Studies on Histoplasmosis in a Rural Community*

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DURING the last 40 years less than 100 proved cases of histoplasmosis have been reported in the world literature. These few cases, nearly all fatal, cannot be considered a significant public health problem. Public health interest is aroused, however, by Christie and Peterson[1] and Palmer,[2,3] who advance the hypothesis that human histoplasmosis may exist in a mild form and may be an important factor in the etiology of calcified pulmonary lesions. The general background of this hypothesis lies in the reports of Gass, et al.,[4] Lumsden and Dearing,[5] Olson, et al.,[6] and others, showing that a large number of persons have pulmonary clacification not associated with demonstrable tuberculosis. Olson, et al.[6] concluded that such calcification must be due to some unrecognized disease of common occurrence other than tuberculosis. Thus, when Christie and Peterson,[1] Palmer,[2,3] and Furculow, et al.[7] found that a large proportion of the population in certain areas gave positive skin reactions to a filtrate of *Histoplasma capsulatum* grown on a synthetic medium, namely histoplasmin,† and also found definite association between the occurrence of positive skin reactions and pulmonary calcification, the above hypothesis was advanced and public health interest aroused.

Of the less than 100 cases of histoplasmosis reported in the world literature, 4, all fatal, occurred in Loudoun County, Virginia. This county lies between the Potomac River and the Blue Ridge Mountains, and its 517 square miles consist of rolling hills covered with farms and dairies. The population, 16,000 white and 4,000 colored, is entirely rural; the only sizable community is Leesburg with a population of 1,700. The first case of histoplasmosis recognized in Loudoun County occurred in 1922 in a 4 year old girl living in the village of Hamilton. The second occurred in 1938 in an 11 month old girl living near Paeonian Springs. The next two cases occurred in 1945 in 6 and 8 year old brothers living in Ashburn. These cases and 2 cases in dogs also from Loudoun County have been reported.[8,10] They represent a disproportionately large number of cases in a small population. Map 1 shows the location of known histoplasmosis infection in Loudoun County. The authors participated in studying the 1945 cases and promptly initiated a search for *Histoplasma* infection in man and animals. This report summarizes observations to date.

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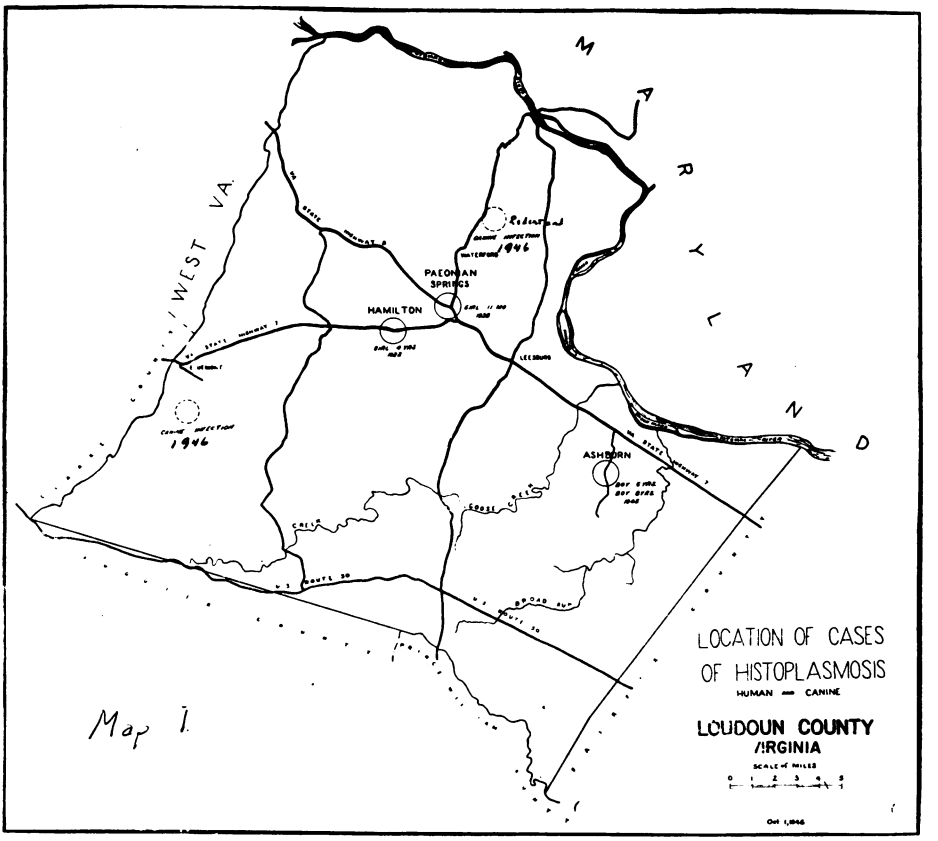
† The histoplasmin skin test is not specific for histoplasmosis in animals and no evidence has been found that the reaction is specific for histoplasmosis in humans.[9]



In the search for human infection, local physicians were interested in the problem and asked to report cases of illness suggestive of histoplasmosis. Cases were hospitalized, when hospitalization was indicated, for clinical and bacteriological study. In January and again in May and October, 1946, clinics were held in the Ashburn School. Fourteen inch by 17 inch chest x-rays and histoplasmin and tuberculin skin tests * were made on people living in the vicinity of Ashburn. A full-time public health nurse, Asst. Nurse Officer (R) Elizabeth K. Dodson, made repeated visits to the homes of all persons tested to secure histories of residence and past diseases in members of the household. One of the authors checked a sample of these histories, obtained detailed histories of all persons who were retested in the May and October clinics, and also obtained detailed histories of persons having suspicious illnesses and the household associates of such persons.

In the search for animal infection the local game warden, veterinarians, and other citizens were informed of the problem and asked to report animals with illnesses suggestive of histoplasmosis. Routine trapping of animals, especially rodents, was instigated particularly in areas where infection was

* The final readings of all x-ray films were made by Dr. R. A. Brown, Consultant in Tuberculosis, of Houston, Tex. Histoplasmin and tuberculin skin tests were performed simultaneously in each forearm by two operators giving 0.1 ml. intradermally. A 1:1,000 dilution of histoplasmin was used from the same lot H3 previously reported.[9] For the tuberculin test a single dose of 0.0001 mg. PPD was used. All reactions were read on the second day (48 hours after injection) by one or more of the authors, and reactions showing edema of 5 mm. or more diameter were classified as positive.

TABLE 1

Animals Captured in Loudoun County and Examined by Autopsy and Culture for Histoplasmosis

Place of Capture	*Animal*	*Number*	*Number with Histoplasmosis*
Near Human Cases (Ashburn)	*B'arina brevicauda*	30	0
	Microtus sp.	72	0
	Mus musculus	114	0
	Peromyscus sp.	4	0
	Pitymys sp.	1	0
	Rattus norvegicus	48	0
	Felis catus	4	0
	Total	273	0
Near Canine Cases [1] (Waterford)	*Blarina brevicauda*	31	0
	Microtus sp.	68	0
	Mus musculus	440	1
	Peromyscus sp.	22	0
	Pitymys sp.	2	0
	Rattus norvegicus	10	0
	Sylvilagus sp.	21	0
	Zappus hudsonius	1	0
	Marmota monax	5	0
	Didelphis virginiana	2	0
	Canis familiaris [2]	1	1
	Total	603	2
Other	*Canis familiaris*	5	0
	Marmota monax	5	0
	Total	10	0
Total		886	2

[1] The two cases reported by Dr. Seibold (not included in this table).
[2] Three additional dogs (still living) were examined by repeated blood cultures but no evidence of histoplasmosis was found.

known to have existed in man or dogs. All captured animals were examined, and the spleen, liver, adrenal gland, bladder, and lungs were cultured for *Histoplasma capsulatum.*

Many cases of illness in animals were studied, and Table 1 shows the result of search for animal infection. To date pathologic and bacteriologic studies have been completed on 886 animals. None of these was found infected with histoplasmosis except one dog that died in July, 1946, and one rodent, *Mus musculus,* captured in November, 1946.*

Two other dogs of Loudoun County died of histoplasmosis in February and May, 1946, respectively. Dr. H. R. Seibold made the diagnosis and reported the first case in detail, and stated that the second dog died of the disease in May.[10] In the literature, only 7 other dogs have been reported naturally infected with histoplasmosis.† [11-16]

These 3 1946 cases are the only known canine cases in Loudoun County, and the authors made a detailed study of the circumstances as being of special interest, particularly since Para [15] recently reported a human case associated with a canine case. In Loudoun County the first dog known to have histoplasmosis died in February, 1946. This dog was

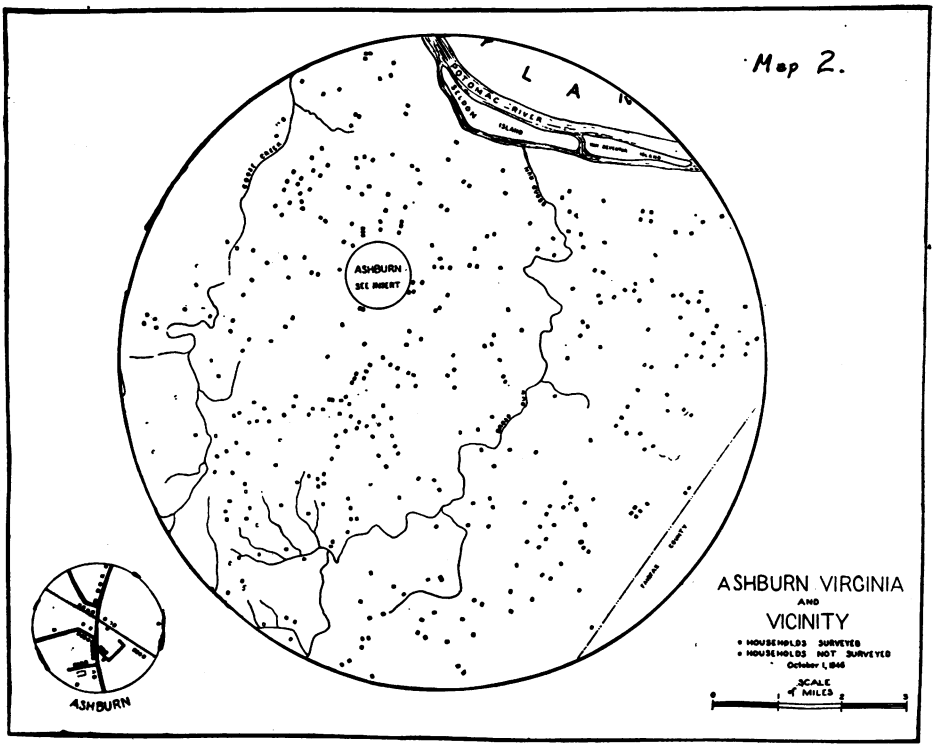
* The rodent was captured November 20, 1946, and found to have histoplasmosis by isolation of the fungus from the liver, spleen, and lungs and by histopathological examination. It was trapped in the basement of the farm home where the two dogs reported by Dr. Seibold [10] and the one reported here were kept.

† Excluding the case mentioned by Parsons.[17]

the sire of 8 pups born in January, 1945. The 2 year old mate has remained healthy. Two female pups of this litter were destroyed at birth; one male died when 1 week old of unknown cause; one female was healthy when destroyed in February, 1946, after giving birth to 9 pups, only one of which has been ill, and careful examination has produced no evidence of histoplasmosis; one male of the original litter died in April, 1946, with symptoms suggestive of histoplasmosis; 2 females died in May, one with proved histoplasmosis and the other with suspicious symptoms; and one male died on July 19 with proved histoplasmosis. This animal was referred for study to Dr. M. Parsons of Arlington, Va. The two dogs dying with symptoms suggestive of histoplasmosis had anorexia and emaciation; no definite diagnosis was established as no material was available for examination. In the three dogs dying of proved histoplasmosis the diagnosis was established by recovery of *Histoplasma capsulatum* from cultures of the spleen and other organs.

The case of the male dog dying of histoplasmosis on July 19 will be the subject of a later report, but a few notes will be included here. Ticks, *Dermacentor variabilis,* were fed on the dog during illness and *Histoplasma capsulatum* was recovered by culture from a ground-up tick soon after feeding. This was not surprising inasmuch as repeated blood cultures showed a heavy infection. Further studies of the relation of ticks to histoplasmosis are under way. On July 2 and again on July 10, two pairs of healthy dogs were placed and remained in continuous contact in the same cage with the natura'ly infected dog dying on July 19. None of these four contacts has shown any evidence of histoplasmosis to date.

Map 1 shows the location of the two



country estates where the infected dogs were kept. They were valuable dogs which were carefully fed and cared for. They were not used for hunting. There were 11 other dogs on one estate, 5 old coon hounds and 6 pups; all have remained well except two which were studied without evidence of histoplasmosis being found. Only one person was found on these estates with an illness suggestive of histoplasmosis; thorough hospital study gave no evidence of *Histoplasma* infection. No evidence of any contact whatsoever could be found between proved canine and human cases of histoplasmosis.

The household studies and physicians' reports yielded several cases of illness in which clinical and laboratory studies produced various diagnoses but no evidence of histoplasmosis. To date, 476 white persons in the vicinity of Ashburn have been satisfactorily x-rayed and skin-tested with histoplasmin and tuberculin (PPD), and their medical histories have been completed. Map 2 shows the geographic distribution of households studied.

Table 2 shows the number of persons having (A) calcified pulmonary lesions, (B) positive skin reactions with histoplasmin, and (C) positive skin reactions with tuberculin (PPD). The histoplasmin reactions are those of initial clinic visits; retests are described later. A tabulation similar to Table 2 has been made showing the presence or absence of calcified pulmonary lesions and the reactions to histoplasmin and tuberculin according to the medical histories of persons tested with respect to pertussis, diphtheria, scarlet fever, pneu-

TABLE 2

Number and Per cent of White Persons Having Pulmonary Calcification and Positive Skin Reaction to Histoplasmin and Tuberculin (PPD) According to Specified Attributes

Attributes		Total Number of Persons	A Persons with Calcified Pulmonary Lesions			B Persons with Positive Histoplasmin Reaction			C Persons with Positive Tuberculon (PPD) Reaction		
			No.	Per cent	"P" in 1,000	No.	Per cent	"P" in 1,000	No.	Per cent	"P" in 1,000
		476	196	41.2		393	82.6		114	24.4	
Age	1– 5	32	3	9		15	47		1	3	
	6– 9	94	41	44		76	81		4	4	
	10–14	121	54	45	6	99	82	<1	17	14	<1
	15–39	148	64	43		136	92		45	30	
	>39	81	34	42		67	83		47	58	
Sex	Male	239	95	40	>300	204	85	107	76	32	<1
	Female	237	101	43		189	80		38	16	
Years residence in Loudoun County	0- 2	75	20	27	5	47	63	<1	16	21	>300
	>2	401	176	44		346	86		98	24	
Tuberculin Skin Reaction	Pos.	114	53	47	186	102	89	26			
	Neg.	362	143	40		291	80				
Histoplasmin Skin Reaction	Pos.	393	170	43	45						
	Neg.	83	26	31							
History of Tuberculosis or in Household with Tuberculosis	Yes	28	20	71	<1	24	86	>300	8	29	>300
	No	448	176	39		369	82		106	24	

"P" = The probability that differences in percentages as great or greater than shown would occur in independent attributes through chance sampling variation.

monia, illness with diarrhea, ascariasis, and malaria. This tabulation shows no significant association between these diseases and pulmonary calcification, and no association with the histoplasmin or tuberculin skin reaction.

Column A of Table 2 shows that 41.2 per cent of the group studied had calcified pulmonary lesions. This was relatively constant in all age groups except 1–5 years where, as expected, it is lower. It is significant that recent residents of Loudoun County are not as apt to have calcified pulmonary lesions as residents of 3 years or longer. Also, individuals with a history of tuberculosis or of having lived in the same household as a person with a history of tuberculosis appear more apt to have calcified pulmonary lesions than individuals without this history. Column A shows no significant association between pulmonary calcification and either sex or the tuberculin reaction.

In the area studied it was impossible to demonstrate any direct association between the occurrence of pulmonary calcification and positive histoplasmin skin reactions. From the crude data in column A one might suspect that if a larger sample were taken a significant association would have been shown. Therefore, the following analysis of the data seems pertinent particularly in view of the association between these attributes reported in other areas.[1–3, 7] The number of persons having pulmonary calcification according to the histoplasmin and tuberculin skin reactions were:

		Per cent
Histoplasmin-positive Tuberculin-positive	48/102	47
Histoplasmin-positive Tuberculin-negative	122/291	42
Histoplasmin-negative Tuberculin-positive	5/12	42
Histoplasmin-negative Tuberculin-negative	21/71	30
Total	196/476	41

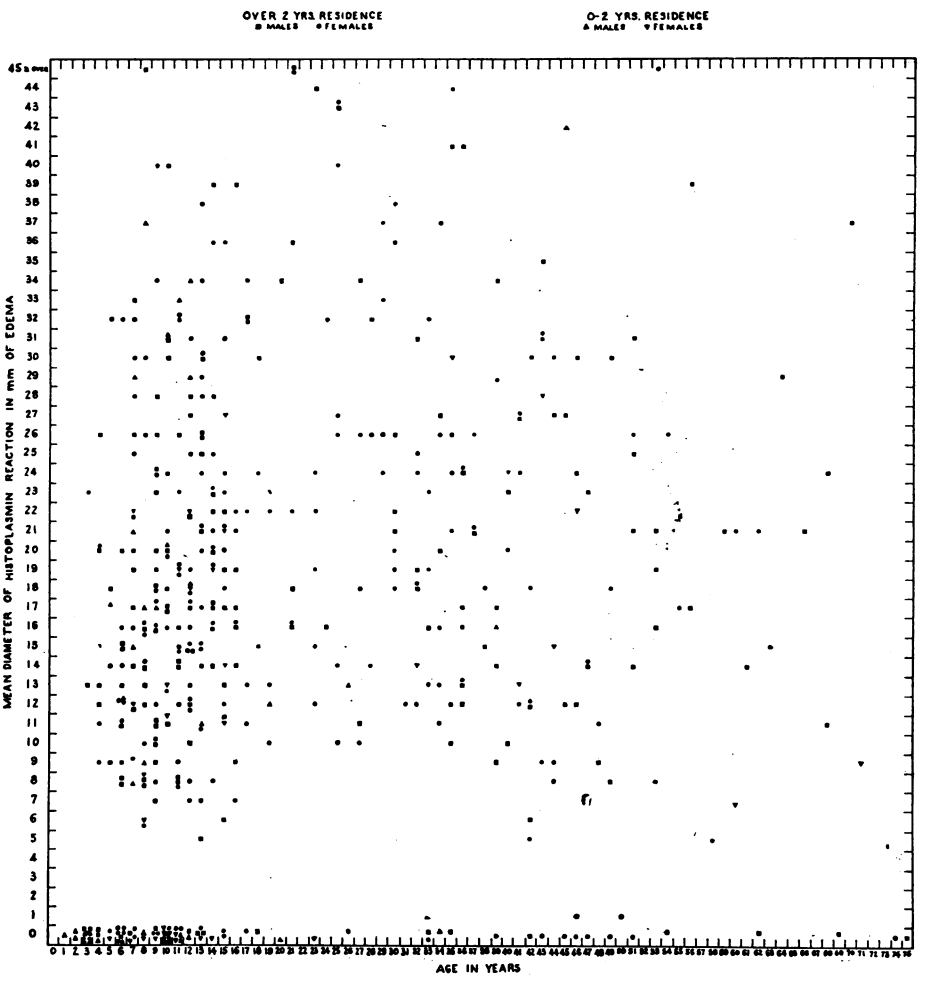
The differences in the above percentages might well be expected by chance sampling variation alone. This suggests that the small difference shown in column A was not due to direct association between the histoplasmin reaction and pulmonary calcification. Thus among the group having pulmonary calcification a comparison of persons positive to both histoplasmin and tuberculin with persons negative to histoplasmin and tuberculin showed that the former included a disproportionate excess of persons who were in the older age group, who resided in Loudoun County 3 years or longer, and who had a history of either having tuberculosis or living in a household with a person with tuberculosis. These three attributes were definitely associated with the occurrence of pulmonary calcification; the first two were associated with the occurrence of positive histoplasmin reactions, and all were associated in the direction that could produce differences similar to those shown above in the percentage of persons with calcified pulmonary lesions, but independent of the histoplasmin reaction. Hence it would appear unlikely that a larger sample, taken from this area where recent, proved cases of human histoplasmosis occurred, would demonstrate any direct association between the occurrence of pulmonary calcification and positive histoplasmin skin reactions.

Column B of Table 2 shows that 82.6 per cent of the group had a positive reaction to the histoplasmin skin test. The incidence of positive reactors is high in all age groups, being 47 per cent in the group 1–5 years old and more than 80 per cent in the older age groups. As with pulmonary calcification, recent residents of Loudoun County are not as apt to have a positive histoplasmin reaction as residents of 3 years or longer. There may be some association between persons reacting to the histo-

CHART 1

SIZE OF HISTOPLASMIN REACTION ACCORDING TO AGE, SEX, AND RESIDENCE IN LOUDOUN COUNTY

INITIAL SKIN TEST IN 476 WHITE PERSONS



plasmin test and those reacting to the tuberculin test. As with pulmonary calcification, the histoplasmin reaction was not significantly associated with sex.

Column C shows that 24.4 per cent of the group gave a positive reaction to the tuberculin (PPD) test. There is a progressive increase with age in the proportion of persons reacting to tuberculin, and males were more apt to be positive reactors than females, otherwise no significant association was found except as mentioned above.

Chart 1 shows the relation between the size of the histoplasmin reaction and age. The chart was prepared to determine whether it would indicate a better criterion for classifying histoplasmin reactions as positive (5 mm. or more diameter of edema was classified as positive). No better criterion was evident.

Three to 8 months after initial test,

40 persons with negative initial histoplasmin skin tests were retested, again x-rayed, and their medical histories including an interim history were carefully checked and studied. Fifty-eight persons with positive initial histoplasmin tests were likewise retested, again x-rayed, and studied medically. Of the 58 persons initially histoplasmin-positive, 24 (41 per cent) had pulmonary calcification; on retest 54 (93 per cent) remained histoplasmin-positive, and of these, 24 (44 per cent) had pulmonary calcification. Of the 40 initially histoplasmin-negative, 20 (50 per cent) had pulmonary calcification; on retest 22 (55 per cent) became histoplasmin-positive, and of these, 9 (41 per cent) had pulmonary calcification. The serial x-ray films of all individuals retested were carefully studied and not a single change was detected between initial and last test. From the medical interviews no evidence of illness could be elicited that could be associated with a change in the histoplasmin skin reaction.

SUMMARY AND CONCLUSIONS

In September, 1945, when the fourth human case of histoplasmosis (all fatal) was recognized among the 20,000 population of Loudoun County, Virginia, the authors began an intensive search for evidence of infection in man and animals. Local physicians, nurses, veterinarians, and others were interested in the problem and they reported cases of suspicious illness in man and animals to the authors for clinical, mycological, and pathological study.

Eight hundred and eighty-six animals have been captured and studied, and among these, only one rodent (*Mus musculus*) and one dog have been found naturally infected with histoplasmosis. Two other dogs of the same kennel have been found naturally infected in Loudoun County and reported.[10]

All infected dogs were associated with each other and the infected rodent was captured in the basement of the home where the dogs which were later proved infected had been kept some 19 months previously. The animal and human cases were distant from each other and no contact between them could be established.

Some 500 persons residing in the area where recent proved infection with *Histoplasma* has occurred, have been studied and to date no evidence of human infection was found. Of the 476 white persons surveyed, 83 per cent had a positive histoplasmin skin reaction at initial test, and 41 per cent had calcified pulmonary lesions. Persons who were histoplasmin-positive were older and had resided in Loudoun County longer than persons who were negative. Persons who had calcified pulmonary lesions were older, had resided in Loudoun County longer, and were more apt to have a history of tuberculosis or to have household association with a person having a history of tuberculosis than persons with no calcified pulmonary lesions. No direct association could be established between the occurrence of positive histoplasmin skin reactions and pulmonary calcifications.

Forty persons initially histoplasmin-negative and 58 persons initially histoplasmin-positive were retested 3 to 8 months after initial test. On retest 7 per cent of the positives became negative, but 55 per cent of the negatives became positive. In all, the individual serial chest x-rays showed no change, and repeated medical studies gave no evidence of any suspicious illness either prior to or during the period of change in skin reaction.

To date no evidence of mild human histoplasmosis has been found in Loudoun County, Va.

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Parent-Teacher Association, and other citizens of the community have facilitated the study in every possible way. The physicians of Loudoun County, especially Drs. J. A. Gibson, J. T. Jackson, and G. H. Musgrave, have given every assistance. The Virginia State Health Department and the Loudoun County Health Department coöperated in the study and furnished nurses, technicians, and x-ray equipment for the clinics.

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