

dations by the various groups interested in milk control, it will have been worth while.

It is believed by the training station personnel and the State health department that a milk sanitarian working in an official health department should know the other activities of the department as well as milk control work. Therefore it is not intended to

enroll sanitarians from official health departments in the 4-week course for dairy fieldmen. Sanitarians in official departments, even though they may be working with milk control, should take the standard 3-month Environmental Sanitation Course. The short course is planned only for milk industry personnel working in the field of milk control.

Histoplasmin Sensitivity among Animals in Central Missouri

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Since 1939, when DeMonbreun (1) first described histoplasmosis in a dog, the disease has been found to involve the cat (2), house mouse (2), rat (2), skunk (2), bear (3), opossum (2), colt (4), and cow (5). Thus far, however, the prevalence of the disease in animals has not been determined. This report presents probable prevalence data in the form of histoplasmin sensitivity rates among cattle, sheep, horses, swine, and fowl for Boone County, Mo. This county is located in the central portion of the State. An analysis of the histoplasmin reactors among the various animals is presented, and a comparison of the histoplasmin sensitivity rates is given. Animal cases of histoplasmosis in Boone County also are mentioned.

MATERIALS AND METHODS

The histoplasmin sensitivity rates were determined by the use of the histoplasmin skin test. The method of testing the various animals has been described previously (6). Histoplasmin lots (KC) 3 and 4 undiluted were used as antigen for all the animals tested.

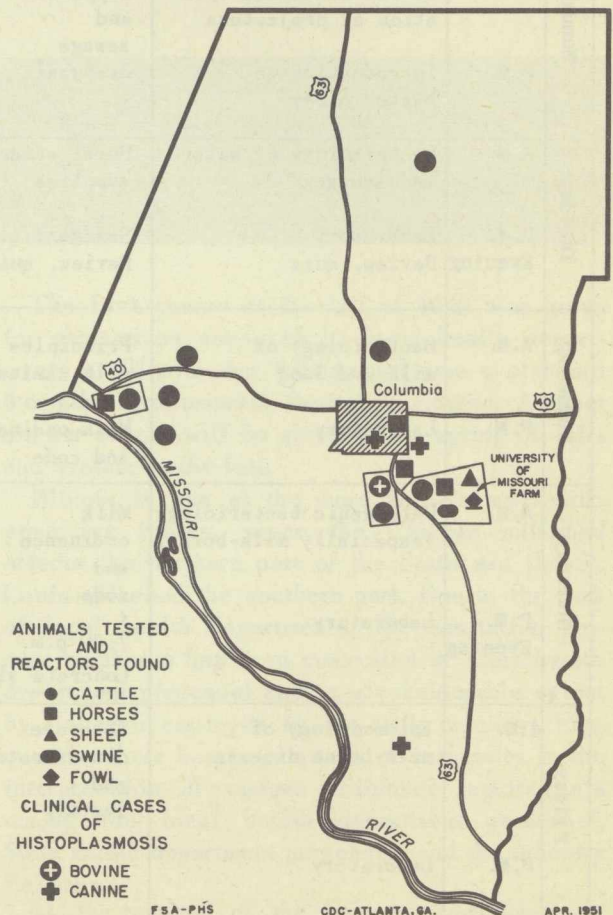
The majority of the animals tested were lifetime residents of Boone County. The location of the farms where the animals were tested is shown in figure 1. A variety of animals were skin tested at the University of Missouri farm, and in addition, cattle were tested at seven farms in the county, and horses were tested at one farm and at two riding stables.

HISTOPLASMIN SENSITIVITY RATES AMONG THE VARIOUS ANIMALS

Cattle. Early work with cattle by Furcolow and

Ruhe (7) showed 4.2 percent histoplasmin reactors in the eastern third of Kansas, 1.5 percent reactors in the central third, and no reactors in the

Figure 1
LOCATION OF ANIMALS TESTED AND
CASES OF HISTOPLASMIOSIS
BOONE COUNTY, MISSOURI



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western third. In one eastern county of Kansas they found that the histoplasmin sensitivity rate increased with age. Cattle under 2 years of age showed 1.8 percent histoplasmin reactors, and cattle 6 and 7 years of age showed 12.5 percent histoplasmin reactors. Many of the reactors were dairy cattle that had been hand milked. A total of 1,909 cattle has been tested in Kansas, and 60 (3.1 percent) histoplasmin reactors found. The cattle were tested on 123 farms, and histoplasmin reactors were found on 34 (27.6 percent) of them.

In Boone County, Mo., the histoplasmin sensitivity rate among cattle was found to be much higher than in Kansas. A total of 382 cattle was tested, and 60 (15.7 percent) histoplasmin reactors found. As in Kansas, the histoplasmin sensitivity rate increased with age. Cattle under 2 years of age showed 2.9 percent histoplasmin reactors, and cattle 6 and 7 years of age showed 33.3 percent histoplasmin reactors. The youngest histoplasmin reactor was a 10-month-old Brown Swiss heifer.

Among the cattle tested there were 349 females — 106 heifers, 191 cows, and 52 calves; and 33 males — 1 steer, 11 bulls, and 21 calves. Since only a few males were tested, no conclusions regarding sex could be reached.

The breeds of cattle tested included Holstein, Jersey, Guernsey, Brown Swiss, Brahma, Aberdeen-Angus, and Hereford. In general, the dairy breeds had higher histoplasmin sensitivity rates than the beef breeds.

Sheep. The first indication that sheep might be involved occurred when six ewes and one ram associated with a proved human case of histoplasmosis were skin tested. Five (71.4 percent) of the seven sheep were histoplasmin reactors. This indicated that sheep might have a high histoplasmin sensitivity rate. In addition, among 39 blood samples from feeder lambs tested using the complement fixation test for histoplasmosis (8), one showed a four plus reaction, one a three plus reaction, and one a two plus reaction. The remaining serums were negative. The feeder lambs were from feeder yards at Omaha, Nebr.

Sheep in Boone County were found to have a high histoplasmin sensitivity rate. A total of 185 sheep was skin tested and 44 (23.7 percent) histoplasmin reactors found. As in cattle, the histoplasmin sensitivity rate increased with age. Sheep under 2 years of age showed 1.5 percent histoplasmin reactors, and sheep 6 and 7 years of age showed 44.1 percent histoplasmin reactors. The

youngest histoplasmin reactor was a 6-month-old (female) lamb.

Among the sheep tested there were 163 females — 140 ewes and 23 lambs; and 22 males — 6 rams and 16 lambs. Since only a few males were tested, no conclusions regarding sex could be reached.

The breeds of sheep tested were Hampshire, Southdown, Shropshire, Dorset, and Western. At age 4 and 5 years, the rates among the Hampshire, Southdown, and Shropshire sheep were quite similar. The data concerning breed, however, were too limited to reach any definite conclusions.

In view of these findings, it appears that cases of histoplasmosis among sheep may be found.

Horse. It is interesting to report that the histoplasmin sensitivity rate among horses in Boone County was exceedingly high. Among 69 horses tested there were 45 (65.2 percent) histoplasmin reactors. As in the other animals, the rate increased with age, although only a few horses under 6 years of age were tested. Horses under 6 years of age showed 50.0 percent histoplasmin reactors, and those 8 years and over showed 73.7 percent histoplasmin reactors.

Most of the horses tested were saddle horses. In addition, some draft-type (Percheron) horses were tested, and a few mules. Among 54 saddle horses tested there were 35 (64.8 percent) histoplasmin reactors. Of 11 Percheron horses tested, 6 (54.5 percent) were histoplasmin reactors; 4 mules were tested and 4 (100 percent) were histoplasmin reactors.

Among the 69 horses, there were 30 males and 39 females. Of the 30 males, 21 (70.0 percent) were histoplasmin reactors, and of the 39 females, 24 (61.5 percent) were histoplasmin reactors.

Thus far only one case of histoplasmosis has been reported in the horse. These findings indicate that the disease is probably much more prevalent among horses than the present records would indicate.

Swine. Histoplasmin reactors also have been found among swine. Nine swine associated with a proved human case of histoplasmosis were skin tested and five (55.6 percent) were histoplasmin reactors.

In Boone County a total of 129 swine was skin tested and 2 (1.5 percent) histoplasmin reactors found. Age again was shown to be an important factor. Of the 129 swine, there were 29 (22.4 percent) under 6 months of age; 98 (76.0 percent) were 1 year old, and 2 (1.6 percent) were 2 years old. The two histoplasmin reactors were found

among 1-year-old Duroc Jersey swine. Both reactors were sows.

Among those tested were 83 sows, 16 boars, 1 stag, and 29 pigs (23 males, 6 females). The breeds included were Duroc Jersey, Hampshire, Poland China, Yorkshire, and Landrace.

Since swine are sent to slaughter at an early age, it probably will be difficult to obtain a true picture of the histoplasmin sensitivity rate according to age among swine.

Fowl. Among the fowl skin tested in Boone County were chickens and turkeys. One reactor (1.0 percent) was found among 98 chickens. Most of the chickens, 83 (84.6 percent), were under 2 years of age. The rate for this age group was 1.2 percent. The histoplasmin reactor was a 1 1/2-year-old Rhode Island Red hen.

Among the breeds of chickens tested were White Leghorn, Hampshire, White Rock, and Rhode Island Red. There were 92 females and 6 males among those tested.

The turkeys tested were 5-month-old Broad-breasted Brauns. Of the 25 tested, there were 21 females and 4 males. No histoplasmin reactors were found.

Fowl present the same problem as swine. They also are slaughtered at an early age, and it will probably be difficult to obtain histoplasmin sensitivity rates according to age.

DISCUSSION

A comparison of the histoplasmin sensitivity rates according to age of cattle, sheep, horses, swine, and fowl in Boone County, Mo., is given

in table 1. The rates for horses are markedly higher than those for cattle and sheep. The rates for cattle and sheep are somewhat similar. The low rate for cattle at the ages of 4 and 5 years cannot be explained.

In the under-2-year age group, the rates are quite low, and somewhat similar for sheep, swine, and chickens. The rate for cattle, although low, is slightly higher than the rates for the other animals. The similarity of the histoplasmin sensitivity rates appears to indicate that all animals may be infected from a common source.

It is interesting to note that before the histoplasmin skin testing survey was completed, cases of histoplasmosis were found. Among these were three canine cases and one bovine case (5). The location of these cases in Boone County is shown in figure 1.

SUMMARY

Histoplasmin sensitivity rates among cattle, sheep, horses, swine, and fowl in Boone County, Mo., are presented. The rates for horses were found to be markedly higher than those for cattle and sheep, and the rates for cattle and sheep were found to be somewhat similar. In the under-2-year age group the rates for most of the animals were quite low and somewhat similar.

The findings suggest that histoplasmosis may be prevalent among domestic animals, and that cases of histoplasmosis may be found among cattle, sheep, horses, swine, and fowl.

Table 1
ANIMAL HISTOPLASMIN SENSITIVITY
RATES ACCORDING TO AGE IN BOONE COUNTY, MO.

Animal	Age in Years																	
	Under 2			2 and 3			4 and 5			6 and 7			8 and Over			Total		
	No.	Pos.	%	No.	Pos.	%	No.	Pos.	%	No.	Pos.	%	No.	Pos.	%	No.	Pos.	%
Cattle	137	4	2.9	102	21	20.6	73	9	12.3	45	15	33.3	25	11	44.0	382	60	15.7
Horse*	2	0	0	5	4	80.0	9	4	44.4	15	9	60.0	38	28	73.7	69	45	65.2
Sheep	66	1	1.5	22	4	18.1	22	5	22.7	68	30	44.1	7	4	57.1	185	44	23.7
Swine	127	2	1.6	2	0	0	0	0	0	0	0	0	0	0	0	129	2	1.5
Chicken	83	1	1.2	10	0	0	4	0	0	1	0	0	0	0	0	98	1	1.0
Turkey	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0
Totals	440	8	1.8	141	29	20.6	108	18	16.7	129	54	41.9	70	43	61.4	888	152	17.1

* Data for the horses under 6 years of age were combined due to the small number tested. The total tested under 6 years of age was 16, with 8 positive (50 percent).

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Enrichment of Loeffler's Medium with Glycerol

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During the early years of World War II diphtheria became fairly prevalent in the high, mountainous regions near Rio de Janeiro, Brazil. Under the direction of Dr. Bretz, the Public Health Laboratory in Petropolis, some 20 km. from the capitol, was faced with the necessity of rapidly arranging laboratory facilities to aid in diagnosis, epidemiology, and control of the disease.

The preparation of Loeffler's medium presented a serious problem because there was no available source of the necessary quantities of serum. In cast about for an appropriate substitute, it occurred to Dr. Bretz that Petragnani's medium (minus the dye) might serve, as it was in good supply, made of readily available materials, and of what might be supposed to be adequate nutrient substances. A small quantity of the medium was therefore prepared without dye and, after slanting and sterilizing was inoculated with a number of different strains of *Corynebacterium diphtheriae* in pure culture. The organisms grew luxuriantly, in 18 hours at 37° C. The results of microscopic examination far exceeded expectations. The organisms, especially when stained by the Albert-Layburn method, presented all of the well-known,

distinctive pleomorphism of *C. diphtheriae*, and the bars and granules were exceedingly large, distinct, and numerous. Other organisms, common in throat cultures (diphtheroids, cocci, and others), were also cultivated on the medium and found to be readily distinguishable from true diphtheria bacilli. In mixed cultures they presented no difficulty or confusion in microscopic diagnosis. This medium and the Albert-Layburn stain were, therefore, adopted for use at Petropolis in the diagnosis of diphtheria, and for some time proved very valuable. The modified Petragnani's medium appeared to be as good as Loeffler's medium.

In 1944 the senior author had an opportunity to continue his studies in Baltimore at the Johns Hopkins School of Hygiene and Public Health, Department of Bacteriology. Large numbers of cultures, both pure and "field," were available for study, as well as an abundance of patients since an epidemic was in progress in a nearby school. It soon became evident that Petragnani's medium, in conjunction with the Albert-Layburn stain, was a valuable diagnostic tool. This medium, however, is expensive and time consuming in preparation.

Because of the usually good morphological differentiation of *C. diphtheriae* on the Petragnani medium, a series of simple experiments was under-

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