boring into the slant. At about this time unfavorable conditions set in in the tubes and multiplication of both ciliates seemed to cease, and at the end of the seventh day, all of both types were dead.

Five tubes were inoculated for the first subculture. Only two of these were positive at the end of 48 hours. In these two the *Troglodytella* seemed to have multiplied considerably at the end of 48 hours and dividing individuals could be seen. The *Balantidia* multiplied much more rapidly, however, than the *Troglodytella* and at the end of 72 hours far outnumbered them. Both types remained active until the end of the sixth day when they all died. Second subcultures were made at the end of 48 hours. In these, twenty-four hours later, the *Balantidia* outnumbered the *Troglodytella,* and, although the latter were alive at the end of 96 hours when subcultures were again made, only the *Balantidia* survived this transfer.

This experiment was repeated several times with the same result and then several other media[5,6,7] were tried but without success. It is possible that the failure of continued cultivation of the ciliate may not have been due so much to any fault of the medium as to the fact that such frequently repeated transfers does not give time for sufficient multiplication as it does in the case of the *Balantidia,* a smaller ciliate. This problem is being worked on at the present time in this laboratory.

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THE EXPERIMENTAL TRANSMISSION OF ANAPLASMOSIS BY DERMACENTOR VARIABILIS

ABOUT a year ago the experimental transmission of bovine anaplasmosis by the brown dog tick, *Rhipicephalus sanguineus,* was reported by the writer[1] from this laboratory. This tick, although very widely distributed throughout all tropical countries of the world, is only known to occur in Texas, Louisiana, Mississippi, Florida, Kansas, Ohio, Pennsylvania and New York so far as this country is concerned, whereas anaplasmosis occurs in an area which, while not yet well mapped, certainly extends not only beyond the zones within which the related disease, piroplasmosis (cattle tick fever), is now confined, but also outside the original range of enzootic bovine piroplasmosis. It was pointed out, therefore, that if anaplasmosis is tick-borne throughout its entire range, it should have, judging from the distribution of ticks in the United States, at least two other species of ticks as its carriers. The experimental evidence presented in the present paper incriminates, as a carrier of anaplasmosis, another tick, *Dermacentor variabilis,* a species important because of its wide distribution and host range. This tick occurs on the Pacific coast and appears to be wide-spread east of a line extending south from the middle of the Canadian border of North Dakota to about Corpus Christi, Texas. It is not known to be present in the Rocky Mountain States. There is grave danger, therefore, that this tick may carry this destructive disease into many areas that hitherto have been considered outside the enzootic range of anaplasmosis.

EXPERIMENTAL CONDITIONS

Since not only the adults but also the larvae and the nymphs of three-host ticks drop to the ground after engorging, it is necessary in experimental work to devise means of retrieving the engorged ticks so that they will not be lost in the stable litter. When available, therefore, bulls have been used in preference to cows since the ticks could be held in a bag which was attached by means of adhesive tape to the bull's scrotum. For the experiments reported in the present paper, the bulls were purchased late in the fall in an area in Colorado where nothing resembling anaplasmosis is known to occur and were shipped immediately to Jeanerette, Louisiana, where they were confined in a screened barn. A description of this barn in which the bulls were kept out of contact with biting flies and other ectoparasites except the ticks used in the experiments has been published by the writer.[1] Bulls that have been held in this barn and not yet used for experiments were checks on the work; during the work herein reported five such checks were held and all of them remained uninfected. Furthermore, during the past three years, 68 head of cattle have been held in this barn, some of them as long as 18 months; except when experimentally transmitted, not a single case of anaplasmosis has been noted.

EXPERIMENTAL PROCEDURE

Many unengorged and partly engorged adults of *Dermacentor variabilis* were taken from cattle in Mississippi and Florida during April and May, 1931, by tick inspectors under the direction of Dr. Hartwell Robbins, of the U. S. Bureau of Animal Iudustry, and by Dr. T. W. Cole, of the U. S. Bureau of Animal Industry. These ticks were sent alive to the laboratory at Jeanerette, Louisiana, where they were used as follows: (1) Adults were allowed to engorge on a bull with clinical anaplasmosis, the females

[5] E. Schumaker, "The Cultivation of *Balantidium coli,*" *The Amer. Jour. Hyg.,* 13: 1, 281–295, 1931.

[6] Sidney Margolin, "Methods for the Cultivation of Cattle Ciliates," *Biol. Bull.,* 59: 3, 1930.

[7] A. Schourenkova and V. Nossine, "Le culture du *Balantidium coli l'ovigen humaine,*" La presse medicale, 10: p. 1686, 1930.

[1] C. W. Rees, "The Experimental Transmission of Anaplasmosis by *Rhipicephalus sanguineus,*" *North American Veterinarian,* September, 1930.

oviposited in the laboratory, and the larvae engorged on two susceptible bulls; (2) the larvae engorged on a clinical case of anaplasmosis and the nymphs from these larvae engorged on two susceptible bulls; and (3) the nymphs engorged on a clinical case of anaplasmosis and the adults from these nymphs engorged on two susceptible bulls. In this way anaplasmosis was transmitted to the last four of the six susceptible bulls, transmission occurring in the moves from larvae to nymph and from nymph to adult. Since the first two bulls have not thus far reacted, the larvae did not "inherit" the infection from their mothers, *i.e.*, the etiological agent of anaplasmosis did not persist in the eggs of the ticks in the step from adult to larva, as is the case with the etiological agent of bovine piroplasmosis.

TEST OF HEREDITARY TRANSMISSION

On May 20, 1931, some unengorged adults of *Dermacentor variabilis* were permitted to engorge on bull No. 47, and on May 25–26 seven engorged females were removed. Bull No. 47, having been splenectomized on May 13, had clinical anaplasmosis during the time of this engorgement. The severity of this attack was demonstrated by the fact that on May 26, 375 erythrocytes per 1,000 were found to be infected with *Anaplasma*. The female ticks oviposited in the laboratory, and from June 30 to July 8 several thousand larvae engorged on a susceptible bull, No. 71. Several thousand other larvae from these females engorged from July 13 to July 20 on another susceptible bull, No. 68. As stated above neither of these bulls has reacted to anaplasmosis. Their susceptibility has not yet been proved by the injection of virulent blood. The blood of bull No. 47 has, however, been proved to be capable of transmitting anaplasmosis by injecting about 40 cc of it on May 25 into a susceptible bull No. 53. No. 53 reacted to anaplasmosis on June 9 and died on June 12.

LARVA TO NYMPH TRANSMISSION

Many of the partly engorged female ticks oviposited in the laboratory without further engorgement; other females were permitted to engorge on rabbits. From the eggs of both lots of these females many larvae were hatched, and on July 7 the larvae were placed in a bag which was attached to bull No. 65. Bull No. 65 had very severe clinical anaplasmosis at this time. He had been inoculated intravenously on June 16 with virulent blood from bull 46 and reacted to anaplasmosis on July 7. On July 13 a count showed that 370 erythrocytes per 1,000 were infected with *Anaplasma*. A blood count on July 17 showed only 1,140,000 erythrocytes per cubic millimeter and there were 19,600 white blood cells.

The larvae engorged until July 14 and molted in the laboratory to nymphs which were divided into two lots. On July 29 one lot was placed on a susceptible bull, No. 62. However, on August 5 no engorged nymphs could be found on this bull, though many remained unengorged in the bag. On this date other nymphs were added to those already in the bag and the latter was reattached to the scrotum. These latter nymphs were from another lot of larvae which had engorged from June 28 to July 5 on bull No. 46. Bull No. 46 failed to react to anaplasmosis after blood injection on April 13. He was, therefore, splenectomized on June 16 and a mild case of anaplasmosis was diagnosed on June 29. There was only a slight rise in temperature and the *Anaplasma* were never numerous but were present in the smears for about six weeks. The virulence of the blood of this bull No. 46 was proven because as stated above he was the "donor" of the blood used on June 16 to inject into bull No. 65.

Only 14 engorged nymphs were removed (August 10 to August 15) from bull No. 62, but many were found to have been crushed in the bag. Bull No. 62 reacted to anaplasmosis on September 2. The incubation period was, therefore, not longer than 35 days.

On August 4, the other lot of nymphs mentioned above as from the larvae that engorged on bull No. 65 were placed on bull No. 67. Thirty of them were removed, engorged, from August 9 to August 15. Bull No. 67 reacted to anaplasmosis on September 4. The incubation period, therefore, was not longer than 32 days. Both bulls No. 62 and No. 67 had irregular temperatures, fluctuating between 102° F. and 106° F., until about September 15. Anaplasma were numerous, as were also the characteristics of severe anemia. On September 12 the blood counts were as follows: Bull No. 62, erythrocytes 2,460,000, leucocytes 14,600 per cu mm; bull No. 67, erythrocytes 1,460,000, leucocytes 14,300 per cu mm. However, neither bull lost his appetite nor had other discernible symptoms of severe illness. The infectivity of the blood of these bulls has not yet been tested by injection into susceptible cattle.

NYMPH TO ADULT TRANSMISSION

Other nymphs of *Dermacentor variabilis* were secured by engorging the larvae on rabbits. On July 14 several hundred of these nymphs were placed on bull No. 65. Seventy-six of them engorged and were removed on July 20 to 22. Bull No. 65 was convalescing from anaplasmosis during this time, having reacted, as noted above, on July 7. He was still weak and very anemic and occasional *Anaplasma* could still be found in the smears. The engorged

nymphs molted in the laboratory to adults and on August 10 about half of them were placed on bull No. 73, the other half on bull No. 74. Between August 17 and August 21, five engorged females were removed from No. 73 and 9 from No. 74. A number of males, not counted, were also removed from each bull. Bull No. 73 reacted to anaplasmosis on September 13, and bull No. 74 on September 14. The incubation periods were, therefore, not more than 34 and 35 days, respectively.

Discussion

The data of the present paper appear to warrant the following general statements:

1. Larvae of *Dermacentor variabilis* may acquire anaplasmosis by engorging on a carrier, *i.e.,* a bovine in which the blood carries the etiological agent of this disease, and nymphs which develop from these larvae may transmit anaplasmosis to susceptible bovines.

2. Nymphs may become infected by engorging on a carrier, and adults which develop from these nymphs may transmit anaplasmosis to susceptible bovines.

In the present experiments the adult female ticks which engorged on carriers did not transmit anaplasmosis to the larvae of the next generation. More experimental work is needed, however, to determine whether or not hereditary transmission by this tick may occur. Had the experiment been tried, the nymphs or the adults of the second generation might have transmitted the infection, even though the larvae failed to do so. Furthermore, it may be possible for females to become infected either from larvae or nymphs rather than from engorging directly on a carrier and to transmit the disease to the larvae, nymphs or adults of the next generation.

It is possible, though not at all probable, that the nymphs and the adults which were used with success in the experiments of the present paper "inherited" their infections from the adults which were sent from Mississippi and from Florida. Be that as it may, the important feature of this work is that *Dermacentor variabilis,* which is one of the most widely distributed ticks in the United States, will serve in the transmission of anaplasmosis. This discovery warrants the serious attention of all those engaged in dairying or in the production of beef cattle because, as stated above, this destructive disease may be spreading at the present time into new areas.

It has been found that ticks of several genera can and do transmit anaplasmosis in various parts of the world, and with this evidence to the effect that the etiological agent of the disease has so little specificity in its requirements for an intermediate host one is safe in assuming that other ticks, not yet known to transmit anaplasmosis in the United States, actually do transmit it. Certain ones of these ticks, if incriminated, would suffice to make the known distribution of anaplasmosis coincide with the known distribution of carriers.

It is of interest to note that *D. variabilis,* convicted here of carrying anaplasmosis of cattle, has recently been incriminated as a carrier of a disease indistinguishable from Rocky Mountain spotted fever in man. These findings bring this tick to the front as a parasite of major importance.

Summary

Under properly checked and controlled conditions, the writer has succeeded in transmitting anaplasmosis by means of *Dermacentor variabilis* as follows: (1) Ticks engorging as larvae on clinical cases of anaplasmosis transmitted the disease as nymphs to two susceptible bulls; (2) ticks engorging as nymphs on a convalescing case of anaplasmosis transmitted the infection to two susceptible bulls.

The test of "hereditary" transmission was negative when the ticks engorged as adults on a clinical case and the larvae of the next generation engorged on two susceptible bulls.

The wide-spread occurrence of *Dermacentor variabilis* in the United States indicates the danger that anaplasmosis may spread into new areas that have thus far been considered as outside the enzootic range of this disease.

The recent incrimination of *Dermacentor variabilis* in the transmission of what appears to be Rocky Mountain spotted fever has focused the attention of the medical profession and of public health officials on this tick, and this finding and the writer's finding indicate the need of further research on it as a tick of major importance in human and veterinary medicine.

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