

SHOPE'S FIBROMA IN ILLINOIS COTTONTAILS

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Shope's fibroma in cottontail rabbits, *Sylvilagus floridanus*, has been found frequently in states east of the Mississippi (Herman, Kilham, and Warbach, 1956) but to our knowledge, this is the first time it has been reported from Illinois. Although experimental transmission of the virus by fleas, (Kilham and Woke, 1953) mosquitoes, (Dalmat, 1959; Dalmat and Stanton, 1958; and Kilham and Woke, 1953) reduviid bugs, (Dalmat, 1959) and bedbugs (Dalmat, 1959) has been accomplished, the life cycle, reservoir and vector complex of the natural disease are not yet known.

To gain a better understanding of potential vectors and other aspects of the epizootiology of fibromatosis, an investigation of the prevalence of fibromas in cottontails from two different habitats was undertaken. The first habitat (in Allerton Park near Monticello, Illinois) was basically sylvan, with thick woods and fallow fields, but with no cultivated land. The second habitat was basically campestral, composed of agricultural fields almost completely devoid of woody cover. It consisted of cultivated fields in Piatt and three nearby counties.

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MATERIALS AND METHODS

This study was carried out between March 1956 and February 1960.

Rabbits from the sylvan habitat were trapped and shot, largely during the fall and winter months. Those from the campestral habitat were collected each month of the year from Champaign, Piatt, McLean and Ford Counties in central Illinois. A few rabbits from the same type of habitat were included from the southern part of the state. Those from the campestral habitat were collected entirely by shooting.

The fibromas were examined histologically and attempts were made to isolate viruses from them. Tumors from both live and dead rabbits were removed aseptically and triturated either in sterile broth containing 2500 units of penicillin and 2500 micrograms of streptomycin per 0.1 ml of inoculum, or in sterile broth without antibiotics; 0.1 ml of this material was inoculated intradermally into the base of the external ears or the scrotums of domestic rabbits (*Oryctolagus cuniculus*) and cottontails; 0.2 ml were inoculated onto the chorio-allantoic membrane of 9 to 11 day-old embryonating chicken eggs.

Portions of tumors from living and dead rabbits were fixed in 10%

TABLE 1.—Fibromas Found in Rabbits Collected from Sylvan Habitats.

Period	Rabbits Collected	Rabbits with Fibromas
March 1, 1956 to February 28, 1957.....	261	1
March 1, 1957 to February 28, 1958.....	344	0
March 1, 1958 to February 28, 1959.....	381	1
March 1, 1959 to February 28, 1960.....	268	7*
Totals.....	1244	9

Prevalence for Period 1956 to 1960: 0.72 per cent.

* Including one rabbit later in 1960.

formalin. Sections were made from these and examined histologically.

Serial passages of the virus were made in cottontails and domestic rabbits as well as in chicken embryos. Tumor materials and membranes from infected chicken embryos were stored at -10°C .

RESULTS

A total of 1,506 cottontail rabbits collected over a 4-year period from the campestral habitats yielded no fibromas. In contrast, 9 infected rabbits were found among 1,244 rabbits collected from the sylvan habitat in the same part of the state (Table 1). The disease was much more prevalent in 1959 than in previous years. A single case was found in 1956, another in 1958, while 7 were found in the winter of 1959-60. While more rabbits were collected from the campestral areas than from the sylvan areas, no fibromas were found in the campestral group.

A study of the seasonal prevalence (Table 2) shows that by far the greatest number of fibromas were found in the fall months. Eight were found during this period, while only one was obtained in the early winter and none in the late winter. The affected rabbits were about equally divided by sex (Table 3). Only one adult female had a fibroma; all others were found on juveniles. Most of the tumors were found on the feet or parts of the legs touching the ground (Fig. 1). One rabbit had a tumor on the left ear and another had one on the nose (Fig. 1). One rabbit had four tumors, two had two each, and the other six had one each.

Serum neutralization tests were carried out on domestic rabbits using convalescent serum kindly supplied by Dr. Richard Shope and also from our own infected rabbits. The supernatant fluid from triturated tumor material centrifuged at 1000 RPM for 10 minutes was allowed to

TABLE 2.—Seasonal Distribution of Fibroma Infections in Rabbits from the Sylvan Habitat.

Season	Rabbits Taken From						Totals	
	Woods		Fallow Fields*		Both Areas		Without Fibromas	With Fibromas
	Without Fibromas	With Fibromas	Without Fibromas	With Fibromas	Without Fibromas	With Fibromas		
								Per Cent Infected
March to July.....	40	0	40	0	80	0	0.00	
August to October.....	162	5	410	3	572	8	1.37	
November and December.....	0	0	408	1	408	1	0.24	
January and February.....	77	0	98	0	175	0	0.00	
Totals.....	279	5 (1.8%)	956	4 (0.4%)	1235	9	0.72	

* Uncultivated fields adjoining dense woods.

TABLE 3.—Age and Sex of Rabbits Affected with Fibroma Tumors and Anatomical Distribution of the Tumors.

Date	Sex	Age	Wt. (g)	No. Tumors	Part Affected
10/24/56	M	Juv.	1219	1	Left front foot
9/3/58	M	Juv.	1389	1	Foot
9/2/59	F	Adult	1276	1	Leading edge, left ear
9/24/59	M	Juv.	794	1	Right front foot
9/25/59	F	Juv.	1191	4	Both hind feet Right front foot Right front knee
10/1/59	1	Hind feet
10/7/59	F	Juv.	1361	1	Left hind foot
11/23/59	F	Juv.	1134	2	Left front leg Left hind leg
9/15/60*	F	Juv.	2	Nose Left front leg

* Collected after the main period of investigation, but included in 1959 and 1960 figures.



FIG. 1.—Natural Case of Shope's Fibroma in the Cottontail Rabbit. There is a lesion on the nose and another on the fore foot, which is held against the rabbit's body.

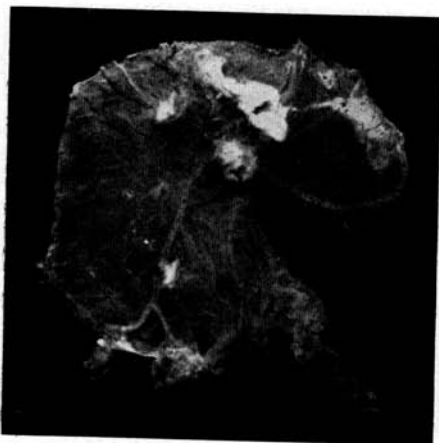


FIG. 2.—Plaques from Shope's Fibroma Virus on the Chorio-Allantoic Membrane of an Embryonating Chicken Egg. (From a color transparency.)

incubate at room temperature with an equal amount of serum for one and one-half hours after which 0.1 ml of the mixture was injected intradermally into the shaven sides of young domestic rabbits. An equal amount of the triturate diluted to the same extent with sterile saline was inoculated into the other side of the rabbit. In addition, rabbits of various ages were inoculated individually with the virus or with the virus-antigen mixture. Additional controls consisted of rabbits inoculated with normal rabbit tissue suspension. Both the homologous serum and serum supplied by Dr. Shope neutralized the virus as shown by the appearance of fibromas on



FIG. 3.—Shope's Fibroma on the Ear of Cottontail. The lesion above resulted from intradermal inoculation of triturated fibroma material into the ear of a cottontail and is typical of first, second and third passage lesions.

all sites where the virus alone was inoculated. No fibromas appeared where the antibody-antigen mixture, as described above, was inoculated or in those rabbits receiving the normal tissue-saline inoculation.

Histologic examinations were made of 2 field cases and three experimentally infected animals. In all cases tissue changes characteristic of Shope's fibroma were found. Numerous inclusion bodies were seen in epithelial cells of the tumor. The virus was transmitted easily to 11 day-old embryonating chicken eggs, but less easily to 9 day-old chicken embryos. Serial passages in 10 and 11 day-old embryonating eggs were made. The virus did not kill the embryos but both large and small plaques formed on the chorio-allantoic membranes (Fig. 2). The infection was readily transmitted to domestic and wild rabbits (Fig. 3). In young domestic rabbits the tumors appeared in 3 to 4 days, reached a peak in 2 weeks and regressed within another 2 weeks. Tumors in cottontails were visible at 8 to 10 days and did not regress for 2 to 3 months or longer. Tumors surgically removed from each species were examined histologically and found to be indistinguishable from those of naturally occurring cases although the gross appearance of the experimentally induced tumors was different from that of the natural lesions (Figs. 1 and 3).

The virus was readily transmitted from either natural or experimental lesions from the cottontail, but not from the domestic rabbit. When experimentally induced tumors were removed from wild rabbits they were quickly replaced by proliferation of

tumor material at the site, but this did not occur in domestic rabbits.

DISCUSSION

The natural mode of transmission of Shope's fibroma remains unknown, although a considerable amount of experimental work has been done on the problem. Analyses of the data, correlated with the findings reported here may give clues for further research. The largest previous investigation was done by the Rose Lake Wildlife Experiment Station in Michigan (Annual Reports, 1940-41, 1941-42, 1942, 1943, 1944, 1945). Lesions were found in 2.7% of 1071 rabbits examined between 1940 and 1945. Herman *et. al.* (Herman, Kilham, and Warbach, 1956) found 4.1% of 359 Maryland rabbits at the Patuxent Research Refuge involved between 1947 and 1953. Reilly (Herman, Kilham and Warbach, 1956) found 8.5% of 174 rabbits from the Three Rivers Game Management Area, Baldwinsville, N. Y. with fibromas in 1953.

From the standpoint of numbers and regularity of collection this investigation was biased in favor of finding more fibromas in rabbits from the campestral habitats. That fibromas were found in rabbits only from the wooded terrain suggests the possibility of a sylvan vector or reservoir.

Dalmat (Dalmat, 1959) found the following mosquitoes capable of transmitting the fibroma virus under laboratory conditions: *Ades aegypti*, *A. triseriatus*, *Culex pipiens* and *C. quinquefasciatus*. *Aedes aegypti* is found only in such sites as warehouses in Illinois and then only oc-

asionally. The other species mentioned are common throughout the state. The flying range of the three species is such that they were probably present in both habitats during the period of study. It is possible that the sylvan habitat might have had larger numbers of *Aedes triseriatus*, the treehole mosquito. Field records show that mosquitoes did not appear to be more numerous in the sylvan habitat than in the campestrial. The bedbug, *Cimex lectularius* L is found throughout the state, but has not been recorded as an ectoparasite of cottontails (Shope, 1959). No triatoma bugs have been reported in central Illinois. The two fleas which were used primarily in the experiments of Kilham and Woke, (1953) *Cediopsylla simplex* and *Odontopsyllus multispinosus*, are found on Illinois cottontails. The former (the common eastern rabbit flea) was far more abundant. It was found in abundance on rabbits of both habitats throughout the year. Cottontails of central Illinois are occasionally parasitized by the following chiggers: *Euschöngastia peromysci* (Ewing), *Trombicula* (*Eutrombicula*) *alfreddugesi* (*Oudemans*) and *Trombicula* (*Neotrombicula*) *whartoni* (Ewing). The species used unsuccessfully in transmission by Dalmat (Dalmat, 1959), *Trombicula splendens*, has not been reported from Illinois cottontails.

The continental rabbit tick, *Hae-maphysalis leporispalustris* (Packard) (Herman, 1938) is another common ectoparasite of Illinois cottontails; it is also the chief carrier of *Pasteurella tularensis*, which has in the past caused mortality among

cottontails of this state (Stannard and Pietsch, 1958). Adults are found on the cottontails largely from March to June. Nymphs have two peak periods, one in May and another in September and October. The peak for the larvae comes in August and September, at which time the cottontails are more heavily parasitized with larvae and nymphs than in the spring. Since Larson *et. al.* (Dalmat, 1958) were able to transmit rabbit papillomatosis virus by nymphs of the rabbit tick, this arthropod should also be considered as a possible vector.

In correlating the experimental findings with this investigation, it would appear that, with the exception of *Aedes triseriatus*, mosquitoes are not the major suspects in spite of their proven capability in the laboratory. The experimental evidence in favor of fleas is much less extensive; from the abundance of fleas on cottontails at all seasons of the year in both habitats, it would appear that they are not the vectors in central Illinois. Population peaks of the rabbit tick nymph and larva as determined by a previous investigation in northern Illinois correlated well with the prevalence of Shope's fibroma in the study reported here. However, few ticks were encountered in these collections and no differences between the two habitats in this respect were recorded. There is a strong possibility that more larval ticks would be found in the sylvan habitat if efforts were made to find them.

The location of the lesions favors the assumption that the vector reaches the rabbit by way of the

ground. The sites of all except two lesions were on the feet or legs as shown in Table 3. One was on the nose and one on the ear. Chigger mites are usually found in Illinois cottontails on, or in, the ears (Stan-nard and Pietsch, 1958). Lesions caused by fleas, mosquitoes and reduviid bugs appeared on other parts of the body in the experimental disease. There is also the possibility that another animal found in sylvan habitat may be the reservoir of the virus. Tumors similar to or even immunologically related to Shope's fibroma have been found on other animals (Herman and Bigchoff, 1950; Herman and Reilly, 1955; and Kilham, Herman and Fisher, 1953), including deer, grey squirrels, fox squirrels, woodchucks and porcupines.

Subdivision of the sylvan habitat into dense woods and the immediately adjoining fallow fields in Table 2, yielded differences which were not so striking as those between the agricultural and wooded areas. Approximately 1.8% of 279 rabbits from the woods had fibromas while only about 0.4% of 956 rabbits from the uncultivated fields were affected. This is another indication that the wooded area might have been the major habitat of the vector or reservoir. The seasonal prevalence of the disease in Illinois supports the hypothesis that an arthropod vector is responsible, since the large majority of cases were found in the fall. There is an abrupt diminution in the number of tick larvae and nymphs found on rabbits after frost (Shope, 1959). The few cases found

in early winter are readily explained on the basis of the time required for the regression of natural lesions, which can be at least 10 months (Shope, 1959). It is also possible that under certain circumstances a few vectors might live into the winter months.

The age distribution of the affected rabbits in Table 3 was one adult to 8 juveniles. This is approximately the proportion of juveniles to adults found in the fall and winter. Ninety-two per cent of the rabbits trapped in these months were juveniles.

The differences between the prevalence of Shope's fibroma in cottontail rabbits on cultivated fields and from heavily wooded habitats indicate that foci of the disease in central Illinois are to be found in the wooded regions.

SUMMARY

An investigation of Shope's fibroma in cottontail rabbits was carried out between 1956 and 1960 in central Illinois. Nearly 3,000 cottontails, largely from four adjoining counties, were examined at monthly intervals. A total of 1,506 cottontails from cultivated fields were negative; in contrast, 0.72% of 1244 rabbits taken during the same period from a heavily wooded area were positive. A much larger proportion of the positives were taken from dense woods than from nearby uncultivated fields. All fibromas except one were found on body parts usually or frequently in contact with the ground. All but one case were found in the fall; the exception was found in early winter.

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