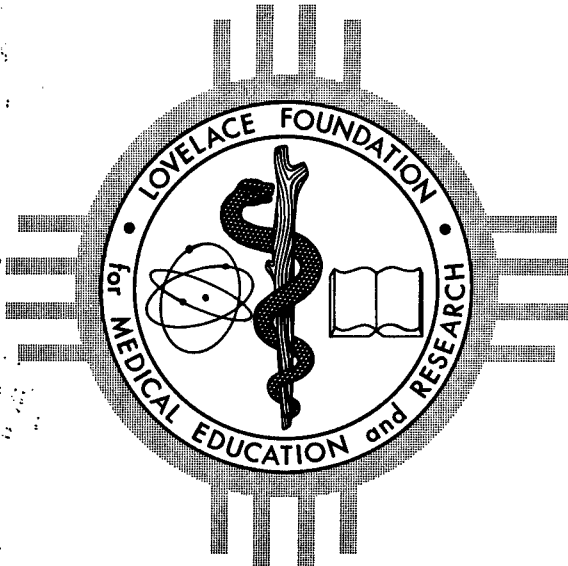


G. Bowen
LF-27
NB-4

LOVELACE FOUNDATION
for Medical Education and Research

AEC RESEARCH AND
DEVELOPMENT REPORT

UNCLASSIFIED



Albuquerque, New Mexico

**INTESTINAL SPIROCHETES
AND DISEASE IN DOGS**

by
F. F. PINDAK
AND
W. E. CLAPPER

September 1965

DISTRIBUTION STATEMENT A
Approved for Public Release
Distribution Unlimited

ATOMIC ENERGY COMMISSION-
LOVELACE FOUNDATION
FISSION PRODUCT INHALATION PROJECT

20000919 016

Reproduced From
Best Available Copy

LF - 27

Biology & Medicine

TID-4500 (44th Ed.)

UNCLASSIFIED

INTESTINAL SPIROCHETES AND DISEASE
IN DOGS

by

F. F. Pindak and W. E. Clapper

Submitted as a
Technical Progress Report

to

The Division of Biology and Medicine
United States Atomic Energy Commission

on

Contract No. AT(29-2)-1013
September 1965

From the Department of Microbiology
Lovelace Foundation for Medical Education and Research
Albuquerque, New Mexico

UNCLASSIFIED

ABSTRACT

Suspensions prepared from fecal samples of beagles were examined by phase microscopy. High numbers of borrelia were consistently found in dogs with diarrhea but seldom in healthy dogs. Other types of spirochetes were randomly distributed in both healthy and diarrhetic dogs. Repeated samplings were necessary to establish the relationship of spirochetes to disease since single specimens were occasionally found to vary from the average in both groups.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr. C.S. White for helpful suggestions in the preparation of the manuscript. They also wish to acknowledge, with thanks, the assistance of Dr. J.H. Sherrod in obtaining the specimens and evaluating the clinical status of the animals sampled.

TABLE OF CONTENTS

	Page
ABSTRACT	i
ACKNOWLEDGMENTS	ii
LIST OF TABLES	iv
LIST OF FIGURES	iv
INTRODUCTION	1
MATERIAL AND METHODS	1
1. Animals Studied	1
2. Collection and Examination of Specimens	2
RESULTS	2
DISCUSSION	7
REFERENCES	12

LIST OF TABLES

Table	Page
1. Incidence of Intestinal Spirochetes in Dogs with and without Diarrhea	3
2. Per cent of Dogs with Intestinal Spirochetes	4
3. Incidence of Intestinal Spirochetes in 4 Dogs with Diarrhea	8
4. Incidence of Spirochetes in High Numbers [4+ and/or 3+] in Diarrhetic and Normal Dogs	9

LIST OF FIGURES

Figure	Page
1. Intestinal Spirochetes (mostly borrelias) in a Fecal Sample of a Diarrhetic Dog	5
2. Intestinal Spirochetes (mostly treponeme) Trapped in Intestinal Mucus of a Diarrhetic Dog	6

INTESTINAL SPIROCHETES AND DISEASE IN DOGS

by

F. F. Pindak and W. E. Clapper

INTRODUCTION

The presence of various types of spirochetes in the lower digestive tract of dogs and other animals has been known since the last two decades of the nineteenth century. However, despite extensive investigation in the fifty years following their discovery, their relation to disease still remains to be established. In recent years only occasional reports concerning these organisms have been published. Lack of satisfactory methods for isolation and growth of these spirochetes has made it impossible to adequately classify them. It has also prevented the application of Koch's postulates and therefore only indirect evidence can be used to determine their ability to produce disease.

Recently, we reported the presence of three basic types of spirochetes in fecal specimens from beagles which had no apparent signs of illness¹.

This report is concerned with a similar study done with beagles suffering from diarrhea.

MATERIAL AND METHODS

1. Animals Studied.

All dogs included in this study were pure-bred, young adult male and female beagles. They were housed singly or in small groups in kennels with concrete floors. They were on a diet consisting of commercial dried food supplemented with raw meat and their drinking water was from the public utility system. The animals were under daily observation by a veterinarian. Occasionally, some of them were found to have diarrhea or loose stools with or without traces of fresh blood in the feces. The duration of the condition varied from two days to a week and had a tendency to reappear at irregular intervals. No other clinical symptoms were present. These dogs made up the experimental group. The control dogs were made up of a group showing none of the symptoms described above.

2. Collection and Examination of Specimens.

Rectal swabs were taken from diarrhetic dogs, and from the normal controls. The fecal material was immediately submerged in approximately 2 ml. of sterile saline. A suspension was made in this fluid and examined in wet preparations by phase microscopy under 400 X magnification. The spirochetes, all motile, were divided into three groups, namely borrelias, treponemes, and large rigid double-contour spiral organisms. Their detailed description was reported elsewhere¹. A minimum of 10 fields per specimen was examined. The numbers of each type of spirochete per field were averaged as follows: none in 10 fields=0; not less than one per 10 fields to one per field=+; 2-10 per field=2+; 11-50 per field=3+; more than 50 per field=4+.

RESULTS

The first group examined consisted of 28 beagles with diarrhea and 16 controls with no clinical symptoms. All were sampled once only and the results of the examinations of their feces are given in Tables 1 and 2.

In the diarrhetic dogs, borrelias were present in all 28 [100%], treponemes in 25 [89.3%], and the double-contour organisms in 27 [96.4%]. In the controls, borrelias were found in 8 [50.0%], treponemes and the double-contour organisms each in 15 [93.7%]. It was observed that some specimens consisted of only scanty amounts of fecal matter, but, upon suspension, were quite turbid and had a milky appearance. As a rule these contained borrelias in exceedingly high numbers, with only few other microorganisms present, [Fig. 1]. Occasionally, an intact fragment of mucus could be examined, [Fig. 2], which showed a large number of treponemes trapped in the dense material.

Our previous experience suggested that correlation of the presence of spirochetes in the lower intestine to disease could be made only by comparing relative numbers and not by the presence or absence of the organisms alone. For this purpose, an arbitrary division was made between

TABLE 1
Incidence of Intestinal Spirochetes in Dogs with and without Diarrhea
28 Dogs Sampled Once

Spirochete	28 diarrhetic dogs		16 normal dogs	
	Number of Dogs	Percent of total	Number of Dogs	Percent of total
<u>Borrelia:</u>				
4+	10	35.7	1	6.2
3+	11	39.3	3	18.8
2+	5	17.9	2	12.5
+	2	7.1	2	12.5
0	0	0.0	8	50.0
Totals	28	100.0	16	100.0
<u>Treponeme:</u>				
4+	0	0.0	0	0.0
3+	1	3.6	2	12.5
2+	16	57.1	5	31.3
+	8	28.6	8	50.0
0	3	10.7	1	6.2
Totals	28	100.0	16	100.0
<u>Double-Contour:</u>				
4+	0	0.0	0	0.0
3+	1	3.6	1	6.2
2+	16	57.1	9	56.3
+	10	35.7	5	31.3
0	1	3.6	1	6.2
Totals	28	100.0	16	100.0

4+=more than 50 spirochetes per field; 3+=11-50 spirochetes per field;
2+=2-10 spirochetes per field; +=1 spirochete per 10 fields to 1 per field;
0 =none in 10 fields.

TABLE 2

Per Cent of Dogs with Intestinal Spirochetes

Spirochete	Positive - [any degree]		4+ and/or 3+ positive	
	Diarrh.	Control	Diarrh.	Control
Borrelia	100.0%	50.0%	75.0%	25.0%
Treponeme	89.3%	93.7%	3.6%	12.5%
Double- Contour	96.4%	93.7%	3.6%	6.2%

28 dogs with diarrhea

16 controls - without diarrhea

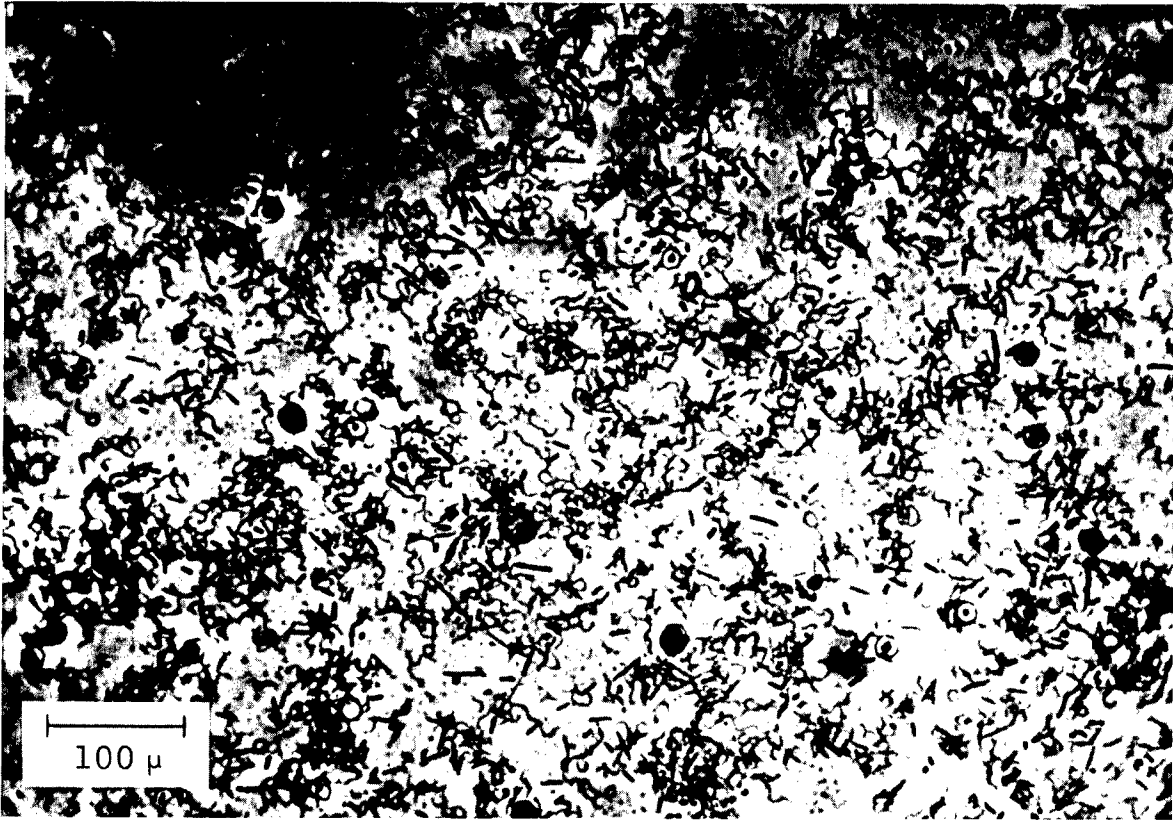


Fig. 1. Intestinal Spirochetes (mostly borrelias) in a fecal sample of a diarrhetic dog.

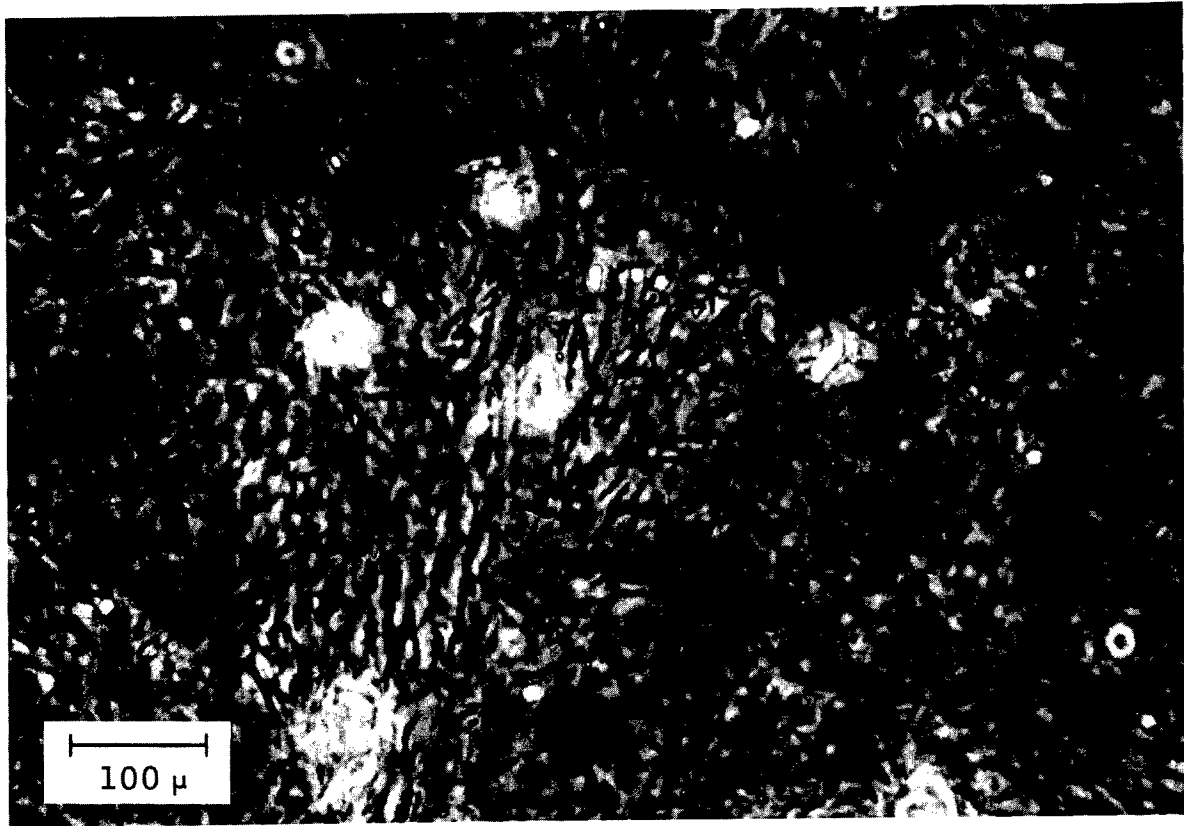


Fig. 2. Intestinal Spirochetes (mostly treponemes) trapped in intestinal mucus of a diarrhetic dog.

dogs with rectal specimens having more than 10 spirochetes per field [3+ and 4+] and those having them in lesser numbers [0-2+]. Table 2 shows that, in diarrhetic dogs, the borrelias were not only found more often than the other two types, but that they were present in high numbers in three times as many of the experimental dogs as in the controls. The validity of these findings was tested by repeated samplings of an additional 17 diarrhetic dogs. Four were examined eight times during 18 days, [Table 3] and 13, two to six times, [Table 4]. As Table 3 shows, in dog number 29, the borrelias were present in high numbers [3+ and 4+] in 7 examinations [87.5%] while both the treponemes and the double-contour organisms were found in high numbers only once [12.5%]. In dog numbers 30, 31, and 32, high numbers of borrelias were found in 50%, 75% and 25% respectively, the treponemes in 0%, 37.5% and 37.5% and the double-contour organisms in 0% in all three dogs [Table 3]. The examinations of the remaining 13 dogs [Table 4] showed borrelias to be present in high numbers in 54.8%, treponemes in 9.5%, and the double-contour spirochetes in 11.9%. The corresponding readings on eight additional control dogs, representing 50 examinations, were 12% for the borrelias, 2% for the treponemes and 4% for the double-contour organisms.

DISCUSSION

Spirochetes in the lower intestine of man and animals were studied by many early microbiologists. Yet, even today, they are not satisfactorily classified, as evidenced by their absence in the 1957 edition of Bergey's Manual. Inability to grow these organisms in pure culture prevents the study of those properties that would make possible a meaningful classification by which various species might be identified. It also prevents the performance of experiments that might clearly indicate their ability to produce disease. Unsuccessful attempts to grow them have been reported by Lim², Jungherr³, Reinhold and Wagner⁴, Weber and Schmittiel⁵, Sherra⁶ and Pindak et al.¹. Recently, Hardy et al.⁷, reported isolation in pure culture of spirochetes from the human oral cavity. Metabolic products of other microorganisms found in the human mouth were necessary

TABLE 3
Incidence of Intestinal Spirochetes in 4 Dogs with Diarrhea

Exam. Date		Spirochete*		Exam. Date		Spirochete*		Exam. Date		Spirochete*		Exam. Date		Spirochete*	
		B	T	B	T	B	T	B	T	B	T	B	T	B	T
		DOG NUMBER				DOG NUMBER				DOG NUMBER					
		30				31				32					
1/25	2+	3+	3+	2+	2+	2/4	2/4	4+	2+	2/4	2+	2/4	2/4	3+	2+
1/27	3+	2+	2+	0	2+	2/5	2/5	4+	3+	2/5	2/5	2/5	2/5	2+	2+
2/5	4+	+	0	2/8	2+	2/8	2/8	3+	2+	2/8	2+	2/8	2/8	2+	3+
2/8	3+	+	2+	2/9	4+	2/9	2/9	3+	3+	2/9	3+	2/9	2/9	3+	2+
2/9	3+	2+	2+	2/10	+	2/10	2/10	+	3+	2/10	2+	2/10	2/10	2+	3+
2/10	3+	2+	2+	2/11	3+	2/11	2/11	2+	2+	2/11	2+	2/11	2/11	0	+
2/11	4+	2+	+	2/12	2+	2/12	2/12	4+	2+	2/12	2+	2/12	2/12	+	2+
2/12	4+	2+	2+	2/15	3+	2/15	2/15	3+	2+	2/15	2+	2/15	2/15	2+	2+

Per cent of all examinations showing 3+ and/or 4+ for each type of spirochete

B		T		D		B		T		D		B		T		D	
87.5	12.5	12.5	0.0	0.0	0.0	75.0	75.0	37.5	37.5	0.0	0.0	25.0	25.0	37.5	37.5	0.0	0.0

*B=borrelia; T=trepone; D=double-contour

TABLE 4
The Incidence of Spirochetes in High Numbers [4+ and/or 3+] in Diarrhetic and Normal Dogs
Multiple Examinations

Dog No.	Borrelia Examinations							Treponeme Examinations							Double-contour Examinations						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
1	0	+						+	3+						0	+					
2	0	0						4+	3+						2+	2+					
3	4+	+						3+	2+						2+	2+					
4	4+	4+						2+	2+						2+	+					
5	+	2+						2+	2+						+	2+					
6	4+	3+		4+				0	2+	+					+	2+	+				
7	+	+		+				2+	2+	2+					+	2+	2+				
8	3+	4+		4+				0	0	2+					0	0	+				
9	2+	3+		4+				+	+	0					+	+	0				
10	3+	4+		4+	4+			+	2+	0	2+				2+	2+	+	+			
11	3+	+		2+	4+	4+		0	2+	2+	0	2+			0	3+	2+	0	+		
12	2+	+		+	0	0		2+	+	2+	2+	2+			2+	3+	3+	2+	2+		
13	4+	4+		2+	4+	4+	4+	+	2+	2+	2+	2+	2+		+	3+	3+	2+	2+	2+	
Total examinations 42																					
4+ and/or 3+ positive 54.8% 4+ and/or 3+ positive 9.5% 4+ and/or 3+ positive 11.9%																					
<u>NORMAL DOGS</u>																					
1	3+	+	+	2+	0	2+		+	2+	+	+	0	2+		+	2+	2+	2+	0	2+	
2	2+	0	0	0	0	0		+	+	+	2+	+	0		+	2+	+	2+	2+	0	
3	2+	3+	+	3+	3+	2+		+	2+	2+	2+	3+	+		+	2+	2+	2+	2+	0	
4	0	+	+	0	0	0	0	+	+	+	2+	+	+	+	+	2+	+	+	+	+	3+
5	0	0	0	0	0	0	0	2+	2+	2+	+	+	+	0	2+	+	+	+	+	+	0
6	2+	+	2+	0	2+	2+		2+	+	2+	2+	+	+	+	+	2+	+	+	+	2+	2+
7	4+	+	0	0	4+	0		0	+	+	0	+	0	2+	+	+	+	+	+	2+	2+
8	0	0	0	0	0	0	0	2+	2+	+	2+	2+	3+	+	2+	+	+	+	2+	2+	+
Total examinations 53																					
4+ and/or 3+ positive 11.3% 4+ and/or 3+ positive 3.8% 4+ and/or 3+ positive 3.8%																					

for this purpose. Our preliminary experience [unpublished] indicates that, at least in the dog, there is a morphological dissimilarity between the intestinal spirochetes and those found in the mouth. Only a thorough day-by-day study of the entire microbial population of the lower intestine can reveal whether a similar dependency exists between these spirochetes of the dog and the remaining intestinal flora. Their periodic incidence in high numbers followed by an almost complete absence seems to favor this possibility.

Macfie^{8,9} described intestinal spirochetes in man, monkeys, dogs, rats, sheep, cattle, goats and pigs. He believed that they were associated with disease. Jungherr³, related similar organisms to enteritis in dogs. Craige^{10,11} believed that certain types of spirochetes in the digestive tract were associated with dysentery in dogs. It is of interest that Reinhold and Wagner⁴ and Sherra⁶ believed borrelia-type spirochetes found in the lower digestive tract to be capable of producing disease in man.

Earlier we [Pindak, et al.¹] reported that the mere presence of intestinal spirochetes in dogs, whether a single type or a combination of all three, should not be interpreted as an indication of disease since 96% of our normal beagles carried at least one type. However, it was thought possible that, if present, in large numbers, they might produce diarrhea. For this reason the present study was directed to those dogs which had the spirochetes in high numbers [4+ and 3+]. The data for the normal dogs, were in agreement with those reported earlier. On the other hand, the incidence of the borrelias in high numbers in the diarrhetic dogs was three times greater than in the normals. This was not found to be true for either the treponemes or the double-contour spirochetes. Essentially the same results were obtained from repeated samplings of diarrhetic dogs. Therefore, it appears that borrelias which we frequently observed in fecal specimens are associated with diarrhea in dogs. Whether these organisms are the primary cause of this condition or whether their presence in the lower intestine in high concentration is secondary to some other type of physiological change cannot be determined from the data at hand. Other than the loose stools sometimes accompanied by intestinal

bleeding, there were no obvious signs of illness in this group of dogs.

It would seem important therefore, to determine the spirochetal population of the lower intestine in any group of experimental dogs exhibiting these signs before attaching significance to them from other causes. Furthermore, studies of changes in microbial flora in irradiated dogs should include the spirochetal population of the digestive tract to have a complete picture of the physiological status of the animal. Finally, if these spirochetes can cause disease in dogs, their epidemiological relationship to humans should not be overlooked.

REFERENCES

1. Pindak, F.F., W.E. Clapper and J.H. Sherrod, "Flora of Healthy Dogs. III. Incidence and Distribution of Spirochetes in the Digestive Tract of Dogs, AEC Research and Development Report, LF-19, November 1964.
2. Lim, R.K.S., "A parasitic Spiral Organism in the Stomach of Cats," Parasitology, 12: 108-112, 1920.
3. Jungherr, E., "Observations on Canine Spirochetosis in Connecticut," J. Amer. Med. Assoc., 91: 661-673, 1937.
4. Reinhold, L. and S. Wagner, "Über einen Fall von Fusospirochätose um Rectum bei einem 12jährogem Kinde," Monschr. f. Kinderheilkunde, 105: 427-429, 1958.
5. Weber, A.F. and E.F. Schmittziel, "Electron Microscopic and Bacteriologic Studies of Spirilla isolated from the Fundic Stomachs of Cats and Dogs," Amer. J. Vet. Res., 23: 422-427, 1962.
6. Shera, A.G., "Specific Granular Lesions Associated with Intestinal Spirochetes," Brit. J. Surg., 50: 68-77, 1962.
7. Hardy, P.H. Jr., Y.C. Lee, and E.E. Nell, "Use of Bacterial Culture Filtrate as an Aid to the Isolation and Growth of Anaerobic Spirochetes," J. Bact., 87: 1521-1525, 1964.
8. Macfie, J.W.S., "A Case of Dysentery in a Monkey," Annals Trop. Med. Parasit., 9: 510-511, 1915.
9. Macfie, J.W.S., "The Morphology of Certain Spirochetes of Man and Other Animals," Annals Trop. Med. Parasit., 10: 305-343, 1916.
10. Craige, J.E., "Spirochetes Associated with Dysentery in Dogs," J. Amer. Vet. Med. Assoc., 113: 247-259, 1948.
11. Craige, J.E., "Intestinal Disturbances in Dogs: Differential Diagnosis in Specific Therapy," J. Amer. Vet. Med. Assoc., 114: 425-428, 1949.