# THE PATHOGENICITY OF GIARDIA (LAMBLIA) INTESTINALIS TO MEN AND TO EXPERIMENTAL ANIMALS. ВΫ

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# INTRODUCTION.

WHILE British troops were in Gallipoli, a number of men contracted various forms of dysentery or diarrhoea, and severe cases occurred. Among the men invalided to England a number were found to be infected with *Giardia* (Lamblia) intestinalis, and some harboured no other dysentery-producing organism. We personally found 187 cases of pure lambliasis among 1,305 patients. Similar cases of pure lambliasis have occurred on the western front, while from the various war zones patients with mixed infections have been common. The pathogenicity of Giardia (Lamblia) intestinalis has been disputed in certain quarters. We have always believed that the organism was pathogenic, and published the same in 1915 and 1916. We here give our personal evidence, based on our observations of pure cases of lambliasis in man and of some experiments with human Lamblia on animals. We may say at once that in both human and animal lambliasis stools, as well as at *post-mortem* examinations, erosion and distortion of the intestinal epithelial cells occurred, owing to the direct suctorial action of the flagellate Lamblia.

# HUMAN CASES.

Recently an account was published by one of us of a numerical study of a series of seven cases of soldiers in whom diarrhoea or dysentery had been reported, and in whom no protozoön other than Lamblia nor dysentery bacillus was found, after repeated examinations, to account for the symptoms produced. These cases were of men who contracted violent diarrhoea or dysentery in Flanders or in Gallipoli, so that two strains have been under constant observation. Experiments have been conducted with these two strains, which we term the "Flanders" and the "Gallipoli" strains. Some variations in virulence between the two strains occurred, the Flanders strain being the more virulent both in man and in subinoculated animals, but the Flanders case was a somewhat more recent infection.

The full history of the men will be found in the paper already mentioned, and need not be given in detail here. As a general statement, all the patients were weak, especially after a little exertion, and one case from Flanders was weak when admitted to hospital, and has been weak ever since. At times most of the men were somewhat constipated. Occasionally the temperature was slightly subnormal, but the charts showed little that was noticeable. There was a distinct increase in the number of mononuclear leucocytes and lymphocytes in the blood.

The stools were sometimes of peculiar colours and con-sistencies; many were sulphur yellow in colour, but in some cases the colour was hardly different from that of normal stools. In the case of one patient who contracted lambliasis in Flanders, the stools were almost always bulky and diarrhoeic in character, and were compared at different times with khaki, mud, putty and whipped cream. At times, blood was voided with the stools.

It has been assumed by some workers that human lambliasis is confined to the tropics. This is not so. *Giardia (Lamblia) intestinalis* is well known in Italy, and lambliasis seems to be endemic there, children being more severely affected than adults. It also occurs in Russia and Germany. Again, lambliasis has been contracted in Flanders by a man who had never left England until he went to Flanders two months before his illness. The assumption as to the tropical localization of lambliasis, therefore, is not really accurate.

A second hypothesis set forward is that in cool countries the virulence of lamblial infections is reduced. This, again, does not seem to be borne out by some of our cases. The most virulent infection with Lamblia in man and in subinoculated animals that we have encountered came С

from Flanders, and the patient, prior to proceeding there, had never left this country. The virulence of the lamblial infection was not reduced in his case. Lamblial diarrhoea in children has been described by Mello Leitao in Rio de Janeiro. Virulencè does not seem to be dependent on geographical distribution, and in our present state of knowledge it is unwise to make generalizations.

# ANIMAL EXPERIMENTS.

Our experiments were made with kittens and with mice. Lamblia is only rarely encountered in carnivores, but is more common in rodents. Before our animals were actually used for experiment we examined their faeces daily for periods up to a month and at times aperients were administered, but no Lamblia was seen. These precautions administered, but no Lamona was seen. These precautions were very necessary in the case of mice, as lambliasis occurs naturally in them, being described as producing "chronic enteritis." We believe that all our experimental animals were quite free from natural lamblial infections before experimentation. The experimental animals were Lamblia cysts or with emulsions of facees likewise parasitized. By repeated decantation and centrifuging of emulsions of facees, Lamblia cysts free from débris were obtained. Control animals were given food contaminated with similar quantities of normal human faeces or with faecal emulsions free from débris.

Daily examinations of the faeces of each of the experimental animals and of the controls were made, as well as full examinations at death. Preparations of the parasites in the fresh state or fixed and stained smears, and sections of the gut were used, the results obtained in each series corroborating those obtained by other methods used. Details of the experiments may now be set forth.

corroborating those obtained by other methods used. Details of the experiments may now be set forth. Experiment I. (H. B. F.) A male kitten, weight 725 grams, was fed with milk containing Lamblia cysts (Gallipoli strain) that had been separated from débris by washing and decantation, followed by centrifuging. On the second day a very few Lamblia cysts were found in the faeces, and as these might merely have passed through the alimentary tract unchanged, a second feed of contaminated milk was given. On the third day the faeces of the kitten were markedly diarrhoeic, of a yellowish-green colour, and contained a few young Lamblia cysts. A similar condition was found on the fourth and fifth days. No parasites were found on the sixth and seventh days, but on the eighth day a few young Lamblia cysts occurred in the faeces, which were very ful. On the twelfth day after the first feed the faeces of the animal were very foul-smelling, and the anus showed signs of inflammation. The kitten did not feed well this day. On the fifteenth day two empty Lamblia cysts were found, but no more parasites were seen until the twenty-ninth day, when a few motile flagellates and a very few cysts were present. No more Lamblia were observed until the thirty-second day, when a few cysts were passed. The same occurred on the thirty-third day. From then till the forty-fourth day the faeces were loose and diarhoeic, and Lamblia cysts and flagellates appeared in them. The kitten forty-seventh, and forty-eighth days some Lamblia cysts were present in the faeces. The kitten seemed ill, vomited, sent up piteous cries, and the animal shivered. On the forty-fifth, forty-seventh, and the animal shivered to the forty fourth day some Lamblia cysts were seen in the faeces. The kitten seemed ill, vomited, sent up piteous cries, and the animal shivered. On the forty-fifth, forty-seventh, and forty-eighth days some Lamblia cysts were seen in the faeces. The kitten seemed ill, vomited, sent up piteous cries, and the animal shiveref. On the forty-fifth,

*Experiment II.* (H. B. F.) A female kitten, weight 712 grams, was fed with milk con-taining Lamblia cysts (Gallipoli strain), freed from débris. On the third and fourth days after the infective feed the faeces were diarrhoeic, and a few Lamblia cysts occurred therein. The faeces were also foul-smelling. The kitten did not feed well from the twelfth to the seventeenth day, when it ate greedily, but became violently diarrhoeic. Lamblia cysts were not found until the twenty-seventh day, when a recurrence took place, a few cysts being voided. On the twenty-ninth day active flagellates were passed in the faeces, and for the next five days a few cysts were found. They were then absent till the fortieth day, when they reappeared, but no more were seen

till the forty-fourth day. The kitten now became much more diarrhoeic, howled a great deal, vomited much, and was restless. The diarrhoea continued, cysts and flagellates being passed in the excrement on the fiftieth, fifty-first, and fifty-second days. The animal then became drowsy, and died on the fifty-third day after the infective feed. At death it weighed 793 grams; the control weighed 965 grams, its weight at the commencement of the experiment being 710 grams. At autopsy, the spleen was large and the blood vessels of the stomach and intestines engorged. The mucous membrane of the duodenum was stripped in parts, whole villi being found in the contents. The caecum was slightly enlarged, and there was much cell débris in the colon. Some flagellates and a few cysts were found in the duodenum and the jejunum. Cysts were also present in the colon. The contents of the rectum contained no parasites. The relative fewness of the Lamblia probably resulted from the constant voiding of them in the diarrhoeic facees.

Experiment III. (H. B. F.) A male kitten, weight 307 grams, was fed on milk containing Lamblia cysts of the Gallipoli strain. On the second and third day the facees were negative; on the fourth, fith, and seventh days Lamblia cysts and a few flagellates were seen. The kitten seemed to weaken a good deal on the sixth day and died on the eighth day. It then weighed 290 grams, the control kitten belonging to the same litter weighing 330 grams. At autopsy, general inflammatory conditions of the intestine were observed with much injection of the blood vessels. Flagel-late Lamblia were present in the proximal loop of the duo-denum, and were more sparsely distributed down the rest of the small intestine. There were some cysts both in the duo-denum and the caecum, while a few were present in the faecal pellets. The control kitten was found to be free from parasites when killed. pellets. The when killed.

Experiment IV. (H. B. F.) A female kitten, weight 325 grams, was fed with cysts of Lamblia (Gallipoli strain) in milk. A control of the same litter and of equal weight was given milk containing normal human faeces. The excrement of the experimental kitten contained Lamblia cysts on the fourth, fifth, and sixth days after feeding. On the third day the kitten became diarrhoeic, and continued so until it diedon the given day. So until it died on the sixth day, its weight then being 305 grams. The control remained healthy, and when killed on the sixth day weighed 339 grams. At autopsy a similar condition prevailed in the experimental kitten to that shown by the kitten of Experiment III, but there were rather more parasites.

Experiment V. (H. B. F.) A male kitten was fed with food contaminated with Lamblia cysts, Gallipoli strain. It weighed 425 grams. The faeces were examined daily, but no Lamblia were found in them. The animal was killed after two months and was found normal at autopsy.

Experiment VI. (A. P.) The strain of Lamblia used in this and in Experiment VII was derived from a patient who contracted lambliasis in Flanders and who had never left England before going to Flanders. Samples of his stools had been kept for some time, and the one used for these experiments had been passed seventy-four days before it was used. Some light is thus shed on the duration of vitality of the cysts of Giardia (Lamblia) intertinglie

on the duration of vitality of the cysts of Granata (Lamonia) intestinalis. A female kitten, weight 400 grams, was fed with food con-taminated with Lamblia cysts from the Flanders strain. Its faeces were examined daily. On the second, third, fifth, and seventh days they were negative, but a few Lamblia cysts were seen on the ninth and eleventh days. After the infective feed, the kitten soon became thinner and showed signs of diarthoea and general disconfort. The diarthoea became more marked the kitten soon became thinner and showed signs of diarrhoea and general discomfort. The diarrhoea became more marked and the animal was noisy, giving forth piercing howls. It died after eleven days. At death the body was somewhat emaciated, the blood vessels of the stomach, intestine, and mesentery were injected, and the walls of the duodenum were thin. The duodenal mucosa was disintegrated, some of the villi being stripped off. Lamblia, both free and attached to epithelial cells, were present in the groximal loop of the duodenum, a fair number occurred in the distal loop and a few were present in the jejunum. In the caecum a few flagellates occurred to gether with some encysted Lamblia, and cysts were found in the rectal contents. The control kitten never showed any sign of infection, remained healthy, and weighed 660 grams at the time of death of the experimental animal, which then weighed 375 grams. 375 grams.

*Experiment VII.* (A. P.) A female kitten, weight 410 grams, was fed with milk con-taining Lamblia cysts of the same strain and age as those used in Experiment VI. The symptoms produced were like those described for the previous subject. On the ninth and eleventh days after the infective feed Lamblia cysts were present in the faeces of the kitten and the faeces were very diarrhoeic. At death, which occurred on the twelfth day after the infective feed, the kitten weighed 375 grams, the loss of weight being thus greater than in the preceding case. At autopsy similar results were obtained to those in the preceding case, but the parasites appeared to be rather less numerous. The control kitten, which weighed 520 grams, though of equal weight at the commencement of the experiment, was found to be quite animal. animal.

Experiment VIII. (A. P.) A male kitten was fed with food containing cysts of Lamblia from a patient from Gallipoli. The animal was under observa-tion for ten weeks, but showed no parasites, nor were any found when the animal was killed.

found when the animal was killed. Experiment IX. (H. B. F.) An adult female mouse was fed on food contaminated with cysts of Giardia (Lamblia) intestinalis, Gallipoli strain, which had been separated from débris. A control mouse, which was of equal weight to the experimental one, was given food to which normal facces had been added. On the second day after feeding, a few empty Lamblia cysts were found in the facces of the experimental mouse, the control being negative, both on this date and onwards. On the sixth day the facces were negative. On the eighth day the facces showed a blood streak along their length, and several Giardia, like those in man, were found in active movement. Flagellates and cysts in scanty numbers were observed on the ninth day. Until the fifteenth day no parasites were seen, but it was noticed that the mouse was growing thinner, and on the fifteenth day fairly numerous Lamblia cysts were voided in the facces. On the sixteenth day the mouse was diarrhoeic and shivered con-siderably. Its coat was staring and eyes protruding. On the nineteenth day after the infective feed it was found dead. *Post mortem* the blood vessels of the mesentery were found to be engorged, and a general inflammatory condition of the intestine was present. Numerous flagellate Lamblia were present in the duodenum. No parasites were found in the caccum or in the large intestine. The mouse weighed 10.6 grams at death, having lost 4.2 grams in weight. The control weighed 20.4 grams. *Experiment X.* (A. P.)

weighed 20.4 grams. Experiment X. (A. P.) An adult female mouse, weight 12.5 grams, was given food contaminated with cysts of Giardia (Lamblia) intestinalis of the Gallipoli strain, its control being provided with food contami-nated with normal facces. On the second day after the infective feed, a number of empty Lamblia cysts were found in the ex-crement of the experimental animal. No more cysts were seen in the facces until the eighth day. On the ninth day no para-sites were present, and none were seen until the fifteenth day, when a few cysts were voided. The mouse showed some signs of distress, and was noticed to be getting thinner. On the six-teenth and seventeenth days cysts were found in the facces, the mouse being alternately constipated and somewhat diarrhoeic. On the eighteenth day the mouse was ill, the coat was staring, and there was constipation. Shivering occurred, and also on the nineteenth day. On the twentieth day the animal was killed in extremis. At autopsy the blood vessels of the stomach, mesentery, and intestine were engorged or injected, and the gut showed obvious inflammation. Shee epithelial cells were attached to them. Duodenal ulcers were present, and the cella of the mucosa to which parasites had attached themselves were also found in the jejunum, where the number of young cysts was somewhat larger. No parasites were found in the ileum. In the caecum a few flagellate Lamblia occurred, together with a large number of mature cysts. The colon con-tained a few flagellates about to encyst and numerous cysts. The rectum contained no flagellates as such, but a few forms in process of rounding subsequent to cyst formation were seen, as well as some fully formed cysts. The mouse weighed 9.4 grams when killed. 9.4 grams when killed.

*Experiment XI.* (A. P.) A male mouse, weight 14.4 grams, was given cysts of Lamblia, Gallipoli strain, in milk. The faces were observed daily for seventy days, but no Lamblia was seen. The mouse was then killed, but was found to be uninfected. It had increased in weight to 20 grams.

*Experiment XII.* (A. P.) A male mouse, weight 14.8 grams, was given food containing Lamblia cysts of the Gallipoli strain. A few normal and several empty cysts were observed in the faeces on the second day after feeding. No more parasites were seen in the faeces until the eighth day, when the faeces contained mucous streaks in which a few flagellate and encysted Lamblia were present. On the ninch day the faeces were howere contained mucous streaks in which a few flagellate and encysted Lamblia were present. On the ninth day the faces were normal in appearance, and no parasites were found. The mouse had been getting somewhat thinner, but not much difference in its condition was obvious. From the sixteenth to the twenty-first day the faces of the mouse were negative, the animal increased in weight and improved in general condition. On the twenty-first to twenty-sixth days a few Lamblia cysts were present. On the thirtieth day some flagellate Lamblia were found, together with young cysts. On the thirty-first day no cysts were seen but rare flagellates occurred. From the thirty-second to the thirty-sixth day the faces were negative. Very few flagellates and cysts were present on the thirty-seventh and thirty-eighth days, and none were present on the thirty-ninth day. A single cyst was found in a preparation of the fresh faces on the fortieth forty-third day a recurrence took place, a very few young flagelforty-third day a recurrence took place, a very few young flagel-lates being shed in the facees. No more parasites were observed until the fifty-sixth day, when some cysts were passed. A negative period followed till the sixty-second day, when the

facces were diarrhoeic and some flagellate forms were seen. From the sixty-second to the seventieth day the mouse grew thinner rapidly, passed diarrhoeic facces, and shivered; it died on the seventieth day. At post-more faces, and shivered it theu of the blood vessels of the stomach, intestines, and mesentery was found; the duodenum contained numerous Lamblia, and there were some parasites sparsely scattered throughout the intestine. This case may be regarded as one of chronic integrine. infection.

Experiment XIII. (A. P.) A male adult mouse, weighing 22.6 grams, was given food, containing cysts of Lamblia of the Flanders strain. The patient from whom the strain was derived had never been abroad except with the Expeditionary Force in Flanders, and then only for a few weeks. The man had chronic diarrhoea, but on no occasion could any bacterial or protozoal infection other than Lamblia be demonstrated in him. This strain proved highly virulent to mice to which it was administered. In the present mouse no Lamblia were found until the third day, when the mouse passed loose faeces containing a few flagellate Lamblia. On the fourth day mucus and shed epithelial cells occurred in the faeces, and a few Lamblia cysts were found. The mucus in the faeces increased in amount on the fifth day, and a few more cysts appeared. The mouse showed distress from the fifth day. It shivered constantly, its coat was staring, and it showed incontinence of urine. On the seventh day the animal died.

At autopsy the body showed emaciation, the weight being 18.9 grams. The blood vessels of the stomach intestine, and mesentery were engorged. The intestinal wall was thin, and a general inflammatory condition was present. Numerous flagellate Lamblia were present in the duodenum, but no cysts were found there. A similar condition prevailed in the jejunum. The caecum was thin-walled, gas was present in the proximal portion, but the appendix contained solid matter. Flagellate Lamblia were present in both the caecum and the colon. A few flagellates and some rounding forms, with a very few cysts, occurred in the rectum, which was thin-walled and inflamed. It is of interest to note that flagellate forms were pre-dominant in this acute, rapidly fatal case, and that relatively few cysts were seen. Also, some of the flagellates were found still living and active in the duodenum two days after the death of the host.

*Experiment XIV.* (A. P.) A male mouse, weight 19.6 grams, was given cysts of Lamblia with its food, the Flanders strain being employed. The facces containing the cysts had been kept for some time prior to being used. The facces of the mouse were examined daily for two months, but were negative. The mouse was then killed, and per provide found of the set more more the set in a strain of the set of the mouse were set in a strain set of the set of t no parasites were found at post-mortem examination.

### Experiment XV. (H. B. F.)

Experiment XV. (H. B. F.) A female mouse, weight 25 grams, was fed with food contain-ing Lamblia cysts of the Flanders strain. On the day following the infective feed an empty Lamblia cyst was found in a pre-paration of the faeces. On the third day two dying flagellate Lamblia were observed, and on the fourth and fifth days a single flagellate form was seen in each of the samples examined. The mouse voided diarrhoeic faeces on the ninth Lambha were conserved, and on the fourth and numbers  $F_{\rm a}$  and  $F_{\rm a}$  objectively, and on the fourth and the number  $F_{\rm a}$  and  $F_{$ 

Experiment XVI. (H. B. F.) A large female mouse, weight 24.5 grams, was given food con-taining cysts from the mouse used in the previous experiment. On the fifth, seventh, eighth, and ninth days after feeding, the faeces contained a very few Lamblia cysts. Then no more cysts were voided, and no parasites were found in the diarrhoeic faeces produced by the administration of an aperient on the fifteenth day. A similar result was obtained on the twentieth D

day. When the mouse was killed, on the twenty-eighth day, no parasites were found and all the organs were normal. It had increased in weight by 3.6 grams.

Experiment XVII. (A. P.) A male mouse, weight 18.4 grams, was fed on a piece of the intestine of the mouse infected in Experiment XIII. This material contained many active flagellates and only a few young cysts of Lamblia. On the third, fourth, fifth, and sixth days after the infective feed a very few Lamblia cysts were found in the faeces of the mouse. A few young flagellates were present on the tenth day, and also some cysts. From the twelfth to the sixteenth days no parasites were found in the faeces, but on the seventeenth day one flagellate and one cyst were present in a preparation. On the eighteenth and nineteenth days no Lamblia was found, and on the twentieth day one cyst only was found in the preparations of the faeces. On the twenty-third day a very few young flagellate Lamblia were recorded, but none were found in the faeces for some time after. The mouse was killed on the fortieth day after the infective feed, and then seemed normal. No parasites were found in it, so that a spontaneous cure of the weak infection may have occurred.

From our experiments it will be seen that Giardia (Lamblia) intestinalis of human origin has been shown to be pathogenic to rodents and to carnivores (kittens). It is, then, possible for such animals to serve as reservoirs of lambliasis, and by contaminating the food of man with their excrement to propagate lambliasis. We are of the opinion that sufficient notice has hardly been taken of the possibility or probability of rodents, such as rats and mice, acting as reservoirs of infection to man, and of carrying lamblial cysts to his food and abodes in the trenches in the fighting area. Mathis (1914) reported cases of lambliasis in Tonkin, wherein the houses of the patients were infested with rats and mice. Noc has reported similar cases. In the war zones where trenches are infested with these rodents it should be remembered that lambliasis may be thus spread, as was pointed out by us in 1915 and 1916. Kittens as pets should not be encouraged, as they may serve as carriers of infection.

### CONCLUSIONS.

From our experiments and observations we conclude that:

1. Giardia (Lamblia) intestinalis is pathogenic to man and is capable of producing diarrhoea, which may be persistent or recurrent.

2. The virulence of the parasite varies, and lambliasis occurs in tropical and non-tropical countries. The Lamblia cysts can remain infective for some time.

3. Lambliasis occurs in rodents, especially rats and mice, and can be of human origin. The influence of rats and mice in the spread of lambliasis has been noted re-cently by Noc and by Mathis. Lambliasis may also be produced in cats. Lambliasis may be conveyed to man from these mammals.

4. As a result, rodents may be reservoirs of lambliasis, and on the western front this should be remembered, as we have already published.

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THROUGH the efforts of the American Hay Fever Prevention Association, supported by the Municipal Board of Health, the city of New Orleans recently passed an ordinance enjoining that the tenant or occupier of any leased or occupied premises, or the owner of vacant premises shall not allow weeds or grass over 2 ft. in height to grow on such premises, or weeds or grass over 1 ft. in height to grow on such premises, of weeds or grass over under a penalty of a fine not exceeding £5 or imprisonment up to thirty days, or both. A similar ordinance was made by the New York Board of Health last year.