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## BALANTIDIUM COLI INFESTATION IN SWINE: HISTOPATHOLOGIC CHANGES AND PATHOGENICITY

B. C. HATZIOLOS

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## BALANTIDIUM COLI INFESTATION IN SWINE: HISTOPATHOLOGIC CHANGES AND PATHOGENICITY

By

BASIL C. HATZIOLOS

Protozoan diseases in animals and man are becoming more important as knowledge is accumulated regarding their nature and prevalence. Balantidiosis of pigs, unlike its severity in man, has been considered to be a harmless entity<sup>2, 20, 34</sup>, and *Balantidium coli*, to be a commensal of normal pig intestine<sup>7, 16, 25, 35, 42</sup>.

However, because *B. coli* occasionally causes intestinal disturbances in pigs, similar to those it produces in man, this organism is regarded by some to be an obligate parasite invading an intestinal mucosa previously injured by some other agent,<sup>4, 15, 23</sup> particularly streptococci, *E. coli*<sup>32</sup>, *Salmonella*<sup>37, 43</sup>, or hog cholera.<sup>8, 34</sup>

Opinions expressing that species variations (var. *suis* and var. *hominis*) account for the pathologic differences in pig and man<sup>1, 31, 41</sup> could not gain ground because of the difficulty in differentiating these varieties<sup>28, 38, 47</sup> and the claim that these varieties are nothing more than the vegetative and cystic forms of the same species, *B. coli*<sup>27</sup>.

A primary etiologic relationship of *B. coli* and intestinal lesions has been demonstrated in experimental animals, including pigs<sup>3, 8, 18, 47</sup>.

The purpose of this paper is to describe the histopathologic changes found in the piglets of a herd free of hog cholera, pathogenic bacteria, and parasites but, nevertheless, heavily infested with *B. coli*. This case, therefore, provides the essential control conditions for an undisputed evaluation of the balantidial pathogenicity in this species.

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CASE REPORT

**Clinical Features:** The herd (HPMF), composed of 150 mixed Hampshire and Yorkshire pigs, 6 - 8 weeks old, had been wormed, vaccinated against erysipelas, and kept indoors. Drinking water came from a drilled well. Housing conditions were satisfactory. Shortly after weaning and despite a well - balanced diet, many of the piglets began to languish, showing slow growth, loss of weight, emaciation, and cachexia. Persistent watery diarrhea and anemia were predominant signs. Body temperature varied between 38.4 and 39.1<sup>0</sup>C. Penicillin, Tylan<sup>(a)</sup>, and vitamins (A, D, and E) were ineffective. Fecal examination revealed heavy *Balantidium coli* infestation with many trophozoites.

**Pathologic Findings:** The euthanatized piglets were dehydrated,

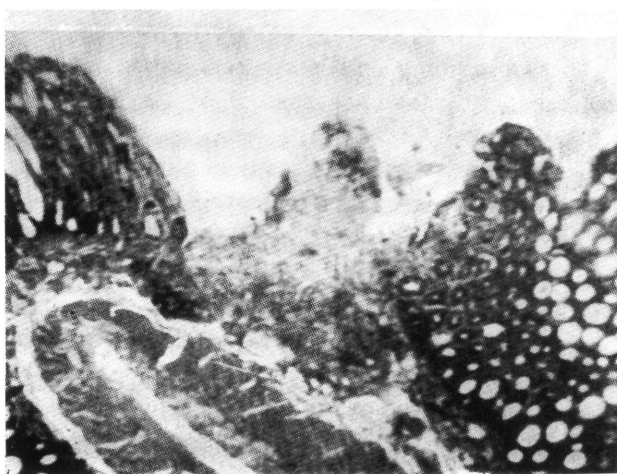


Fig.1. Ileum. Destruction of mucosa and ulcer formation. Note atrophy and dilatation of Lieberkühn glands, W - shaped coagulum, and inflammatory reaction in submucosa.  
H & E  $\times$  63

thin, and emaciated. Their muscles were atrophied and pale. The heart was flabby and had discolored spots. The liver was red with some light discolored areas, but no parasitic scars. The spleen was pale red and slightly enlarged, with variations depending on the degree of anemia. The kidney was light red and had small yellowish spots. The lung appeared to be normal. The stomach mucosa was red and edematous. The intestine was congested; in many areas the mucosa was edematous

(a) Tylan, (tylosin), Elanco Products Co., A Division of Eli Lilly & Co., Indianapolis, Indiana.

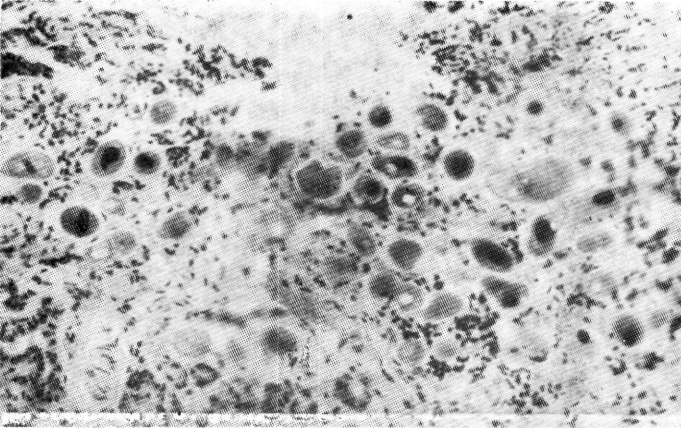


Fig.2. Higher magnification of above section. Note numerous balantidia in axillary area of coagulum preparing to penetrate into lamina propria. H & E  $\times$  120

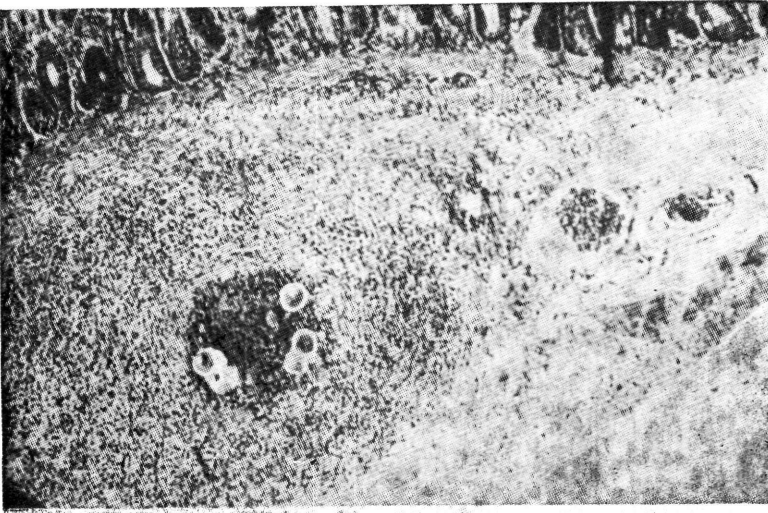


Fig. 3. Ileum. Large nodule in submucosa with balantidia surrounded by cellular debris in center of nodule. Note marked perinodular, round cell infiltration, wide outer zone formed by various inflammatory cellular elements and macrophages; also many newly formed small vessels and zone of fibroblasts separating normal tissues. H & E  $\times$  120

on the tips of the folds. There were fluffy, whitish, scab - like accumulations over the congested areas. Characteristic was the presence of numerous yellowish bodies, the size of a millet seed, dispersed along the intestinal tract in the thickened walls. They were particularly numerous



Fig. 4. Mucosal ulcer in communication with submucosal nodule. H & E  $\times 63$

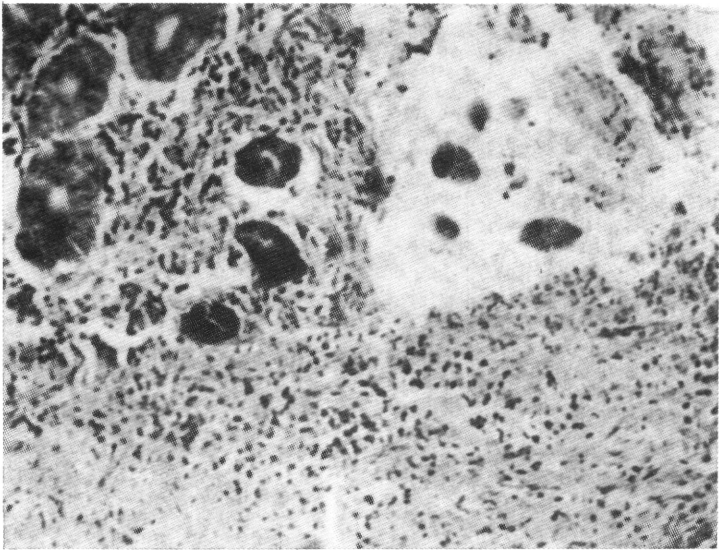


Fig. 5. Higher magnification of Fig. 4. Note balantidia close to lamina propria and bacterial colonies in eosinophilic coagulum filling missing mucosal strip. Also, balantidia (hardly distinct) in nodule along limits with reactive zone. H & E  $\times 120$

in the large intestine. The brain had congested meninges and a pale parenchyma.

Selective blocks of tissue from all organs, including brain, were fixed in 10% buffered formalin solution, processed, sectioned and stained, as required.

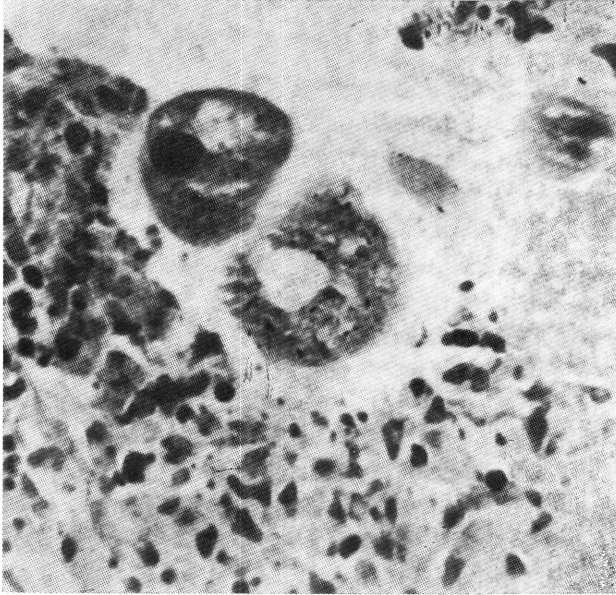


Fig. 6. Ileum. Balantidia, some of which have injected bacteria, close to edges facing ulcerated area at penetration point. Note numerous Gram - positive bacteria, identified in distal part of mucosa (toward lumen). McCallum - Goodpasture  $\times 400$

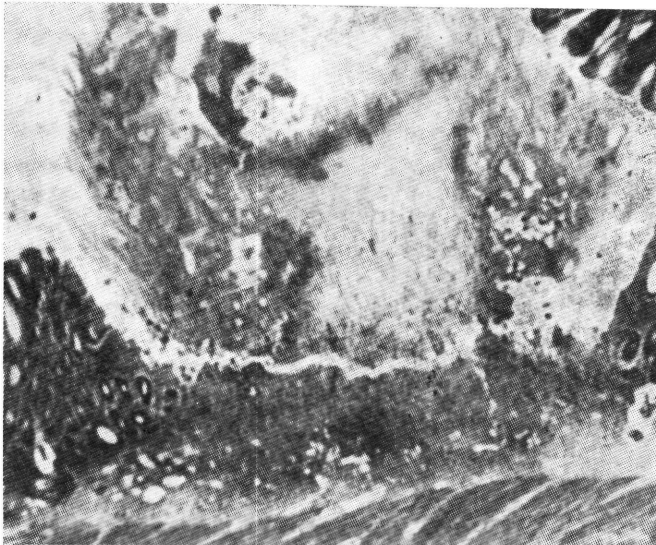


Fig. 7. Large intestine. Mucosal ulcer similar to that of ileum (Fig. 1). Note numerous balantidia at edges in contact with eroded mucosa; also marked inflammatory reaction in muscularis mucosa, vascular dilatation and thickened submucosa. H & E  $\times 63$



Fig. 8. Colon. Nodular formation in muscularis mucosa and submucosa with ulceration of mucosa above. Balantidia clusters, hardly seen, in center of muscularis mucosal nodule and bacterial colonies at distal part of coagulum; far below an unusually dilated tubule of Lieberkühn gland with mucopurulent material. H & E  $\times$  120

Microscopically the gastric mucosa was edematous and the submucosa congested. In the nonaffected segments of the small intestine, the mucosa was congested and moderately infiltrated by granulocytes, primarily eosinophils, and by macrophages having engulfed erythrocytes. The villi were intact; the submucosa was congested and edematous in places. Many balantidia were free in the lumen.

In the areas affected early, erythrocytic extravasation, goblet-cell increase, erythrophagocytosis, and inflammatory reaction were marked. Nuclear pyknosis and necrosis appeared in spots close to the balantidia nests. Sloughing of cells and progressive destruction of the mucosa resulted in ulceration. Balantidia were present only in areas of erosion and ulceration of the mucosa. Bacterial flora was noted in the lumen.

Noted elsewhere was deeper ulcer formation in the mucosa with eosinophilic coagulum filling the gap created by necrosis (Fig. 1). In addition, there were cellular necrosis and fragmentation, atrophy and

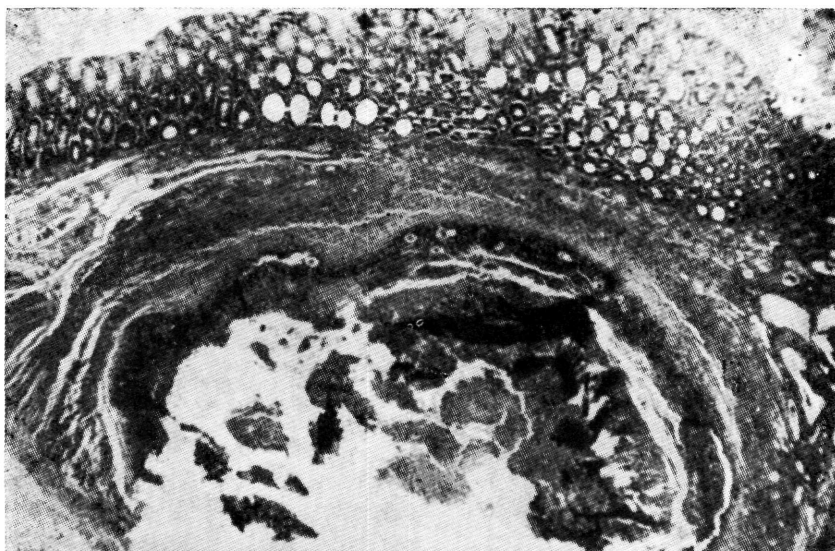


Fig. 9. Colon. Large nodule in submucosa and t. muscularis. Note caseated material with balantidia in center but mostly aligned at periphery in dark zone, probably representing a band of lymphoid cells, encircled by fibrotic layer. H & E  $\times$  120

dilatation of the Lieberkühn glands, and marked inflammatory reaction of the submucosa. At the edges of the ulcers, numerous balantidia tended to penetrate the mucosa (Fig. 2). In a cross section of the ileum, at least 4 such ulcerated areas were noted.

Elsewhere, balantidia formed nodules in the submucosa (Fig. 3). They were surrounded by inflammatory elements, separating the muscularis mucosa from the tunica muscularis. In the center of the nodule, the balantidia were in nests surrounded by caseated material. Marked round cell infiltration without neutrophils encircled the nodule. Characteristic also, was the conspicuous dilatation of the vessels in the adjacent areas..

A complex of ulcer - nodule formation was often noted (Fig. 4). Again, the balantidia were close to the lamina propria, whereas numerous bacterial colonies grew either within or behind the eosinophilic coagulum, replacing missing mucosal strips (Fig. 5). Special staining revealed numerous Gram - positive bacteria in the distal part of the mucosa, toward the lumen, and balantidia scattered close to the edges of the ulcerated areas (Fig. 6).

Also in instances with a submucosal nodule, no bacteria were present at the points of contact of the balantidia with the necrosed cellular





Fig. 10. Colon. Nodule in tunica muscularis splitting layers. H & E  $\times$  63



Fig. 11. Higher magnification of above (Fig. 10). Note protective zone, including fibroblasts, extending even to longitudinal layer of t. muscularis and balantidia in center of nodule. Also, pycnosis of muscular fibers and increased cellularity in serosa. H & E  $\times$  160

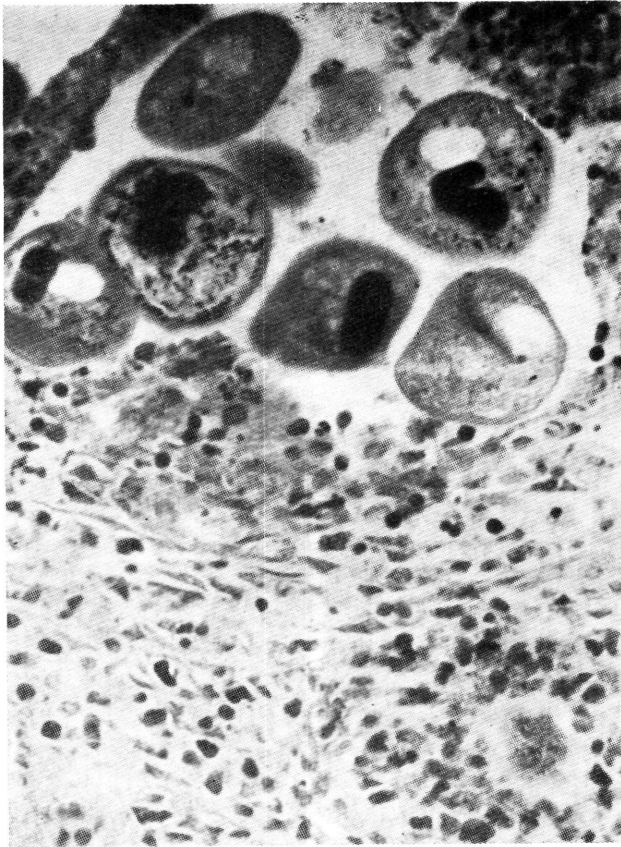


Fig. 12. Colon. Balantidia, some having injected bacteria, penetrating m. mucosa. Note bacteria absent from eroded mucosa, and present only in distal part. Note also cellular reaction around balantidium, penetrating deeper into submucosa, absence of granulocytes. McCallum - Goodpasture X 400

elements. The bacteria were always the rearguard of the aggressive ciliates. The fibrous capsule surrounding the nodule was wide and the protective inflammatory reaction, narrow.

In the colon, the lesions were similar, although more extensive and more often encountered. The mucosal ulcers were wide and in their periphery had numerous balantidia in contact with the eroded mucosa (Fig. 7). Here again, with special staining, bacteria were, as in the small intestine, always absent from the area between the balantidia and the destroyed mucosa. Often, balantidia were noted containing bacteria in their digestive apparatus, and surrounded by inflammatory reaction with round cells but without granulocytes (Fig. 12).

A combination of mucosal ulceration and nodular formation in

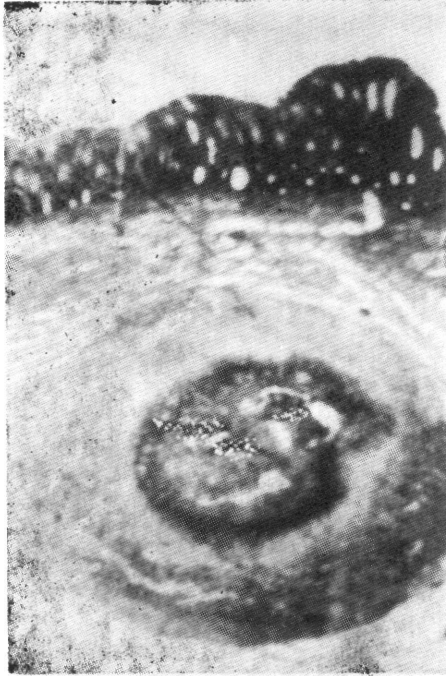


Fig. 1c. Colon. Nodule deep in t. muscularis, affecting circular layer, and intact—except for glandular dilatation—intestinal mucosa, lying above. H & E X 63

the muscularis mucosa and submucosa was often noted. Here, the balantidia were numerous—over 2 dozen noted in a high—dry magnification microscopic field arranged in nests along the muscularis mucosa and in the center of the nodule (Fig. 8).

Large nodules containing balantidia were also in the submucosa (Fig. 9), or in the tunica muscularis, splitting its layers (Fig. 10). The protective inflammatory zone was conspicuous and contained fibrous cellular elements. At certain points, it extended even to the longitudinal layer of the tunica muscularis (Fig. 11).

In other places, the nodular formation affected deeper layers, although the superimposed mucosa remained intact (Fig. 13). Balantidia were noted, as in the small intestine, always facing ulcerated intestinal walls and cellular elements with nuclear pycnosis and absence of bacteria. Usually, the latter formed colonies at the distant part of the damaged walls, toward the center of the nodule (Fig. 12, 14).

The liver showed early lobulation, dilatation of the centrilobular

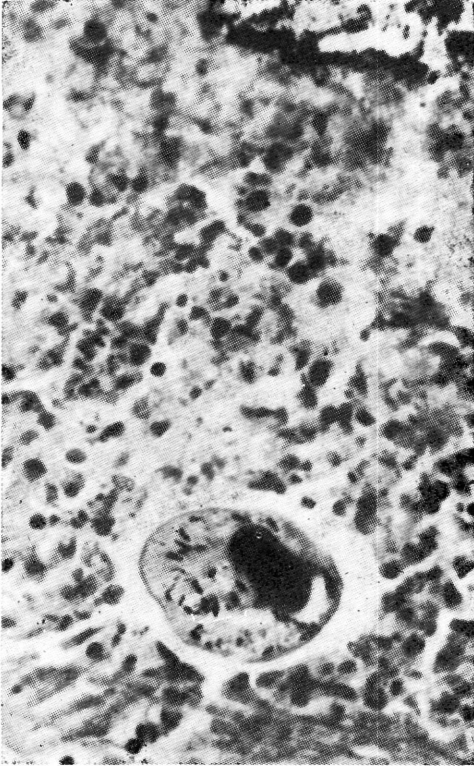


Fig. 14. Colon. Balantidium, containing Gram + bacteria, burrowed in intestinal walls. Note aggregate of bacteria lined only in distant part of damaged walls. McCallum Goodpasture X 400.

sinusoids, and moderate fatty changes. In the kidney, glomerulitis, casts in the convoluted tubules, and calciferous deposits in the collective tubules were noticeable. Noted were ballooned Purkinje muscular fibers and interstitial edema in the heart and partial lymphocytic depletion of malpighian follicles and increased hematopoiesis in the spleen. The brain was free of specific lesions. F. A. T. was negative for hog - cholera, as were cultures for pathogens from the organs.

#### DISCUSSION

The lesions observed in the small and large intestines were extensive and severe characterized by catarrhal or hemorrhagic necrotizing inflammatory reaction. They were followed by ulceration in the mucosa and nodular formation in the submucosa and tunica muscularis. These changes were associated with the protozoa arranged close to the edges of the ulcerated areas, facing the necrosed mucosa at the penetration point. The bacteria were at the distal part of the mucosa, toward the lu-

men. In the nodules, the balantidia were surrounded by necro - caseated material formed by cellular debris and round cell response at the periphery, and few, if any, granulocytic leukocytes. In the micro - abscesses formed in the intestinal walls, bacteria were absent, as has been previously observed<sup>47</sup>. This arrangement and cellular response strongly indicated that bacterial activity and infection were not prerequisites to the production of the lesions. Moreover, it demonstrated the primary etiologic relationship of *B. coli* to the intestinal lesions, thus supporting the observation that this protozoon can produce the same type of lesions in pigs as it does in man.<sup>1, 19, 47</sup>

The presence of *B. coli* in the walls of both intestinal segments is the result of this organism's capability to penetrate the tissues<sup>18, 22, 25, 36, 40, 45, 47</sup>. The organism may achieve this penetration in a combination of ways, such as mechanical irritation or rupture of the host's mucosa<sup>1, 25, 47</sup> elaboration of cytolytic<sup>18, 22, 37</sup> or necrotizing substances<sup>40, 47</sup>, hyaluronidase production<sup>26, 46</sup> and even changing its own shape<sup>18, 36</sup>.

These means enable balantidia to burrow deep and migrate locally in the intestinal walls<sup>1</sup> and to lodge, preferably, in the lymphoid follicles<sup>37, 47</sup>, the muscular layers, and the subserosa<sup>3, 13</sup> and to pierce the blood vessels and lymphatics<sup>1, 3, 5, 6, 9, 25, 36, 37, 45, 47</sup> in man and various animals. Division and multiplication of balantidia in the tissues have been suspected<sup>3, 18</sup>.

The penetration of the organism in the vessels may lead the protozoon, by hematic transportation, to distant areas, which may explain the unusual presence of *B. coli* in the mesenteric lymph nodes<sup>1</sup> and the uterus<sup>14, 21</sup> in pigs, and in the mesocolic lymph nodes<sup>6</sup> and the urinary apparatus<sup>30</sup> in man. In the latter, also erratic forms have been reported in the liver<sup>44</sup> (a) and lung (b). Balantidia also have been found in the peritoneal cavity, apparently through perforation of the cecum<sup>6, 48</sup> in man, and in the submucosa of the pharynx and respiratory tract, through an undetermined channel, in a heifer<sup>19</sup>.

These potential pathogenic capabilities, however, are activated only under certain conditions, but the determining factors are still little understood. In experimental animals, aggressiveness of the balantidia has been related to special nutritional conditions created in the intestinal tract of certain hosts, under which the protozoon develops a predilection for proteins instead of carbohydrates<sup>3, 11, 12, 16, 25, 33, 37</sup>.

This relationship of the host's diet with infectivity is now also

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(a) Manson, (b) Winogradow, mentioned by Neveu - Lemaire.

recognized in pigs. Although starch is universally believed to be an important item of food for *B. coli*<sup>17, 31, 36, 39</sup>, blood cells are equally desirable as food to the parasite<sup>3, 10-12, 24, 33</sup>. A great avidity of *B. coli* for erythrocytes, even to the point of cannibalism under starved conditions experimentally produced, has been observed<sup>31, 33</sup>.

A comprehensive study, therefore, concerning the factors presumably related to drastic changes in the feeding habits of the *B. coli* in the intestine, which might result in changes from its parasitic to its aggressive state, may shed greater light on the pathogenicity of this organism and may explain the paradox of its usually being harmless to one species and pathogenic to others. The necessity for reopening the question of pathogenicity of *B. coli* in the pig is quite obvious.

#### SUMMARY

A herd of 150 mixed breed pigs, 6-8 weeks old, kept under control conditions for hog-cholera, pathogenic bacteria, and parasites, and given a well-balanced diet, manifested diarrhea and sluggish growth shortly after being weaned. Weight loss, emaciation, anemia, and cachexia followed. Antibiotics and vitamins were ineffective. Euthanatized piglets had enteritis and numerous yellowish nodules the size of a millet seed in the intestinal walls throughout the intestinal tract.

Microscopic examination showed a diffuse inflammatory reaction, vilus necrosis, and ulceration of the mucosa with eosinophilic coagulum and numerous protozoa at the edges of the damaged mucosa of the small and large intestine. In addition, nodules containing protozoa and necro-caseated material were lodged in the submucosa and even in the tunica muscularis of both intestinal segments. Granulation tissue and a thin capsule encircled the nodules. Bacterial activity was absent or remote. Cultures and F. A. T., and brain sections for hog cholera, were negative

### ΠΑΡΑΣΙΤΩΣΙΣ ΧΟΙΡΩΝ ΕΚ *BALANTIDIUM COLI* ΙΣΤΟΠΑΘΟΛΟΓΙΚΑΙ ΑΛΛΟΙΩΣΕΙΣ ΚΑΙ ΠΑΘΟΓΕΝΕΙΑ

Ἰπὸ

Β. ΧΑΤΖΗΘΑΟΥ

ΠΕΡΙΛΗΨΙΣ

150 χοιρίδια ἀποτελοῦντα ἀγέλην διασταυρωμένης φυλῆς, ἡλικίας 6—8 ἑβδομάδων, ἐκτρεφόμενα ὑπὸ ἐλεγχόμενας συνθήκας ὡς πρὸς τὴν πανώλην, παθογόνα μικρόβια καὶ παράσιτα καὶ καλῶς διατρεφόμενα, παρουσίασαν διάρροϊαν καὶ βραδείαν ἀνάπτυξιν ὀλίγον μετὰ τὸν ἀπογαλακτισμόν. Ἐν συνεχείᾳ παρετηρήθη ἀπώλεια βάρους, ἀπίσχνασις, ἀναιμία καὶ καχεξία. Ἐφαρμοσθεῖσα θεραπευτικὴ ἀγωγή ἐξ ἀντιβιοτικῶν καὶ βιταμινῶν ἀπέβη ἄνευ ἀποτελέσματος.

Τὰ εὐθανατωθέντα χοιρίδια παρουσίαζον ἐντερίτιδα καὶ πολλὰ ὄζιδια κιντρίνης χροιάς μεγέθους κόκκων κέχρου εἰς τὰ ἐντερικά τοιχώματα καθ' ὅλον τὸ μήκος τοῦ ἐντερικοῦ σωλήνος.

Κατὰ τὴν μικροσκοπικὴν ἐξέτασιν παρατηρήθη διαχύτως φλεγμονώδης ἀντίδρασις, νέκρωσις τῶν λαχνῶν καὶ ἐξέλκωσις τοῦ βλεννογόνου καλυπτομένου ὑπὸ ἠωσινοφιλοῦς πηγματος ὡς καὶ πολυαριθμῶν πρωτοζῶων εἰς τὰ ὄρια τοῦ τρωθέντος βλεννογόνου τοῦ λεπτοῦ καὶ παχέος ἐντέρου. Ἐπιπροσθέτως, ὄζιδια περιέχοντα πρωτόζωα καὶ νεκρώδη τυρσοειδοποιημένην οὐσίαν ἐλάχουον εἰς τὸν ὑποβλεννογόνον ἔτι δὲ εἰς τὸ μυϊκὸν στρώμα ἀμφοτέρων τῶν ἐντερικῶν τμημάτων. Τὰ ὄζιδια περιεκλείοντο ἐντὸς κοκκώδους ἴσπευ καὶ λεπτοῦ (εὐλακος) ἢ μεμβράνης.

Ἡ βακτηριδιακὴ δραστηριότης ἦτο ἀποῦσα ἢ ἐλαχίστη. Καλλιέργειαι, καὶ ἀνοσοφορισμὸς καὶ ἱστοπαθολογικαὶ ἐξετάσεις ἐγκεφάλου διὰ πανῶλην ἀπέβησαν ἀρνητικαί.

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