SOME LABORATORY METHODS FOR PARASITOLOGICAL INVESTIGATIONS [1]

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INTRODUCTION

The post-mortem examination of the viscera of animals for parasites constitutes a large part of the work of a laboratory of parasitology, and the efficiency of the work depends largely on the effectiveness of the methods used in such examinations. Owing to the general prevalence of parasites in the digestive tract, the examination of this tract is of special importance.

The method of examining the digestive tract which is most widely employed in laboratories is substantially as follows: The esophagus is removed, slit with the indispensable enterotome, and examined by reflected light for evidence of parasites superficially visible and by transmitted light for parasites embedded in the tissue and not superficially visible. The stomach is opened into a jar of water or physiologic saline solution, the contents washed out, the stomach itself examined internally, externally, and if possible by transmitted light for parasites present, and the stomach contents repeatedly sedimented and washed until the supernatant water or saline solution is clear, the sedimented contents then being examined a bit at a time after being poured into wide, shallow glass dishes. The small intestine or its component portions, the cecum, and the large intestine or its component portions are examined in the same manner as the stomach.

This method has long been in use and is, as a rule, a fairly satisfactory procedure. It is best suited for examining the digestive tract of such animals as dogs. In the case of a larger animal, such as a horse, cow, or sheep, the contents of the stomach and large intestine are so bulky that this method of examining is unsatisfactory, and as a rule the examination is confined to the region of the mucosa and the contents near the mucosa, the interior of the mass of ingesta or fecal material receiving little or no attention in many cases. This was the method employed by Looss [2] in examining horses for parasites. In some cases the contents of the large intestine (cecum and colon) of the horse are examined by pressing the material into balls and picking these apart by hand. This was the method employed by Hall, Wilson, and Wigdor [3] and they used the same method for examining the manure of horses for worms passed after anthelmintic treatment.

THE USE OF SCREENS

It has occurred to the writers that the use of the graduated set of screens which they find indispensable in examining feces for parasite eggs and for worms passed after anthelmintic treatment in the case of dogs and similar small animals, might be of value in post-mortem examination as well. Various other writers, such as Stiles, Cobb, Telemann, Bass, and Garrison, had advocated the use of a screen or sieve of metal, gauze, or bolting cloth in the examination of feces for parasite eggs; but the advantage of a set of metal screens of graduated sizes, set in a rack in the order of size with the largest-mesh screen at the top and the smallest-mesh screen at the bottom, appears to have been emphasized first by Hall.[4] This set of screens was also used in examining feces for worms passed after anthelmintic treatment in critical testing of anthelmintics by Hall and Foster.[5] The usefulness of assorted screens for the separation of mixtures of objects of various sizes is generally recognized in industrial procedures. Feces and gastro-intestinal contents are mixtures of this sort.

[1] Received for publication June 30, 1924; issued June, 1925.

[2] LOOSS, A. THE SCLEROSTOMIDAE OF HORSES AND DONKEYS IN EGYPT. Rec. Egyptian Govt. School Med. 1901: 25–138, illus. 1901.

[3] HALL, M. C., WILSON, R. H., and WIGDOR, M. THE ANTHELMINTIC TREATMENT OF EQUINE INTESTINAL STRONGYLIDOSIS. Jour. Amer. Vet. Med. Assoc. (n. s. 7) 54: 47–55. 1918.

[4] HALL, M. C. A COMPARATIVE STUDY OF METHODS OF EXAMINING FECES FOR EVIDENCES OF PARASITISM. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 135, 36 p., illus. 1911.

[5] HALL, M. C., and FOSTER, W. D. EFFICACY OF SOME ANTHELMINTICS. Jour. Agr. Research 12: 397, 447, illus. 1918.

In their experiments, the writers have substituted the use of screens for the sedimentation and washing method in examining the digestive tract in all cases where such a substitution saved time. The screens used were the same ones used by Hall and by Hall and Foster. These screens were made by taking round tin pans with a bottom diameter of about 6.5 inches and a slightly greater top diameter, with sides 2 inches high, and with a projecting flange rim at the top, cutting out most of the bottom of the pan, but leaving a small flange projecting inward, and soldering brass screening of assorted sizes to the bottom flanges of the various pans. Some of the pans were enameled and some shellacked to prevent rusting. The enamel and shellac have never been renewed since the first coat, and these screens—very cheap affairs to begin with—are still serviceable after about 14 years of constant use. The expense of screens is therefore a very small matter, and even poorly equipped laboratories can easily afford them.

A much better set of screens for the purpose has been described by Hall.[6] These screens (fig. 1) are of copper and are about 7⅞ inches (20 cm.) square in inside dimensions. They are made of two copper strips, swaged together at two diagonal corners, the top half-inch of the metal being bent over and doubled back against the side on two opposite sides to form a reinforced flange ¼-inch wide which carries the screen in a rack. The copper sides are 2 inches high. On the bottom of the screens the metal is bent in to form a flange ⅜ inch wide for the attachment of the brass screening, which is soldered to this flange. The screens described had mesh apertures of 6, 8, 10, 14, 16, 20, 60, 100, and 120 to the inch, the latter being about the finest screen that will permit of the passage of the eggs of practically any worm parasite. Each sieve has a number stamped in the front side to show its number of mesh apertures to the inch. A solid-bottomed copper pan, of the same shape and dimensions as the screens, completes the set. A rack with grooves in its solid board sides or with transverse pieces on the sides and back of a skeleton construction of upright pieces is provided, and the screens are supported by the grooves or transverse pieces when they are set up in the rack. The number of screens used in any operation will vary with the nature of the material examined and the judgment of the operator.

The material which the writers have examined post mortem by means of screens consisted mostly of the contents of digestive tracts of dogs killed at the end of experiments in critical testing of anthelmintics, and of digestive tracts of wolves, coyotes, lynxes, and one bear sent in by the field service of the Biological Survey of the Department of Agriculture in response to a request by the Zoological Division for this material. The viscera of the dogs were fresh. The viscera of the wild carnivores were sent from the field wrapped in borax or in cloth saturated in formaldehyde solution, some of them coming from as far as California, and were often a week or ten days old when received.

THE SCREENING METHOD

The method of screening the contents of practically all portions of the digestive tract, including the stomach, small intestine, cecum, and large intestine, was first tested, each of these portions being opened separately into a jar of water and each screened separately. For this purpose the three screens having 6, 12, and 24 mesh apertures to the inch were generally used. The finest of these screens shows some little variation in its mesh apertures and may have in places 21 or 22 mesh apertures to the inch. It is not so fine as could be desired for the final screen, and the 60-mesh screen was sometimes used as a final screen. One with a mesh aperture of perhaps 40 to the inch should probably be added to the set. The number of screens and the mesh sizes to use are matters of individual judgment. The screens should be used in a rack for various reasons, one reason being that this results in cutting down the water pressure on the last screen and diminishing the likelihood of washing worms through that screen by high-water pressure.

The advantages of this method over the sedimentation and washing method are very evident. As soon as the contents of a jar are poured on the screen, a hose which is attached to a water tap having a foot-pedal control for the flow is used to wash the material on the top screen until it is clean and comminuted as much as possible. The pressure of the water and the amount of washing are matters of individual judgment. The water runs

[6] HALL, M. C. APPARATUS FOR USE IN EXAMINING FECES FOR EVIDENCES OF PARASITISM. Jour. Lab. and Clin. Med. 2: 347-353, illus. 1917.

through to the lower screens and washes the contents which pass through from the top screen. Most of the unpleasant odor of the ingesta and fecal material (and the odor of viscera packed in borax for 10 days is decidedly unpleasant) is associated with the fine material which passes through the last screen, and this is promptly washed down the sink in which the rack is set. The less unpleasant material, with whatever parasites may be present, remains on the screens and is transferred to shallow glass dishes either by washing or by immersion in water in the dishes and tipping to remove all or part of the contents at a time. In either case the screens are placed in water in the dishes and examined for parasites that may be held on them or in their meshes.

In most cases this method of examination shows the following advantages over the sedimentation and washing method: There is a great saving of time, as washing through the screens is a much quicker process than sedimenting and washing repeatedly to remove the supernatant discolored fluid and flocculent material. The parasites are washed out of the ingesta, making their recognition and collection easier and more certain. The malodorous material is removed much more promptly and the entire process of examination is much pleasanter.

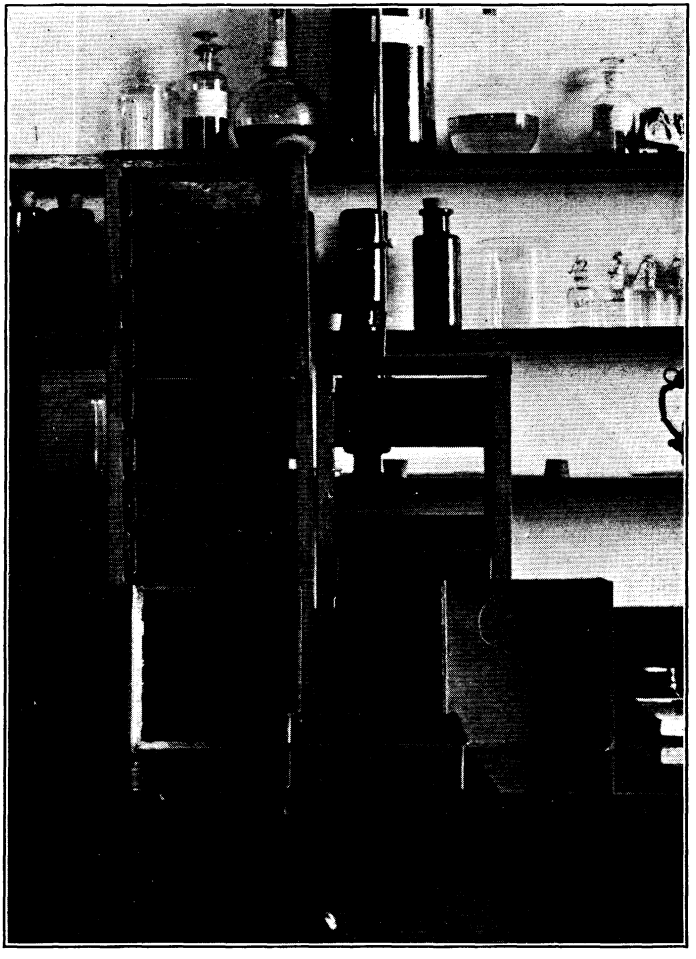

FIG. 1.—A set of copper screens, some on skeleton rack with transverse pieces at left, some in solid board rack with grooves at right, one on edge at bottom of left-hand rack, and one, seen partly from above, on the table in the center; solid copper pan on edge at right. (From Hall, 1917.)

The disadvantages of this method of screening are mostly theoretical and generally follow errors of judgment. In the first place, it was found that in practice time could not always be saved by substituting the screening method for the sedimentation method. These cases were usually those in which washing itself was unnecessary. Such cases include the examination of the practically empty stomach of a fasted dog or the almost empty cecum of individual carnivores of any sort. In these cases the organs in question could be slit open into a jar, the material allowed to settle for a few minutes, the supernatant fluid decanted, and the small sediment examined immediately, or after one washing, in a glass dish. This is a quicker procedure than screening and examining the screens, and there is little in the way of ingesta or fecal material to conceal parasites or afford unpleasant odors.

Another objection is on the score of possible injury to parasites by screening and washing. For the practical purposes of routine examination for and collection of parasites, this objection is mostly theoretical, although in special cases it would be sound. In general, worm parasites are fairly resistant structures as met with in the field of human and veterinary medicine. Such worms as the ascarids are fairly tough structures, and *Ascaris lumbricoides* has been kept alive in Kronecker's solution for 26 days, which shows that it is not readily damaged by being removed from its host and placed in an alien environment. It is true that there are some very fragile worms that are very susceptible to mechanical injury and the destructive action of osmosis in such fluids as tap water, but worm parasites of the digestive tract are rarely of this sort. It is also true that the easily lost hooks of some tapeworms may be lost by screening.

Where worms are being collected for very careful studies of their morphology a more refined technic is desirable; but for practical routine purposes the writers find the screening method superior to the sedimentation and washing process, provided one uses good judgment, and believe that the contents of the small and large intestines may be screened to advantage in almost all cases and that screening of the contents of the stomach and cecum should be carried out when of sufficient quantity to warrant it or omitted if of such small quantity that little or no washing is necessary. The screening method would be of value for the examination of swine viscera and to some extent for the examination of the viscera of such animals as sheep, cattle, and horses.

In connection with the subject of laboratory technic it is also noted that it is advisable to slit the larger air passages of the lungs and then wash and squeeze the lung in a dish of water or saline solution in order to detect worms which may be overlooked in slitting and examining without this washing. The urinary bladder, gall bladder, and similar structures also should be slit in a dish of water or saline solution and examined in the same way and for the same reason.

CONCLUSIONS

A set of metal screens of assorted mesh apertures with a suitable rack to hold them is a very valuable and almost indispensable part of the equipment of a parasitological laboratory. The screens are of service in examining feces for parasite eggs and for worms passed after anthelmintic treatment and in examining the contents of the digestive tract post-mortem. If used with judgment in post-mortem examinations they save time, make the detection and collection of parasites easier and more certain, and make the work less unpleasant by removing the malodorous portions of ingesta and fecal material more rapidly than the sedimentation and washing method does. There appears to be little damage done to parasites, as a rule, by the use of screens and washing. The lungs, urinary bladder, gall bladder, etc., should be slit open in a dish of water or saline solution and examined in this way for parasites present.