the ileum near the ileocaecal junction. Similar ligatures are tied around the duodenum at

\* Department of Parasitology, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, Onderstepoort 0110

Received 15 May 1981—Editor

the pylorus and around the oesophagus cranial to the stomach. A cut is made between the double ligatures to divide the gastro-intestinal tract into the:

- (i) stomach
- (ii) small intestine
- (iii) caecum
- (iv) ventral colon
- (v) dorsal colon
- (vi) descending colon, rectum and anus.

The organs are placed in plastic containers.

With the exception of the caecum, which harbours approximately 10% of adult *Cyathostominae*, almost all these worms are present in the ventral and dorsal colon. The ventral colon is therefore opened first and the ingesta emptied into the large bin. The wall with the adhering worms and ingesta is placed in a 15 l bucket. This process is repeated for each organ so that there are finally 6 bins of ingesta (stomach, small intestine, ventral colon, dorsal colon, caecum, descending colon and rectum) and 6 buckets of gastro-intestinal walls.



FIG. 1 Apparatus for washing intestinal wall to collect the helminths.

Worms and ingesta adhering to the walls of these organs are washed off with a water hose fitted with a spray attachment into a collecting apparatus which is made of galvanized sheeting 2–3 mm thick (Fig. 1). The dimensions of the apparatus are:

1. upper washing area 700 × 400 × 200 mm
2. drainage channel with a floor 440 mm long, and a top 400 mm wide with a slope of approximately 1 in 3 narrowing to a

3. drainage pipe 130 mm in diameter, 270 mm in length
4. the stand made of square (25 mm<sup>2</sup>) steel tubing 2 mm thick, 760 mm high where washing takes place, sloping to 730 mm at the entrance of the drainage channel.

The water from the drain-pipe is collected in a bucket and 5 ml, of 45% iodine solution\* is added to kill the worms before they are fixed in 10% formalin.

After washing, each gut wall is placed in a heavy duty plastic (polyurethane) bag marked with a felt pen, the neck tied with string, and the bag stored in a refrigerator at 4 °C.

After the worms in the wall washings have been killed and fixed, the specimens are poured on to sieves (apertures 150 μm), washed, and the residue transferred to wide-mouthed 1 l glass jars to which 10% formalin is added as a preservative.

The wall of the descending colon and rectum is examined macroscopically for adult worms, e.g. *Cylindropharynx* spp. in zebra and larvae of *Gasterophilus* spp., and then discarded. The ingesta in the latter 2 organs are broken up manually into small pieces and any worms present collected before the ingesta are discarded.

The stomach ingesta are thoroughly mixed manually and random samples taken until  $\frac{1}{4}$  by mass has been collected. The small intestinal ingesta, being more fluid, are vigorously stirred with a soup ladle before being transferred in small amounts  $\frac{1}{10}$  by mass to a bucket. Only  $\frac{1}{10}$  by mass of either caecal, ventral or dorsal colon ingesta is collected after thorough manual mixing.

All worms in the aliquots are killed, fixed, sieved and preserved as described above. The balance of the ingesta is examined macroscopically for the larger worms, which include trematodes, cestodes, *Parascaris equorum*, *Oxyuris equi*, adult *Strongylus* spp., *Triodontophorus* spp. and the larger *Cylicocycylus* spp. These are then transferred to specimen jars.

The gastro-intestinal walls of the various organs are examined for worms. If these organs cannot be examined immediately, they may be frozen (−6 °C) and examined at a convenient time.

The gut wall is examined for helminths as follows:

#### 1. Stomach

All *Gasterophilus* larvae are removed and preserved in 70% alcohol. The glandular stomach (*corpus* and *pars pylorica*) is palpated to locate hard swellings caused by *Draschia megastoma*. These swellings are cut open, the worms removed and fixed in 10% formalin. The mucosa and muscular layers of the glandular stomach are scraped from the serosa and the former digested in pepsin/HCl (Reinecke & Brooker, 1972).

#### 2. Small intestine

The larvae of *Gasterophilus* are removed manually from the wall of the duodenum and then the entire small intestine is cut into pieces 10–15 cm long. With the aid of a diamond sorting lamp† each piece is examined macroscopically for larvae of *Cyathostominae*, which can be numerous in the zebra. These larvae are either black dots in small nodules

\* The iodine solution is made up by dissolving 7–9 kg of KI in as little hot distilled water as possible, adding 4.5 kg of I crystals and then adding water to make a final volume of 10 l

† Dazor, MFG Corporation, St Louis, USA

2 mm in diameter or red worms curled up in nodules 1 cm in diameter. Each piece is spread on a flat surface, e.g. blotting paper, the wrinkles smoothed out and a piece of black silk used to divide the gut into a grid. If available, 50 nodules containing larvae of different sizes are cut out and examined under a stereoscopic microscope. A larva that is alive at the time of the animal's death has a complete cuticle and the mouth parts and gut cells are intact.

### 3. Caecum

This is cut into pieces of 10 cm<sup>2</sup>. Tucks tend to form adjacent to the bands (taeniae) and these are cut with scissors to eliminate them. Each piece is spread out, wrinkles smoothed out and counting done as described for the small intestine. If there are many thousands of worms present in the nodules, the entire wall is cut into pieces of 10 cm<sup>2</sup>, the mass determined and  $\frac{1}{4}$  to  $\frac{1}{10}$  by mass (depending on the mass and total number of worms) examined and the worms counted.

### 4. Colon

There are more larvae in the ventral colic than in the dorsal colic wall. These are counted in exactly the same manner as described above. In both the caecum and colon, about 50 nodules are cut out and checked with the stereoscopic microscope to determine the accuracy of the counts.

### Helminth and arthropod identification

The descriptions of Lichtenfels (1975) are used to identify most of the helminths, and those of Theiler (1923) for *Crossocephalus* and *Cylindropharynx*. The identification of oestrid and *Gasterophilus* spp. larvae follows the descriptions of Zumpt (1965).

### DISCUSSION

Previously, we used the modified Baermann apparatus to recover worms from the gastro-intestinal tract (Reinecke & Brooker 1972). Having to place

specimens twice in traps in physiological saline meant that there were 3 specimens of residue and 1 of filtrate. The filtrate contained 66.6–86.5% of the strongyles and as few as 55.8% of the *Probstmayria vivipara* present in the ingesta. The extra labour involved in preparing these specimens is not justified if high percentages are retained in the residues, as these must also be examined for worms. In anthelmintic trials this is unacceptable if accurate assessment of efficacy is desired.

A disadvantage of washing the walls of the gut is that the worms thus obtained are morphologically distorted. Distortion may be overcome if physiological saline rather than tap water is used to wash the wall. The size of the different aliquots collected is governed by the number of worms present in the ingesta. Worm burdens in the stomach and small intestine are low and a larger aliquot is essential for accurate estimation of the total number of worms present, while the massive worm burdens in the caecum and colon give more accurate results, even if only  $\frac{1}{10}$  of the mass is examined.

### REFERENCES

- LICHTENFELS, J. R., 1975. Helminths of domestic equines. Illustrated keys to genera and species with emphasis on North American forms. *Proceedings of the Helminthological Society of Washington*, 42, Special issue p. 92.
- MALAN, F. S., REINECKE, R. K. & SCIALDO, ROSINA, C., 1981. Recovery of helminths postmortem from equines. I. Parasites in arteries, subperitoneum, liver and lungs. *Onderstepoort Journal of Veterinary Research*, 48, 141–143.
- REINECKE, R. K. & BROOKER, DEIDRE, 1972. Recovery of parasitic nematodes from the gastro-intestinal tract of a mule at necropsy. *Onderstepoort Journal of Veterinary Research*, 39, 51–58.
- THEILER, GERTRUD, 1923. The strongylids and other nematodes parasitic in the intestinal tract of South African equines. 9th & 10th Reports of the Director of Veterinary Education and Research, Union of South Africa. 603–773.
- ZUMPT, F., 1965. Myiasis in man and animals in the old world. A textbook for physicians, veterinarians and zoologists. London: Butterworths.