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DISSEMINATION OF TUBERCULOSIS BY THE MANURE OF INFECTED CATTLE.

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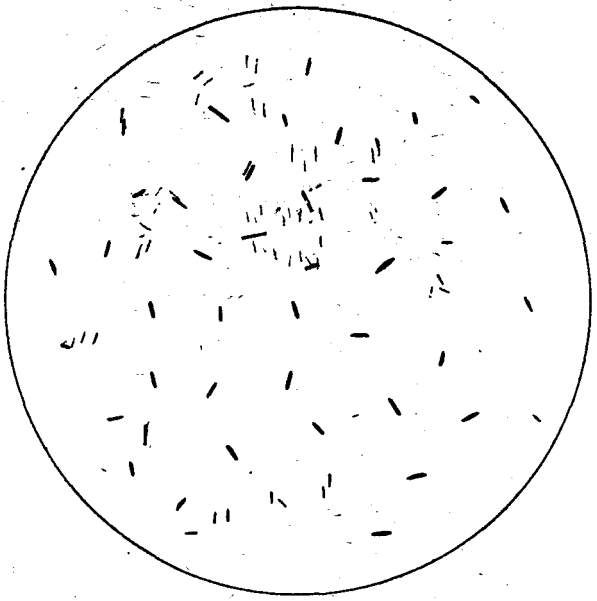

Figure A.—ACID-FAST BACILLI.
Presumably of Tuberculosis in Cover Glass Smears from the Intestinal Mucous Membrane of the Cow, Reddic.

DISSEMINATION OF TUBERCULOSIS BY THE MANURE OF INFECTED CATTLE

BY

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AND

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OBJECTS OF THE WORK HEREIN REPORTED.

The general purpose was to secure information concrning the ways in which tuberculin reacting cows pass off tubercle bacilli aside from bronchial secretion by coughing, and in milk. For this purpose the following was undertaken:

First. To study the manure of tubercular cattle.

Second. To study nasal secretion of tubercular cattle.

Third. In case the work with either manure or nasal secretion or both gave positive results, to determine whether such discharge of tubercle bacilli is constant, or intermittent and irregular.

Fourth. To determine if possible whether there is any clinical evidence associated with discharge of tubercle bacilli in the manure, particularly with reference to an accompanying diarrhea.

When this work was begun there was an abundance of information concerning methods by which infection of tuberculosis might be taken into the animal or human body, and such information is still accumulating rapidly. There was very little information that could be considered reliable concerning the methods by which the infection may be given off by the tuberculous animal, aside from spraying of bronchial discharges in the act of coughing and through milk.

Comparatively few tuberculous cattle cough to an unusual extent, and, there seems slight probability that there may be serious spread of tuberculosis among mature cattle from virulent milk, yet, rapid spread of this disease in the stable is a matter of commonest occurrence. If there are

other means of discharging the infection: for instance, in the manure, or urine, or nasal secretion, then these become at once matters of serious concern; serious alike for confined animals, and for exposed people. Our work has been under consideration for several years and was actively begun early in March, 1905.

It is not pleasant to estimate what may easily happen if tubercular cows pass virulent manure, and are present in a city dairy, in a breeding herd, or in any herd of milk cows. Manure readily becomes smeared upon the tail, thence, upon the udder and flank, where it becomes dried. Particles are broken off and fall into the milk pail, and the milk has become seriously contaminated. To make a serious case it is not necessary to suppose that such manure reaches the udder or flank. It may become dried on the floor, and particles may then float as dust in air currents, caused by animals and people walking about the floor or air currents from open doors and windows. Such particles may easily settle into milk pails or calf pails or upon the food of the older cattle. Persons in the stable may inhale such dust. Virulent manure from cows may still be considered infectious even if it be shown that there is slight danger from dried, human sputum.

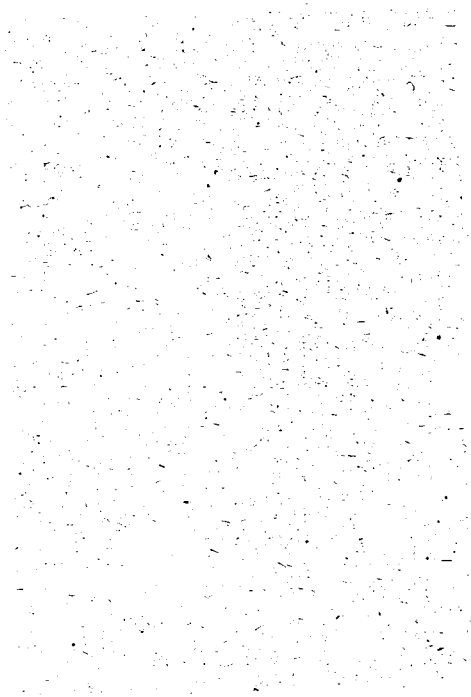
The possible contamination of past ures with virulent manure appears to be an important consideration. The Experiment Station has recently completed certain experiment work, undertaken with a view of estimating the probability of outdoor transmission of the disease, which work will be reported in the near future.

There are other reasons emphasizing the importance of such investigation. We cannot intelligently consider the spread of this disease unless probable sources of infection are known. The impression seems to be gaining general acceptance that infection frequently occurs very early in life and by the way of the digestive tract. If this opinion be correct, then tuberculous manure becomes at once a serious consideration for young children; and for calves that are raised upon dams whose udders and teats and the milk itself may easily become contaminated and dangerous.

Frequent and thorough disinfection of the floors in a stable where tubercular cows are kept, and where it is important to reduce the area of infection and to disinfect all

parts as rapidly as possible becomes a more important consideration, in view of the possibility of such infected manure contamination.

In view of the possibility of dry particles of contaminated manure resting upon the surface of the floor and bodies of confined cattle, sunshine becomes a still more important factor than is commonly supposed. The importance of sunshine is still further emphasized, in view of abundant evidence that it is very destructive to tubercle bacilli.



WORK DONE BY OTHERS.

Dr. Repp reported a very interesting case in a paper before the American Veterinary Medical Association, in 1903, where tubercle bacteria occurred abundantly in the intestinal mucosa and in the smears from the surface of this membrane. Ulcers were present on its mucous surface. Imagine this cow, or a cow with tubercular ulcers on the mucosa like that shown in a certain cut used in Dr. Moore's Pathology of Infectious Diseases of Domestic Animals, being used in any herd of milk cows, especially in a dairy herd.

Schroeder and Mohler[1] fed two healthy cattle a virulent culture of tubercle bacilli, and at the same time fed these cattle heavily on corn, so that corn would be passed in considerable quantity in the manure. This manure was given to an experimental pen of four pigs. Three out of the four hogs became infected with tuberculosis.

In another experiment the same workers used manure from two cattle which were known to be tubercular. This manure was thrown into an experimental pen of four hogs. One of the hogs showed lesions of probable tuberculosis on postmortem. Bearing in mind the fact that hogs are very susceptible to tuberculosis by ingestion, this is seen to be an important experiment upon the custom of running hogs after cattle in the feed lot.

Some time before beginning the experiment reported in this bulletin, one of us (Reynolds) had found innumerable acid-fast bacilli resembling in every way bovine tubercle bacilli in the manure of a cow named Reddie on the University Farm. These bacilli were found also in scrapings from the surface of the rectal mucosa, and in smears from bits of that membrane. (See Fig. A.) This cow, Reddie, had reacted to tuberculin some time before, and had chronic diarrhea which was supposed to be of tubercular origin. It is of course possible that this may have been a case of Johne's disease; and the cow may have been affected with tuberculosis and this other disease, simultaneously, granting that the latter is distinct from tuberculosis.

[1] Bureau of Animal Industry, Bulletin 88.

The cow Reddie was tested with tuberculin as shown by the following records:

(I.) Weight, 1450; dose 3.5 c.c.; bureau tuberculin; injected at 9 p. m., Jan. 29, 1902.

	6 a.m.	8 a.m.	10 a.m.	12 m.	2 p.m.	4 p.m.	6 p.m.	8 p.m.
Jan. 29			101.2		101.1	101.2		101.3
Jan. 30	105	102	105	106	105	105	104	

Diagnosis, tuberculosis.

(II) Weight, 1,430; dose, 3.75 c.c.; bureau tuberculin; injected at 9 p. m., April 8, 1902.

	6 a.m.	8 a.m.	10 a.m.	12 m.	2 p.m.	4 p.m.	6 p.m.	8 p.m.
Apr. 8		100.1	101.1		101.5		101	101
Apr. 9	102.5	104.8	106.2	105.3	105	103.3	103.8	

Diagnosis, tuberculosis.

(III) Weight, 1,300; dose, 3.8 c.c.; bureau tuberculin; injected at 9 p. m., May 27, 1903.

	6 a.m.	8 a.m.	10 a.m.	12 m.	2 p.m.	4 p.m.
May 27			100.9		101.4	101
May 28	105.4	105.2	104.6	104.7	104.8	104.4

Diagnosis, tuberculosis.

HISTORY AND AUTOPSY OF COW REDDIE.

This was a grade short-horn cow brought to the university farm in 1901, from Toronto, Can., where she had taken sweepstakes prize. She was tested for tuberculosis on the following dates, and reacted each time: Jan. 29, 1902; April 29, 1902; May 28, 1903;

This cow was a good breeder and very valuable, and was therefore retained in the herd for a time for this purpose.

Her general conditions remained good until June, 1903, when she began to decline. The cow gradually lost flesh and general condition until about October, 1903, when she commenced to gain back the lost weight, making a gain of about 150 pounds in 3 months. After this there came another decline. By the middle of February, 1904, she had begun a noticeable decline in general health and failed rather rapidly. She died March 10, 1904. The first symptom of the second decline was a severe diarrhea which persisted until death. Her temperature ranged during this time from 105 to 107, with fast and weak pulse. Edematous, swellings occurring behind the left elbow, under the brisket, and between the jaws. The appetite remained fairly good until the latter part of her illness.

Autopsy.—March 11, 1904, Reddie, 7 to 8 years old, weight about 1,400 pounds, still in fairly good flesh. She was last tested with tuberculin on May 28, 1903, and reacted. The post pharyngeal, mesenteric, and mediastinal glands, also one supra mammary lymph gland and several bronchial glands were all more or less badly involved with tuberculosis lesions. Both small and large intestines showed small nodules which were supposed to have been tuberculous, but were not tested for virulence or for bacilli. The spleen and liver showed plain lesions of tuberculosis, also the costal, diaphragmatic, and visceral pleurae (membrane lining the chest cavity) were all involved. The lungs were very generally affected, the disease involving both lungs and all the lobes.

Microscopic examinations of a considerable number (16) of cover glass smears were made from various affected structures and areas. Examination of these indicated a very general and very active infection. The word positive in the following list means that bacilli of tuberculosis were found on microscopic examination.

(1) Serum from cut surface of tubercle in active development on costal pleura, positive.

(2) Fluid from calcareous tubercle on costal pleura, negative.

(3) Serum from cut surface of large tubercle in costal pleura, positive.

(4) and (5) Forceps tissue smears from inflamed capsule of large tubercle on costal pleura, positive.

(6) Surface smear from the inflamed capsule of another large tubercle in costal pleura, negative.

(7) Surface smear from another small active tubercle near 6, positive.

(8) Forceps tissue smear from inflamed areas of peritoneum, right side, positive.

(9) Forceps tissue smear from inflamed capsule of tubercle in the lung, positive.

(10) Same as 9, negative.

(11) Same as 9, positive.

(12) Loop smear from surface of intestinal mucous membrane, positive.

(13) Loop smear from intestinal mucous membrane at another point, positive.

(14) Heart blood, negative.

(15) Loop smear intestinal contents, positive.

(16) Forceps tissue smear from intestinal mucous membrane from nearly the same point as number 13, positive.

The forceps tissue smears mentioned above were made by simply tearing out a bit of tissue and making direct smear upon the cover glass.

These bacilli were acid-fast and showed the peculiar staining reaction of tubercle bacilli; they correspond with tubercle bacilli in all details afforded by microscope; came from a cow that had reacted repeatedly to tuberculin, and showed extensive lesions of tuberculosis upon post mortem. They were not tested for virulence, but we are presumably

justified in saying that in all reasonable probability they were bacilli of bovine tuberculosis.

Schroeder and Cotton[2] also have undertaken to secure information concerning methods by which tubercle bacilli are passed off by tubercular cattle. They first made microscopic examinations of manure of tubercular cattle, also of scrapings of the rectal mucous membrane.

Smears made from the manure and rectal scrapings of five out of seven tuberculous cattle examined and of two other healthy cattle which were fed cultures of tubercle bacilli, showed bacilli which were not distinguishable from bovine tubercle bacilli, and were more numerous in the rectal scrapings than in the manure. These preliminary examinations were checked by similar studies of the manure of non-tubercular cattle which did not show such bacilli. The virulence and identity of these bacilli were determined by guinea pig inoculations.

Of seven natural cases, five showed bacilli in the manure on microscopic examination, as mentioned. The manure of these five was tested by inoculation and found infectious in three cases. Three healthy cows were fed culture of virulent bacilli and passed manure containing tubercle bacilli detected by microscopic examination and subsequently proven virulent. They conclude from this experiment that tubercle bacilli pass through the entire digestive tract of the cow without losing virulence.

The same experimenters also did work with saliva, nasal discharge, and urine of two cows, and, with the milk of three, making microscopic examinations in these cases and guinea pig inoculations. Microscopic examinations were all negative except for the saliva of one of the two cows examined in this way. The milk as shown by guinea pig inoculations was free from virulent tubercle bacilli. They conclude that cows rarely or never pass tubercle bacilli in the urine unless the genital or urinary organs are involved. Their results with the nasal secretions both by microscopic and inoculation tests were negative.

These writers estimate that with an average cow passing about thirty pounds of fresh manure per day and calculating upon the basis of the average number of bacilli in each microscopic mount, and the weight of feces smeared upon the covered glass, that such average cow

[2] Bureau of Animal Industry, Bulletin 99.

passing tubercle bacilli would give off 37,800,000 per day that could be demonstrated by the microscope, and it will be easily understood by anybody familiar with such work that there would be several times this number which could not be so demonstrated. They found uniformly that the tubercle bacilli were evenly distributed through the manure.

Schroeder and Cotton[2] are convinced that tubercle bacilli do not from any other source have a wider distribution in a fresh and virulent state than those that enter milk with the fresh manure of tubercular cows. If we remember that practically everybody uses milk every day, and remember the enormous number of tubercle bacilli which a single cow may pass in a day, and that milk may be easily contaminated by indirect as well as direct methods, this conclusion does not appear unreasonable as the reader would naturally conclude at first thought—providing a considerable proportion of tubercular cattle pass virulent manure.

These same writers, Schroeder and Cotton[2], contaminated fresh milk from healthy cows with the manure of cows which were known to be passing tubercle bacilli in this way. They endeavored to make the experiment compare as closely as possible with conditions which would develop in natural stable contamination. The amount of feces used for the samples of the milk was very small, estimated as not more than might easily gain access where average cleanliness in milking was observed.

In some cases the contaminated milk was injected immediately, and in other cases it was first strained through linen cloth. The experiment was properly controlled with similar milk not so contaminated. Five tubercular cows which were known to be passing tubercle bacilli were used for this work. The unstrained milk contaminated by the manure of four of these five cows produced tuberculosis of guinea pigs by injection into the abdominal cavity. The milk contaminated by the feces of these five cows and strained, proved infectious in three cases.

WORK BY THE MINNESOTA EXPERIMENT STATION AND THE LIVE STOCK SANITARY BOARD.

The material for this work was obtained from 45 head of cattle that had previously reacted to the tuberculin test. Twenty-two of these were pure bred, and grade Short-horn, Aberdeen Angus, and Hereford cattle that had been rejected from the University herd on account of their having reacted to the tuberculin test. Some of these animals had been kept in isolation for several years since they first reacted. They had been tested at intervals with tuberculin, and had at all times given reactions. The remaining 23 animals were pure bred Short-horns and grades which were kept upon a stock farm about 25 miles from the experiment station. The majority of these animals had first reacted to the tuberculin test about two years previous to the time this investigation was started. There were a few of these, however, that reacted six months after the first test, and were then stabled with the other tuberculous animals. Some of the cattle from which samples were taken were presumably badly affected as most of them had been known to be tuberculous for about two years, and during this time they had been confined in a barn for at least six months of each year.

Method.—In order to prevent extraneous infection much precaution was taken. In most cases the manure was removed from the rectum after the anus was carefully disinfected and the disinfectant wiped off with cotton. The small experimental animals were guinea pigs, most of which had been previously used for testing diphtheria antitoxin.

The original outline of our work was as follows: One guinea pig to be inoculated each month for four months from each reacting cow; smear preparations to be made at the same time, and stained for tubercle bacilli. After a lapse of six weeks, the guinea pigs were to be killed and a careful post mortem examination held to ascertain whether or not the cow was affected with tuberculosis. As the work progressed, it was found impossible to adhere closely to this plan.

When this work was first started much difficulty was encountered in obtaining manure uncontaminated by other bacteria, etc. The first plan tried was to go to the barn in the morning while the animals were lying down, and obtain the manure as they were made to rise. Usually we were able to obtain a few samples by this method, but much difficulty was experienced in getting samples from the desired animals.

The next method tried was to first wash the anus and surrounding parts with soap and water, then disinfect with alcoholic bichloride, 1-500. The excess of bichloride was wiped off, and a spoon shaped apparatus, see Figure 7, inserted into the rectum for about 10 inches. This was revolved around once or twice and withdrawn. Usually we could obtain the requisite amount of manure by one or two insertions of the spoon. The manure was then scraped from the spoon into a sterile test tube with a glass rod. These samples were taken to the laboratory, smear preparations made from them to stain for tubercle bacilli, and the remainder was used for making a suspension.

During the early part of the experiment 10 c. c. of sterile water was poured into the test tube and about 2 grams of manure triturated with it. The manure was then allowed to settle and a few cubic centimeters of the fluid drawn off by means of hypodermic syringe. This method was found unsatisfactory on account of the large amount of suspended material. We therefore decided to filter it through cotton. In order to do this, the following method was devised: Seven-eighth inch test tubes were heated over a large Bunsen burner until the bottom could be drawn out to a point. The point was then broken, and a small piece of cotton put into the tube and pushed down into the drawn out point. The top of the tube was plugged with cotton and the tube sterilized in the dry air sterilizer at 140-160 degrees C. The suspension of sterilized water and manure was then poured into this funnel and allowed to filter down into another test tube as shown in Fig. 6. By this method it was found that the liquid passed through rapidly and all coarse particles were removed so that the filtrate could be drawn up into a hypodermic syringe through the needle without causing obstruction.

During the early part of the work 5. c. c. of this suspension was injected subcutaneously into guinea pigs, but

TABLE OF ANIMAL INOCULATIONS AND MICROSCOPIC EXAMINATIONS.

Animal's Number	Animal's Name	G. P. Inoculation		Death		Microscope	G. P. Inoculation		Death		Microscope	G. P. Inoculation		Death		Microscope	G. P. Inoculation		Death		Microscope	G. P. Inoculation		Death		Microscope
		Date	Dose	Date	Cause		Date	Dose	Date	Cause		Date	Dose	Date	Cause		Date	Dose	Date	Cause		Date	Dose	Date	Cause	
		1905	C. C.	1905			1905	C. C.	1905			1905	C. C.	1905			1905	C. C.	1905			1905	C. C.	1905		
1	Golden Prince.	Mar. 11	2.	Mar. 13	Sept.	—	Mar 16	2	July 6	P. M.—	—															
2	Black Bess.	" 16	2.	July 6	P. M.-	—	May 17	1.	" 27	P. M.—	—	July 3	.5	Oct 12	P. M —											
2½	Rosella of Glendale	April 26	2.	" 6	Secondary Inoc. Made P.M. † Peritonitis	—	" 17	1	" 20	†††	—	July 3	.5	Sept 2	P.M.†††		July 27	5	Oct 4	Tuberc.	—					
3	Hawthorne I.	Mar. 25	2	May 26		—																				
4	Bonnie Wild Rose	May 24	1	July 17	P. M.—	—						Sept 26	5	Dec 1	P M.-		Aug. 4	.5	Oct 12	P. M.—		June 30	.5	Oct. 12	P. M.—	
4½	Golden Queen.	April 26	2.	May 5	Sept.	—	May 12	1	May 14	Sept	—	May 17	1	July 26	" —	-	June 30	.5	July 31	P. M.—		Sept. 26	.5	Dec. 5	P. M.—	
5	Dairy A.	Mar 25	2	Mar 27	"	—	April 1	2.	April 5	"	—	April 6	2	April 9	Sept	-	May 12	2.	May 16	Sept	—	May 17	1.	July 6	" —	—
													1	" 16	"	-										
5½	Dairy B.	" 25	2	" 26	"	—	" 1	2	" 5	"	—	" 6	2	" 8	"	—	" 12	2.	" 16	"	—	" 24	1.	May 26	Sept.	—
6	Gipsy Girl 2nd.	May 26	1	May 29	Sept.	—	June 6	1.	June 7	"	—	June 20	.5	July 24	P. M.-		Sept 26	.5	Dec 5			Aug. 4	.5	Oct. 12	P. M.—	
7	Nellie Keillor 2nd.	" 26	1.	July 20	P. M.—	—	Aug. 4	.5	Nov 18	P. M —		" 30	5*	Nov. 18	P M -											
7½	Mint Drop 8th	May 26	1	Oct. 12	P. M.—	—	July 3	5	Oct 12	P. M —	—	Aug 4	.5	" 18	P M -											
8	Bonnie Wild Rose 2nd	May 24	1.	May 26	Sept.	—	July 3	.5	July 12	Sept		May 31	1.	July 26	P M.- -	—	Sept 26	5	Dec. 4	P M.—		Aug. 4	.5	Nov. 18	P. M.—	
8½	Poppy	" 24	1	" 25	"	—	May 26	1.	May 27	"	-	June 6	1	June 11	Sept.	—	June 20	5	July 24	P M —		" 4	.5	" 18	P. M.—	
9	Topsy.	Mar 16	2.	July 6	P. M.—	—	" 24	1	July 20	P M —	—	July 3	.5	Nov 18	P. M —											
9½	Winsome Beauty	May 24	1	May 24	Sept	—	" 26	1	May 27	Sept	—	May 31	1	July 19	" —	—	July 3	5	July 8	Sept		Sept 26	.5	Dec. 8	P. M.—	
10	Royal Lake Edward	June 6	1.	June 7	"	—	Sept. 26	.5	Nov 24	P M.—		Aug 4	5	Oct 12	P M —	—	June 20	1.	Oct. 12	P M —	—					
11	Euroma 4th.	April 13	2	July 6	P M.—	—	Mar 10	2	July 6	" —	—															
12	Houston	Mar 10	3.	" 6	" —	—	April 13	2	" 6	" —	—															
13	Hellen.						Mar 13	2	" 6	" —	—															
5	Dairy A.	April 20	2.	" 6	P M.—	-											April 12	2	July 6	P. M —	—					
5½	Dairy B.	" 20	2.	" 6	" —	—																July 3	.5	Sept. 21	Septico-pyemia	
6½	Hawthorne II.	—	—	—	—	—	May 26	1.	July 30	P. M.—	—	June 30	5	Nov 18	P M —		Aug 4	.5	Nov 18	P M.—						
5½	Dairy B.	May 24	1.	May 26	No.P.M.	—	May 31	1	Oct. 12	P M —																
8½	Poppy	Sept. 26	.5	Dec 8																						
0	Rose H..	Nov. 25	.5	" 16	Pneumonia																					
9½	Winsome Beauty	Aug. 4	.5	Oct. 12	P. M.—																					

Key to Table: —No Tuberculosis. †–Tuberculosis slight. ††–Moderate. †††–Extensive. G. P.—Guinea Pig. Sept.—Septicemia.

Animal's Number	G. P. Inoculation		Death		Microscope	G. P. Inoculation		Death		Microscope	G. P. Inoculation		Death		Microscope	G. P. Inoculation		Death	
	Date	Dose	Date	Cause		Date	Dose	Date	Cause		Date	Dose	Date	Cause		Date	Dose	Date	Cause
	1905	C. C.	1905			1906	C. C.	1906			1906	C. C.	1906			1906	C. C		
27	Nov. 24	.5	Dec. 15	P. M.—		Feb. 12	.5	June 7	P. M.—		April 28	.5	July 9	No P.M.		Aug. 16	.5	Nov. 14	P. M.–
28	" 24	.5	" 14	Sept.		" "	"	" "	" "		" "	"	April 8	P. M.—					
29	" 24	.5	1905 Dec. 21	P. M.—		" "	"	June "	" "		" "	"	July 24	" —					
30	" "	.5	" 22	" "		" "	"	April 26	Pneumonia		April 28	.5	April 29	Sept.		" 16	"	" "	" "
31	" "	.5	" 3	Sept.		" "	"	June 7	P. M.—		" "	"	" "	"		" "	"	" "	" "
32	" "	.5	1906 Jan. 3 1905	P. M.—		" "	"	" "	" "		" "	"	" "	"					
33	" "	.5	Dec. 3	Sept.		" "					" "	"	" "	"					
34	" "	.5	" 5	"							" "	"	" 8	P. M.—		" "	"	Aug. 18	Sept.
35	" "	.5	" 2	"		Feb. 12	.5	June 7	P. M.—		April 28	.5	April 30	Sept.		" "	"	Nov. 14	P. M.–
36	" "	.5	" 18	P M.—		" "	"	April 18	Pericarditis		" "	"	" 8	P. M.—					
37						" "	"	May 1	P. M.—		April 28	.5	May 3	Sept.		" "	"	Sept. 4	" "
38	Nov. 24	.5	Dec. 6	Sept.		" "	"	June 7	" "							" "	"	Nov. 16	" "
39	" "	.5	" 23	P. M.—		" "	"	May 26	Pneumonia		April 28	.5	May 1	Sept.					
40	" "	.5	" 22	" "		" "	"	" 2	Internal Hæmorrhage		" "	"	" "	"					
41	" "	.5	" 8	Sept.		" "	"	June 7	P. M.—		" "	"	" 2	"		" "	"	Aug. 18	Sept.
42	" "	.5	" 13	P. M.—		" "	"	" "	" "		" "	"	" 1	"		" "	"	Nov. 14	P.M.–
43	" "	.5	" 1	Sept.		" "	"	May 18	Pneumonia		" "	"	April 29	"		" "	"	" "	" "
44	" "	.5	" 21	P. M.—							" "	"	April 8	P. M.—		" "	"	" "	" "
45	" "	.5	" 25	" "		Feb. 12	.5	June 7	P. M.—		April 28	.5	April 30	Sept.					
46	" "	.5	" 27	" "		" "	"	" "	" "		" "	"	" 8	P. M.—					
47	" "	.5	1906 Jan. 27 1905								" "	"	" "	" "					
48	" "	.5	Dec. 12	P. M.—		Feb. 12	.5	June 7	P. M.—		April 28	.5	May 6	Sept.		" "	"	" "	" "
49																			
50						Feb. 12	.5	May 29	Pneumonia										

in all cases they died with septicaemia. We then decided to reduce the size of the dose, and it was not until we had reduced it to .5 c. c. that we succeeded in getting a reasonable number of guinea pigs to live. Occasionally small lesions that somewhat resembled tuberculosis were found present in guinea pigs that were killed for post mortem purposes or that died. When these conditions were encountered other guinea pigs were inoculated from the suspected tissue or pieces were fixed, inbedded, cut, and stained to see if tubercle bacilli could not be demonstrated.

A study of the above table shows that one animal, Rosella No. 2½ (2.08%), was found to pass manure that contained virulent tubercle bacilli. It is to be observed also that this animal did not pass such bacilli in large enough number that they could be found by microscopical examination, although they were present in sufficient abundance to produce tuberculosis in the experimental animals by inoculation.

Nov. 8, 1905, this cow, No. 2½, was killed (see Autopsy Records, page 53) and guinea pigs were inoculated from the filtrate obtained by mixing 5 c. c. of water with 1. gram of the contents of the paunch, small intestine, and rectum. The guinea pigs inoculated from intestinal and rectal material filtrates died in a short time from septicaemia, and hence gave no evidence in the matter of tuberculosis. The one inoculated from the paunch contents lived for about two weeks. Upon examination post mortem many small apparently caseous areas were found present in the liver. A guinea pig was inoculated from small pieces of the liver of this animal, but unfortunately died before it was time for tuberculous lesions to develop. From the lesions found in the original guinea pig inoculated from the paunch contents it was quite evident that it was affected with tuberculosis.

As will be observed upon reading the post mortem notes on this case (No. 2½) no intestinal ulcers were found. It seems quite safe to assume that the tubercle bacilli present in the manure came from the lungs by way of the gullet. Attention should be called to the fact that the lungs were badly affected containing abscess cavities. These cavities might easily furnish bacilli in enormous numbers to infect the paunch contents, which were proven virulent.

AUTOPSY RECORDS.

It should be said in explanation of the following meagre records, that the cattle were killed by Swift & Co., at South St. Paul, Oct. 31, 1905, under federal inspection, and that the killing was done so rapidly that there was scant opportunity for examination post mortem.

Hawthorn II. The Lungs, bronchial, and mediastinal lymph glands were badly affected with tuberculosis.

Gipsy Girl. The posterior lobe of the right lung was moderately involved.

Black Bess. The submaxillary lymph glands contained a considerable quantity of pus. The left lung was also slightly involved, and nearly all the thoracic lymph glands showed involved areas. The intestinal lymph glands were normal.

Poppy. Both lungs showed areas that contained cheesy pus. These areas were quite uniformly distributed throughout both lungs.

Bonnie Wild Rose. Both lungs were slightly affected. The mediastinal and bronchial glands were also involved.

Mine Drop VIII. No post mortem notes obtained.

Rosella, No. 2½. Aged Angus cow in prime condition, killed Nov. 8, 1905. (See Figs. 1, 2, 3, 4, 5.) The udder and supermammary glands are found to be unaffected. The superficial inguinal lymph glands are enlarged and contain many miliary tubercles, some of which are partly calcified.

The omentum is thickly studded with tubercles varying in size from that of a millet seed to that of a small chestnut. (See Fig. 2.) Along the line of attachment of the great omentum to the paunch there is a large mass of grape-like tubercles which is from 3 to 7 inches broad. and extends along the attachment for about 3 feet. A few small tubercles are present on the peritoneum covering the fat around the rectum and kidneys.

A large number of tubercles are noticed on the peritoneal surface of the liver. Upon cut surface, the liver tissue is found to be normal with the exception of an abscess about the size of a walnut in the apex of the left lobe. This abscess contains a small amount of thick pus encapsulated by a thick fibrous wall. The peritoneum covering the spleen contains many tubercles but the spleen

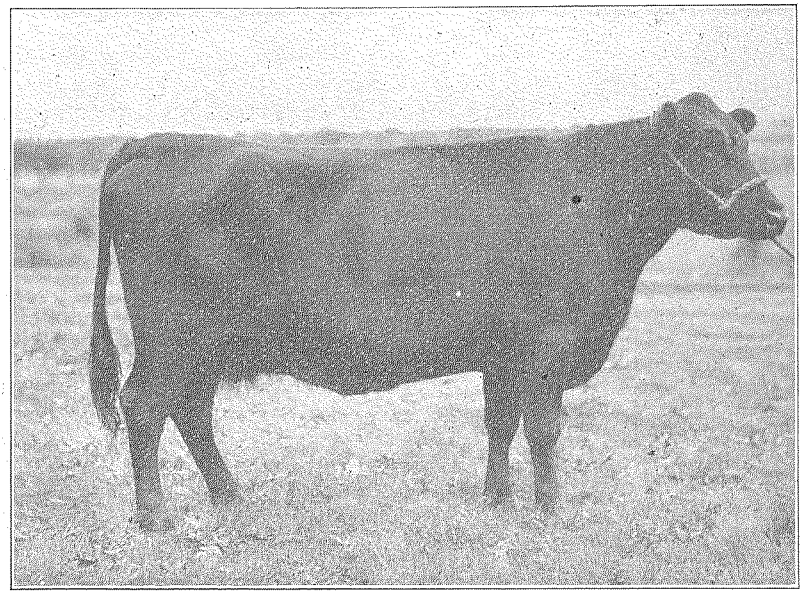


Figure 1.—ROSELLA, Animal No. 2½.
This animal passed virulent manure and also virulent nasal secretion.

tissue appears normal upon cut section. No ulcers of the intestinal mucosa are found upon a very careful examination of the entire intestine. Two glands in the colic mesentery are studded with miliary tubercles and one lymph gland near the ileocecal alve contains caseated areas. The kidneys are normal in appearance on the surface and upon the cut section. A few tubercles about the size of a small pea are scattered over the surface of the bladder and also over the peritoneal surface of the uterus. Several small grayish white nodules, which contain pus are observed in the mucosa of the horns and body of the uterus.

The right ovary contained an elongated cavity about 1¼ inches in length and ½ inch in diameter, filled with thin pus. Several small calcified tubercles are also observed.

The diaphragm is very thickly sprinkled with flattened tubercles from 1 mm. to 2 cm. in diameter.

One of the posterior mediastinal lymph glands is enlarged to about the size of a man's fist. Upon cut section

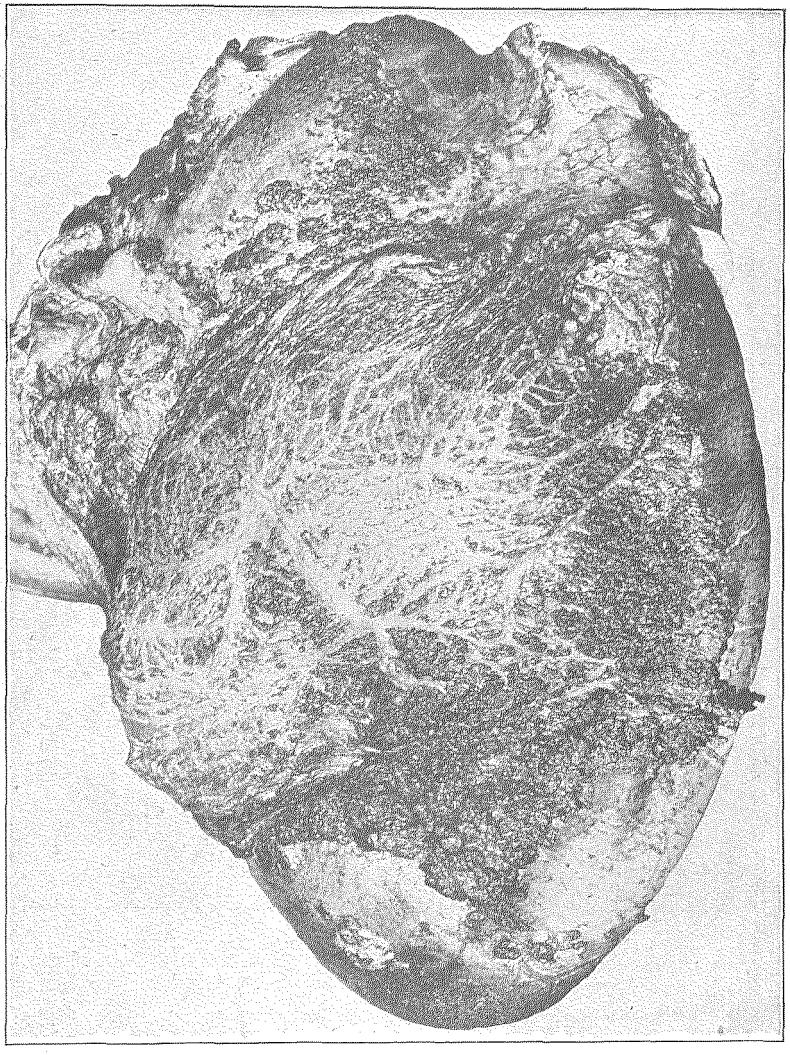


Figure 2.—PAUNCH AND OMENTUM OF THE COW, ROSELLA.
Note the extensive involvement. These tubercles vary in size from that of a millet seed to large masses.

it reveals a broken down caseous center which grates when cut. Two of the bronchial glands are enlarged to about the size of turkey's egg. Upon cut section they show a broken down caseous center which contains calcified granules. Glands around the supernumerary bronchus are not affected.

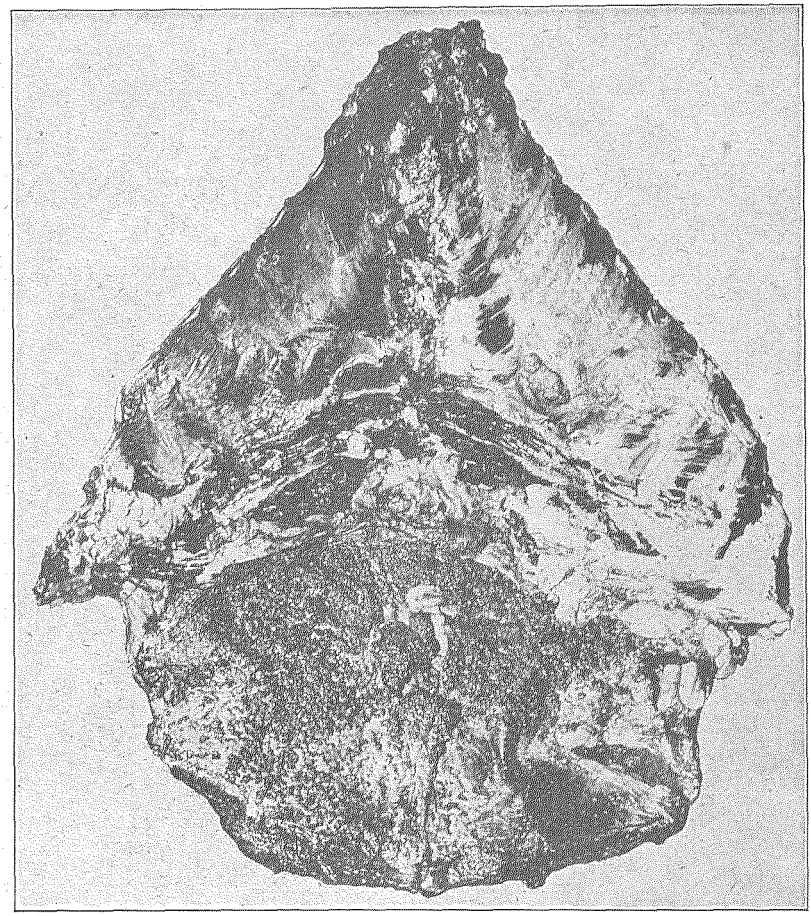


Figure 3.—Bottom of Chest and Abdomen of Rosella.
Note masses of tubercles in the latter cavity. The short sections of the ribs will enable the reader to locate these parts.

Both lungs contain many abscesses varying in size from that of a chestnut to that of an egg. Some of these contain thick, viscid pus, while others were caseous and partly calcified.

Both retro-pharyngeal lymph glands contain caseous areas.

Submaxillary lymph glands are normal.

The peritoneal lining of the abdominal wall is thickly studded with tubercles, particularly on the left side and the floor. (See Fig. 4.)

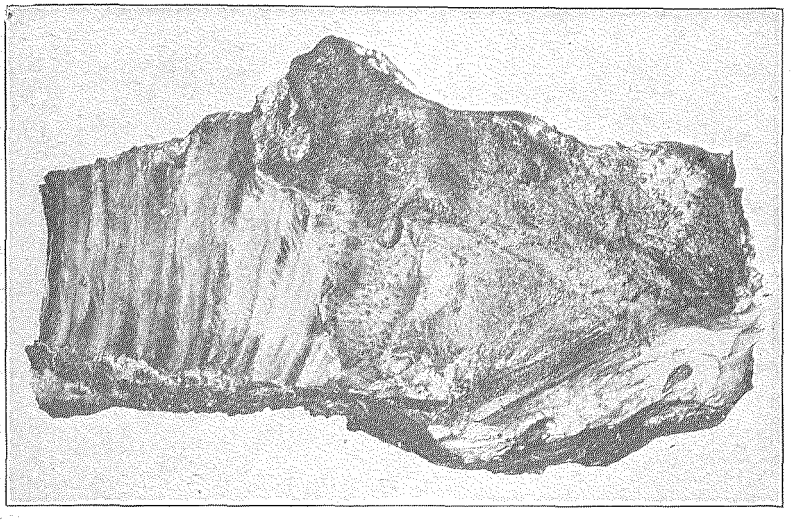


Figure 4.—Section from the Chest and Abdominal Wall of Rosella Showing Extensive Disease.

Hawthorne I. No. 3. Aged grade shorthorn ,calved March 14, 1905. Did well until March 18, when a slight swelling of entire udder was noticed. The cow was first examined March 19, with following result: Temperature 107, pulse rapid, respiration rapid and shallow. Posterior half of udder much swollen and hard.

Diagnosis, Garget. Temperature remained at 107 for several days and then dropped to 103. Bowels loose, udder hard, no improvement. March 23, a thin, dark, bloody discharge from right hind teat. Died during the forenoon of March 24.

Autopsy, March 24, 1905.

Autopsy.—Upon opening the thoracic cavity, the mediastinum was found covered with tubercles varying in size from that of a millet seed to that of a pea. The cephalic lobe of the right lung was badly affected. The left costal and viceral pleurae contained many miliary tubercles. The diaphagmatic pleura also showed many small tubercles.

The liver tissue was of a brownish yellow color. This was probably due to bile infiltration as other tissues exhibited a similar color. The gall bladder was very much enlarged, and contained about two quarts of greenish yellow bile. The spleen pulp was very soft, and of an almost

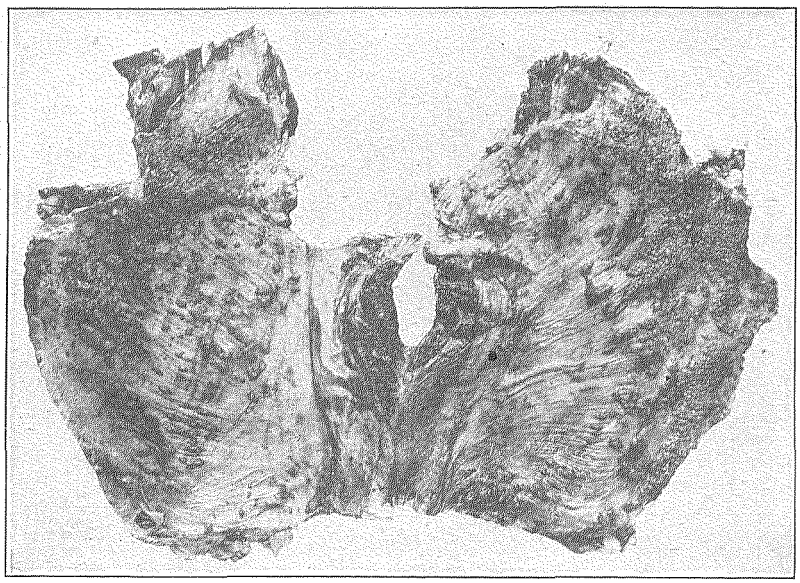


Figure 5.—Diaphragm of Rosella.
Note tubercles.

semi-liquid consistency. The stomach and intestines were apparently normal.

Winsome Beauty. Autopsy Nov. 3, 1906. Aged shorthorn. The intestines were badly decomposed as the animal had been dead for about 36 hours and it was therefore thought unwise to cut them open and make examination for ulcers. The intestinal lymph glands were all normal in appearance. The liver and stomach were also apparently normal. Both kidneys were very friable, and the parenchyma contained many gas bubbles. The left pleural side of the diaphragm, the costal, and visceral pleurae were thickly studded with tubercles varying in size from that of a No. 6 shot to that of a pea. The post mediastinal lymph glands were completely decomposed, but some caseous material was found in the vicinity of the posterior mediastinum, so it seems fair to suppose that they may have been tuberculous. There was also a very marked acute pleurisy on both sides of the cavity.

The parietal and visceral pericardia were adherent to each other. A small amount of effusion was also present in the pericardial sac.

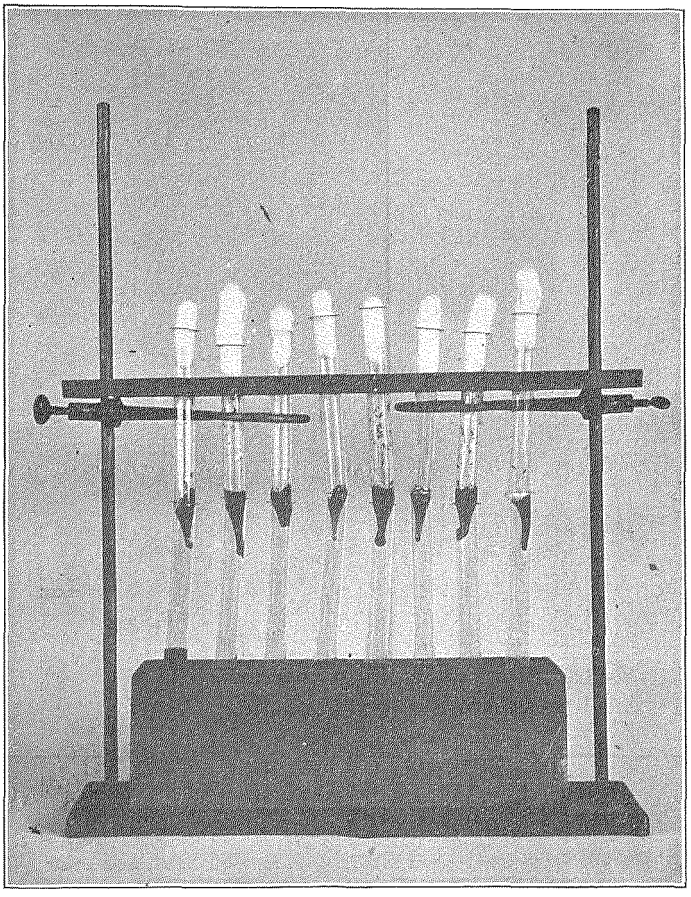


Figure 6.—Method of Filtering the Manure Suspension.

VIRULENCE OF NASAL SECRETION.

In view of our almost negative results in studying manure of tuberculous cattle as to virulence, only one cow being shown to pass virulent manure out of 45 cows examined, the question arose how then were these remaining 44 cows passing off tubercle bacilli, if at all? Coughing was not conspicuous in either herd, and yet here were 45 tuberculous cows, some of which, judging from common experience, must have been excreting virulent tubercle bacilli. It is a matter of common observation that given tuberculous cattle confined in stable with others, a large number

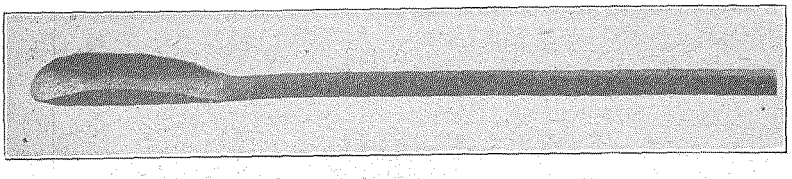


Figure 7.—Rectal Spoon.

of mature animals may become infected during a single winter, and it has been shown that outdoor transference of infection is not by any means impossible.

In view of these considerations it seemed probable that there must be some other method by which tuberculous cattle commonly pass off the bacilli of tuberculosis aside from coughing and in milk. We therefore decided to undertake a study of the nasal secretion. This seemed a quite plausible source of infection in view of the very common habit which cows have of licking their feed boxes, mangers, and each other's faces.

Tuberculous cattle were available at the university farm for this purpose, and the work was undertaken which is here briefly reported.

In conjunction with the work on manure, inoculations were also made from the nasal mucous secretion. Material was obtained in the following manner: Absorbent cotton was sterilized and small pieces of it were grasped with long sterilized forceps and pushed up into one of the nasal cavities of the animal for 2 or 3 inches, while its head was held by an assistant. Usually the animal would become very much excited and breathe rapidly and forcibly, which tended to expel adherent secretion so that usually a sufficient quantity of material would be obtained in this manner.

In most cases, material was obtained from both nostrils. The cotton was then removed from the forceps and placed in a sterile test tube. Five c. c. of water was poured into each tube. The tube was thoroughly shaken, and the cotton removed. Two c. c. of this suspension was injected subcutaneously into guinea pigs. The guinea pigs were allowed to live at least six weeks, and were then killed by chloroform, and examinations post mortem held.

Inoculations were made from the nasal mucous secretion once from each of the following animals, 14 in number:

Nos. 2½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½ and 13. Two inoculations were made from No. 2½ with positive results each time. All other inoculations were negative. This shows a very small percentage, only 6.6 %.

SUMMARY, AND COMPARISONS OF RESULTS.

It will be obsered that we have had 45 animals under experiment. Of these, we have tested for virulence the manure of 16 cattle three times; 1 cow 5 times; 2 cows 4 times. The feces of 14 animals were tested for virulence 2 times, and 7 animals were tested for virulence once. Of the entire lot but one, Rosella, of Glendale, feces experiment 2½, gave virulent manure, and it is intesesting to note that in this case the nasal secretion was uniformly virulent also.

Nasal secretion of 14 cattle was tested for virulence, and but one, the same cow which gave virulent feces, was found to give a virulent nasal secretion. This seems somewhat remarkable in view of the considerable number of tuberculin reacting cattle whose manure and nasal secretion were examined in our work, and the apparently conflicting results obtained by others quoted.

The results obtained by Schroeder and Mohler[1] and Schroeder and Cotton[2], see page 46, seem to differ very radically from results which we have obtained in similar work. There does not appear to be any features in the reported work to account for such radical differences. Either our cattle were remarkably free from lesions which would result in contamination of the manure, or Schroeder and Mohler, and Schroeder and Cotton experimented with animals in which such lesions were quite common. These experimenters found that a large proportion of their seven experimental animals gave virulent manure and none of them virulent nasal secretion.

The manure of 45 tubercular cattle was tested for virulent baçilli by the methods described. One animal (2.08%) gave positive results. Nasal secretion of 14 was tested for virulence as described in previous pages. One of these (6.6%) gave positive results, this being the same cow which gave virulent manure.

Schroeder and Cotton[2] found tubercle bacilli by microscopic examination in the manure of 5 of 7 tubercular

cattle. Of these 5, three cattle passed manure which was proven virulent by inoculation.

In view of our widely differing results, it is highly important that more work be done along this line for it is a serious problem.

It is evident that virulent bacilli may pass through the entire alimentary canal and remain virulent. In the one case where we found a cow passing virulent manure, we also found virulent bacilli in the paunch. This has been even more positively shown in the work of Schroeder and Mohler[1] and Schroeder and Cotton[2].

If a considerable percentage of tuberculous cattle are passing virulent bacilli in the manure, then this method of dissemination is a serious one for both bovine and human.

If but few cows pass virulent manure, it is important to remember that those few are dangerous and seriously objectionable factors in any herd.

Until further information is available, and considering the disastrous results which have been shown to be possible from virulent manure, it becomes evident that tuberculin reacting cattle should not be allowed to remain in any stable from which milk is taken for food purposes or where they are associated with healthy cattle, even though the milk of the reacting cows be discarded.

Microscopic test for the presence of tubercle bacilli in manure is not reliable. We were unable to demonstrate them in the case of cow No. 2½ though this cow was passing virulent manure. Nor does it appear feasible at present to differentiate by microscopic examination between the bacilli of Johne's disease, or other acid-fast bacilli resembling tubercle bacilli, and virulent bovine tubercle bacilli.

The method of inoculation by injection of filtrate from a suspension of manure is fairly satisfactory as described. Five-tenths c. c. (.5 c. c.) of such filtrate appears to be approximately the maximum dose which it is practicable to use on account of the development of septicaemia in the inoculated animals.