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RECOVERY OF PARASITIC NEMATODES FROM THE GASTRO-INTESTINAL TRACT OF A MULE AT AUTOPSY

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ABSTRACT

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An autopsy was carried out on an aged mule and the gastro-intestinal nematodes recovered in a modified Baermann apparatus in a waterbath. Ingesta were initially placed on fibreglass gauze (apertures 1,5 × 1,1 mm) and the filtrate subsequently poured on to a double gauze platform in traps. The upper platform had nylon grit gauze with an aperture of 700 micron, while the lower platform had gauze with an aperture of 500 micron. As many as 56,7% *Probstmayria vivipara* and 75,2% of the other nematodes migrated through all three layers of gauze into the filtrate, which constituted less than 5% of the total ingesta. Although 12,9% of the strongyles were washed off the caeco-colonic wall, none were recovered when this gut wall was subsequently digested.

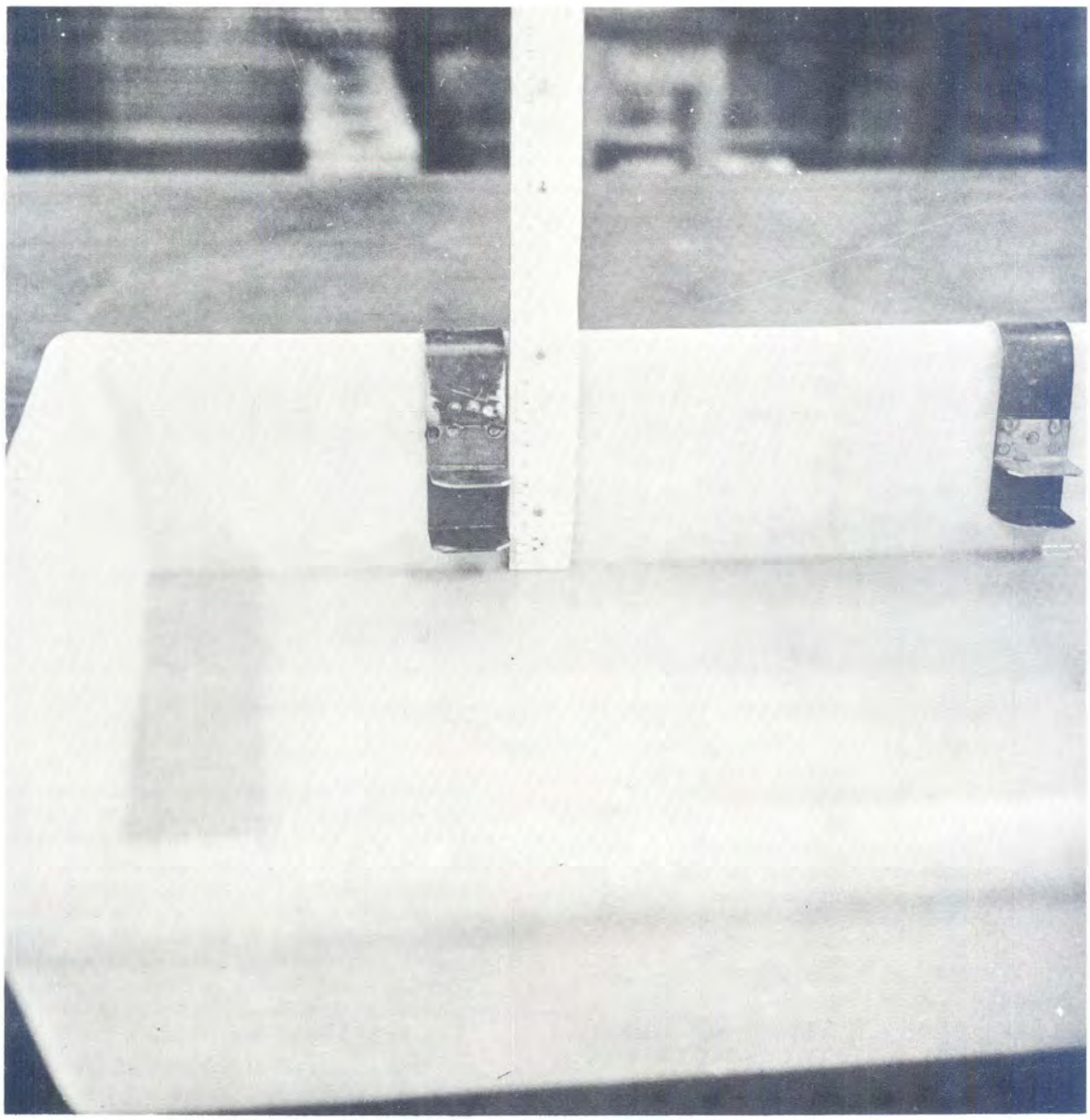

FIG. 1 Steel supports suspended from the edge of a plastic tray. (Scale metric)

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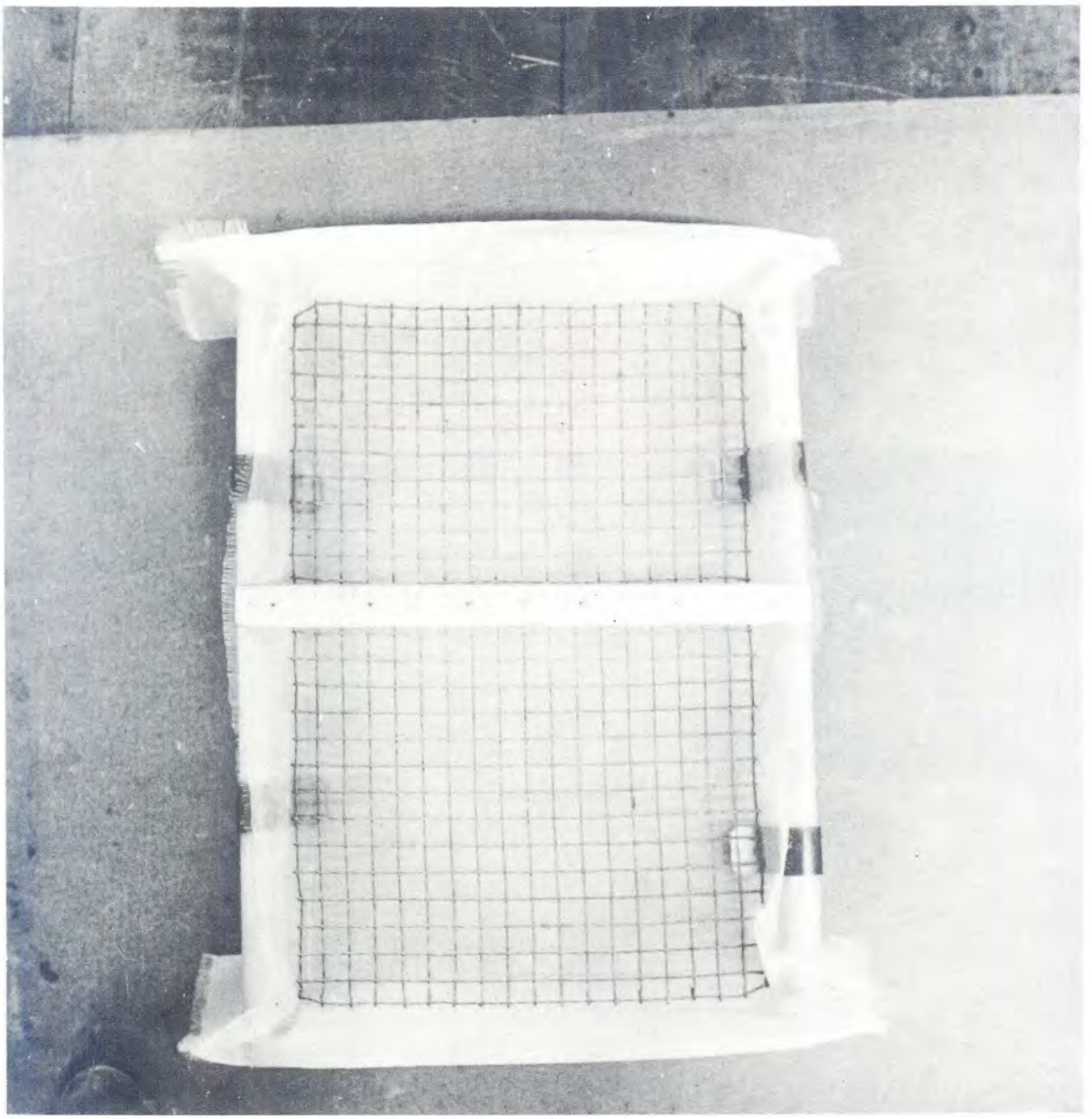

FIG. 2 Nylon grit-gauze covered with wire mesh resting on steel flanges in the plastic tray. (Scale metric)

INTRODUCTION

In equines the majority of parasitic nematodes in the gastro-intestinal tract are mixed with a vast mass of ingesta in the ascending colon and caecum. This paper describes a method of concentrating these parasites in a small quantity of ingesta.

MATERIALS AND METHODS

A. *Apparatus*

Waterbaths; The three waterbaths used were 207 × 135 × 25cm, 171 × 136 × 32,5 cm and 120 × 100 × 23 cm in size. Hot water was poured into them and the thermostat adjusted to 40°C in the large and medium waterbath. The temperature in the small waterbath was maintained at 50°C for digestion of the gut wall with pepsin/HCl. The minced gut wall and pepsin/HCl were aerated to facilitate digestion (Reinecke, 1972).

Traps: These were plastic trays which measured 61 × 41 cm at the top and tapered to 57 × 38 cm at the base; they were 11,5 cm deep (Fig. 1). Flat steel strips (4 cm wide × 1,5 mm thick) were hung from the upper rim down the inner slope of each trap to within 2 cm of its base. At right angles to the strips two flanges, 2 cm wide, were welded, one at the base and the other 2,5 cm above it. In each tray four of these strips acted as supports for wire netting to form a single or double platform. This netting had apertures of 2 cm^2 and was 57,5 cm by 37,5 cm in size (Fig. 2).

Mesh: Three types of mesh were used to line the trays:

1. Fibre-glass gauze — aperture 1,5 × 1,1 mm
2. Nylon grit-gauze — aperture 700 micron
3. Nylon grit-gauze — aperture 500 micron

Labels and glass-ware: Plastic clip-on labels, wide-mouthed glass jars for digestion and specimen jars were marked beforehand as follows:

Specimen	*Code*	*Clip-on labels*	*Digest jar* (4 l)	*Specimen jar* (0,9 l)
Stomach -				
residue	StR	1	0	2
filtrate	StF	1	0	1
digest	StD	0	1	1
Small intestine -				
residue	SIR	1	0	2
filtrate	SIF	1	0	1
digest	SID	0	2	1
Caecum -				
wall washings	CeWW	0	0	1
supernatant	CeSup	0	0	1
residue 1	CeR_1	3	0	5
residue 2	CeR_2	2	0	3
residue 3	CeR_3	1	0	3
filtrate	CeF	1	0	2
digest	CeD	0	4	1
Anterior colon -				
wall washings	ACWW	0	0	2
supernatant	ACSup	0	0	1
residue 1	ACR_1	7	0	*
residue 2	ACR_2	4	0	5
residue 3	ACR_3	2	0	4
filtrate	ACF	2	0	3
digest	ACD	0	8	2
Posterior colon -				
wall washings	PCWW	0	0	1
supernatant	PCSup	0	0	1
residue 1	PCR_1	4	0	6
residue 2	PCR_2	3	0	4
residue 3	PCR_3	2	0	3
filtrate	PCF	2	0	2
digest	PCD	0	4	1

*This was placed in plastic bags instead of specimen jars (see below).

Miscellaneous apparatus: The gut-washing apparatus was tested (Reinecke, 1972). Plastic buckets (20 l), stainless steel buckets (15 l), saucepans (10 l), Endecott sieves 400 mesh (aperture 39 micron) and 100 mesh (aperture 150 micron), graduated glass measuring cylinders and buckets graduated in litres were cleaned. Balances and spring scales as well as butane gas cylinders and ring burners were tested.

B. *Reagents*

As a preliminary test showed that 300 ml 1% m/v pepsin 10 N HCl 3% v/v took 5 hours to digest 100 g minced gut, the concentration of pepsin was increased to 3% m/v, which digested the gut in the normal period of 2 hours. Adequate quantities of 40% formaldehyde and NaCl were acquired and 25 l formol-saline prepared.

C. *Autopsy*

A large (18 hands) aged mule with an egg count of 350 eggs per gram (e.p.g.) was selected. The animal was starved for 2 days but allowed free access to water during this time. It was shot with a captive bolt pistol and bled. The thorax and abdomen were opened, a ligature tied around the oesophagus, and the gastro-intestinal tract removed and placed in a plastic bin (80 × 40 × 25 cm). The mesentery was cut off close to the intestinal wall. Tight double ligatures were tied with string around the following organs:

1. the oesophagus at the entrance to the stomach,
2. the pylorus,
3. the ileum adjacent to the ileo-caecal valve,
4. the caecum at its junction with the colon,
5. the ascending colon, at its junction with the transverse colon adjacent to the right kidney,
6. the rectum 10 cm cranial to the anus.

The gut was severed between these ligatures and separated into:

(a) the stomach,
(b) the small intestine,
(c) the caecum,
(d) the anterior colon, i.e. the ascending colon including the right ventral, sternal flexure, left ventral, pelvic flexure, left dorsal, diaphragmatic flexure and right dorsal colon,
(e) the posterior colon i.e. the transverse colon, descending colon and rectum.

The sequence in which the organs were handled was anterior colon, caecum, posterior colon, stomach and small intestine. These were treated as follows:

1. *Anterior colon:* This had a mass of 35 kg while that of its ingesta was 27 kg. After it was opened, the ingesta were poured into a plastic bin and the gut wall placed in a separate container. Glass fibre mesh was placed on the wire gauze supports in the lower flanges of six traps. Physiological saline (0,85% NaCl solution) was poured into the traps to cover the gauze. The ingesta were thoroughly mixed and evenly spread over the gauze to a thickness of 2 to 2,5 cm. These were labelled ACR_1 and placed in the large waterbath at 40°C for 1 hour.

After an hour the ingesta remaining on the glass fibre gauze (ACR_1) were poured into buckets and the worms heat-killed as described by Reinecke (1968). They were washed on to a 100 mesh Endecott sieve and placed in a plastic bag in the refrigerator.

The filtrates were poured into buckets and allowed to sediment for 10 minutes before the supernatant was transferred to other buckets (ACSup). This supernatant was processed as described above but washed on to a 400 instead of a 100 mesh Endecott sieve and placed in the specimen jar labelled ACSup.

The sediment was placed on nylon grit gauze in three traps. Nylon grit gauze with an aperture of 500 micron was placed under wire netting on the lower platform and grit gauze with an aperture of 700 micron under netting on the upper platform. The grit gauze was fixed to the edge of the traps with foldback steel clips (paper clips) and masking tape. The traps were placed in the water bath and the base of a petri dish, 10 cm in diameter, placed upside down on the upper platform. The sediment remaining in the buckets was mixed and slowly poured on to the bottom of the petri dish so that it spread evenly over the gauze. Each trap was filled with ingesta until the latter covered the upper platform. These were labelled ACR_2.

After an hour in the waterbath, the trap was removed and the upper grit gauze (700 μ) and residue were placed in a bucket and labelled ACR_2. The ingesta on the lower gauze (500 μ) were placed in another bucket labelled ACR_3.

The ingesta lying on the nylon gauze were washed into their respective buckets and any worms remaining on the gauze removed. The filtrate in the traps (ACF) as well as the residues ACR_2 and ACR_3 were heated to 60°C to kill the worms which were then fixed in the usual way and sieved on 400 mesh sieves before being placed in specimen jars and preserved.

The wall of the anterior colon was thoroughly washed and rinsed a few times with saline (40°C) in two buckets. These specimens, (ACWW) were fixed, sieved on a 400 mesh sieve and placed in a specimen jar.

2. *Caecum*: The mass was 8,8 kg, of which the ingesta were 5,2 kg. The methods used were identical to those described for the anterior colon but only two traps were used in the first stage of extraction.

3. *Posterior colon:* The mass was 7,9 kg, consisting of 6,7 kg ingesta and a wall of 1,2 kg respectively. Because both the large and the medium-sized waterbaths were fully occupied with the anterior colon and caecum, the first stage of the extraction process on the fibre-glass gauze took place in three traps placed in the sunlight for an hour. The temperature was about 25°C (in the shade). Apart from this modification the rest of the process was identical to that described for the anterior colon.

4. *Stomach:* The mass was not determined. The relatively small quantity of ingesta was placed on nylon grit gauze (500 micron apertures) in a single trap in the waterbath for 1 hour. The residue (StR) and filtrate (StF) were processed as already described.

5. *Small intestine:* The ingesta were processed in the same way as those of the stomach.

Digestion: The gut was minced in an electric mixer.* First it was coarsely minced (plate apertures 10 mm) and then finely minced twice (plate apertures 3 mm). Approximately 1 kg of this homogenate was placed in each digest jar (4 l capacity) and 3 l freshly prepared pepsin/HCl (3% m/v pepsin and 3% v/v 10 N HCl) added to it. These jars were placed in the small waterbath (50°C) and aerated to mix the enzyme with the homogenate (Reinecke, 1972). Digestion of the stomach was completed in an hour but the rest of the intestinal tract required 2 to 2,5 hours. Thereafter the specimens were treated as described above for the filtrates.

Worm counts: The residue of the anterior colon (ACR_1) stored in the refrigerator had a mass of 7,5 kg. It was thoroughly mixed and 750 g, i.e. 1/10 removed and placed in a specimen jar to which formalin was added. Water was added to the other specimens to form a thin suspension and the volume adjusted to the nearest litre. Each specimen was well mixed by stirring and bubbling air through it, while the requisite number of aliquots were transferred to measuring cylinders using wide-mouthed pipettes as advocated by Clark, Tucker & Turton (1971). In the case of *Probstmayria vivipara,* however, a single 5%, 1% or 0,1% aliquot was used to estimate the total number in each specimen.

The digested gut wall and aliquots of *P. vivipara* were examined microscopically. Negative aliquots were checked microscopically. The aliquots of the remaining specimens were examined macroscopically on black plastic trays (45 × 27 × 5 cm). Thereafter total macroscopic counts were carried out to assess the validity of the sampling methods of Clark *et al.,* (1971). If available, 100 worms per specimen were identified and this percentage converted to estimate the total of each genus in the specimen. The descriptions of Ihle (1922), Theiler (1923) and Skrjabin, Shikabalova, Schulz, Popova, Boev & Delyamure (1952) were used to identify the Strongylata; Theiler (1923) was used for the identification of *Habronema* spp. and *P. vivipara.*

Results

The following species were identified:
Probstmayria vivipara
Delafondia vulgaris
Habronema muscae
Habronema megastoma
Habronema microstoma

The following genera were also present:
Craterostomum
Oesophogodontus
Trichonema
Cylicocyclus
Poteriostomum
Schulzitrichonema

Although there was a massive infestation of 2 621 240 *P. vivipara,* the total of all the other nematodes was only 6 575 (Table 1).

The distribution of the worms in the various specimens is expressed as a percentage in Table 2. Most of the *P. vivipara* (93,4%) as well as the other helminths (88,0%) were recovered from the anterior colon. The majority of *P. vivipara* (56,7%) and other nematodes (75,2%) migrated into the various filtrates. Although relatively few *P. vivipara* (7,4%) and other nematodes (12,9%) were present in the wall washings most of the *D. vulgaris* adhered to the wall of the caecum. With the exception of the residue (ACR_1) in the anterior colon, from which a few *P. vivipara* (1,1%) were recovered, these specimens were consistently negative. The digestion of one gut wall sample had a negligible number of *P. vivipara* (<0,1%) and was negative for other species.

The sampling method of Clark *et al.,* (1971) was satisfactory in all but two specimens. In the stomach filtrate 91 worms were present in four 5% aliquots and a further 106 worms in an additional five 5% aliquots:

*Model A 200 mixer, Hobart Mfg. Co. Ltd., London.

TABLE 1 *Worms recovered at autopsy*

Organ and specimen	*Probstmayria vivipara*	*Delafondia vulgaris*	*Craterostomum*	*Oesophagodontus*	*Trichonema*	*Cylicocyclus*	*Poteriostomum*	*Schulzitrichonema*	*Habronema muscae*	*Habronema megastoma*	*Habronema microstoma*	Total (excluding *P. vivipara*)
Stomach												
residue	20	0	0	0	0	0	0	0	26	9	19	54
filtrate	40	0	0	0	0	0	0	0	72	101	173	346
digest	140	0	0	0	0	0	0	0	0	0	0	0
Small intestine												
residue	0	0	0	0	6	0	0	0	0	0	0	6
filtrate	120	0	0	0	11	1	0	0	0	0	0	12
digest	0	0	0	0	0	0	0	0	0	0	0	0
Caecum												
wall washings	4 300	37	0	0	123	6	3	0	0	0	0	169
supernatant	660	0	0	0	0	0	0	0	0	0	0	0
residue 1	0	0	0	0	0	0	0	0	0	0	0	0
residue 2	16 000	2	0	0	8	0	0	0	0	0	0	10
residue 3	3 300	0	0	0	13	0	0	0	0	0	0	13
filtrate	31 100	0	0	0	154	10	3	4	0	0	0	171
digest	300	0	0	0	0	0	0	0	0	0	0	0
Anterior colon												
wall washings*	177 300	0	0	0	554	90	34	0	0	0	0	678
supernatant	108 100	0	1	0	11	0	0	0	0	0	0	12
residue 1	30 000	0	0	0	0	0	0	0	0	0	0	0
residue 2	618 000	0	13	0	312	53	13	0	0	0	0	391
residue 3	135 900	0	5	4	228	40	18	0	0	0	0	295
filtrate	1 379 000	0	44	0	3 749	485	132	0	0	0	0	4 410
digest	1 900	0	0	0	0	0	0	0	0	0	0	0
Posterior colon												
wall washings	11 400	0	0	0	0	0	0	0	0	0	0	0
supernatant	2 180	0	0	0	0	0	0	0	0	0	0	0
residue 1	0	0	0	0	0	0	0	0	0	0	0	0
residue 2	18 700	0	0	0	8	0	0	0	0	0	0	8
residue 3	6 240	0	0	0	0	0	0	0	0	0	0	0
filtrate	76 500	0	0	0	0	0	0	0	0	0	0	0
digest	40	0	0	0	0	0	0	0	0	0	0	0
GRAND TOTAL	2 621 240	39	63	4	5 177	685	203	4	98	110	192	6 575

*The specimen jar containing this specimen broke and some of the worms were lost

TABLE 2 *Worm distribution expressed as a percentage*

Organ and specimen	*P. vivipara*		Other nematodes	
	Percentage of the specimen	Percentage of the total	Percentage of the specimen	Percentage of the total
Stomach—				
residue	10,0	< 0,1	13,5	0,8
filtrate	20,0	< 0,1	86,5	5,3
digest	70,0	< 0,1	0,0	0,0
Total No. of worms	200	—	400	—
Small intestine—				
residue	0,0	0,0	33,3	< 0,1
filtrate	100,0	< 0,1	66,6	0,2
digest	0,0	0,0	0,0	0,0
Total No. of worms	120	—	18	—
Caecum—				
wall washings	7,7	0,2	46,6	2,6
supernatant	1,2	< 0,1	0,0	0,9
residue 1	0,0	0,0	0,0	0,0
residue 2	28,7	0,6	2,7	< 0,1
residue 3	5,9	< 0,1	3,6	0,2
filtrate	55,9	1,2	47,1	2,6
digest	0,5	< 0,1	0,0	0,0
Total No. of worms	55 660	—	363	—
Anterior colon—				
wall washings	7,2	6,8	11,7	10,3
supernatant	4,4	4,1	0,2	0,2
residue 1	1,2	1,1	0,0	0,0
residue 2	25,2	23,6	6,8	5,9
residue 3	5,6	5,2	5,1	4,5
filtrate	56,3	52,6	76,2	67,1
digest	< 0,1	< 0,1	0,0	0,0
Total No. of worms	2 450 200	—	5 786	—
Posterior colon—				
wall washings	9,9	0,4	0,0	0,0
supernatant	1,9	< 0,1	0,0	0,0
residue 1	0,0	0,0	0,0	0,0
residue 2	16,2	0,7	100,0	< 0,1
residue 3	5,4	0,2	0,0	0,0
filtrate	66,5	2,9	0,0	0,0
digest	< 0,1	< 0,1	0,0	0,0
Total No. of worms	115 060	—	8	—
GRAND TOTAL	2 621 240	—	6 575	—

this gives 438 as the estimated total but only 346 were present when all the worms were counted. Moreover, in the anterior colon wall washings the actual total was 678 but from the aliquots 852 should have been present.

DISCUSSION

This experiment showed the value of the modified Baermann apparatus for concentrating the worms of equines in a minimal amount of ingesta. The filtrates which had less than 5% of the ingesta contained 56,7% of the *P. vivipara* and 75,2% of the other nematodes. The very coarse material on the fibre-glass gauze can be discarded unless large worms such as *Parascaris equorum, Oxyuris equi* and *Alfortia edentatus,* which may remain in this residue, are present. Should their presence be important, e.g. in anthelmintic trials, all this material can be rapidly examined macroscopically because these species are large and easily seen. (Reinecke & Le Roux, 1972).

In aged animals such as this mule the gut wall need not be digested because the specimens were negative except for *P. vivipara,* of which less than 0,1% were present.

Only 8 strongyles (<0,1%) were recovered from the posterior colon. Even 115 060 *P. vivipara* represents only 4,2% of this species in this animal. Therefore, the ingesta of the descending colon and rectum need only be examined macroscopically for the presence of *O. equi*, which is large enough to be easily recognizable.

The sampling method of Clark *et al.,* (1971) is remarkably accurate, but very time-consuming. The inaccurate results in two specimens in this experiment are probably due to poor sampling methods.

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