Observations Regarding the Presence of Psittacosis and Related Viruses in the Northwestern States

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The other than pathologist reader of this paper will come away from it impressed first, with the scientific stamina of the researchers who venture into the study of virus diseases, and second, with the prodigious amount of painstaking work called for in each forward step in any rickettsial or viral research.

✠ An investigation into the presence of psittacosis and related viruses in the western states, particularly in the northern Rocky Mountain area, was initiated as an adjunct to extensive field studies on Q fever. The similarity in clinical symptoms between Q fever and psittacosis made it advisable to look for psittacosis among patients having a clinical diagnosis of Q fever but who failed to show Q fever antibodies in complement-fixation tests.

Attention was first directed to the occurrence of diseases of the psittacosis-lymphogranuloma group in human beings. It was later extended to include studies of the presence of these diseases in domestic animals. Interest in this latter phase was stimulated by recent reports of four new members of the group, two having been isolated from cattle and two from sheep.

In the first section of this paper, the results of serologic studies on human and animal sera are presented. Complement-fixation tests revealed that antibody to psittacosis virus was more prevalent in these sera than had been previously suspected. The second section of this paper considers the source of viruses which gave rise to this antibody. Special emphasis is placed on the new members of the psittacosis-lymphogranuloma group isolated from cattle, including two strains from cattle in western Montana.

Materials and Methods

The majority of the human sera tested by complement fixation were submitted by physicians throughout the western states. As a diagnostic service to them, the sera were tested with a variety of rickettsial and viral antigens, including psittacosis antigen. The remainder of the human sera were collected from groups representing specific occupations. All of the sheep and cattle sera were collected in the field from herds of animals. Upon arrival at the laboratory, sera were removed from the blood clots and either frozen or stored at 5° C. until they could be tested.

For the complement-fixation tests, antigens were prepared from either yolk sacs or allantoic fluid of embryonated eggs infected with one of these three strains: (1) the D383 strain of ornithosis virus isolated from a pigeon

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in our laboratory, (2) the Gleason strain of psittacosis virus, or (3) the "L" strain of bovine encephalomyelitis virus isolated in 1949 by Dr. G. S. Harshfield at Brookings, S. D.[1] Two general methods of antigen preparation were employed. Either the elementary bodies of the respective virus were concentrated and purified by differential centrifugation or crude yolk-sac suspensions without purification were used. Heat treatment, either by boiling for 10 minutes or by exposing to live steam for 30 minutes, was applied universally to all antigens and, as reported previously by other workers,[2] was found capable of increasing the complement-fixing titer about eightfold.

In the complement-fixation test itself, the procedure proposed by Plotz[3] was employed, fixation being allowed to proceed for 18 hours at 4–6° C. Complement was standardized according to the method of Mayer and co-workers.[4] The unit of complement was defined as the amount necessary to produce 50 per cent hemolysis of sheep red blood cells. In each test, four units were employed. In all complement-fixation tests, care was used to prevent misinterpretations due to nonspecific reactions. Several known positive and negative control sera were included in each test. Whenever doubt remained, tests were repeated with several antigens of the same disease agent.

For isolation of ornithosis virus, a method described by Davis[5] was preferred. Mice were given a massive dose of the suspected tissue intraperitoneally. Seven days later, liver and spleen were passed to other mice intracerebrally. One blind passage of brain was made when necessary. This method of isolating the virus has been found to be highly sensitive, more so than either direct brain or nasal inoculation. It has the further advantage of permitting studies on contaminated material without the use of antibiotics for suppression of bacteria.

Experimental

Serologic Tests

As a result of complement-fixation tests on human sera received early in 1952, two cases of psittacosis were encountered in southern Idaho. They were among a group of individuals originally considered to have Q fever. Sera from both of these patients showed a titer of 1:128 against psittacosis antigen. The clinical history was likewise consistent with the diagnosis of psittacosis.

In the same year, another case of human psittacosis was encountered at Wenatchee, Wash. The illness occurred in a 70-year-old male. The source of infection was probably a parakeet which the man had owned until the recent death of the bird.

Following the discovery of these three cases of psittacosis in the northwestern states, psittacosis antigen was used routinely in the complement-fixation tests on all human sera submitted to our laboratory. One thousand nine hundred and fifteen specimens were examined for psittacosis antibody during the 18-month period ending in August, 1953. Thirty-one showed antibody titers of 1:16 or 1:32, five gave titers of 1:64, and 12 had titers greater than 1:64. In summary, 48 or 2.5 per cent of the 1,915 sera tested were positive for psittacosis antibody in a titer of 1:16 or greater. Seven of the individual patients from whom blood specimens were received probably had had psittacosis; this decision was based on the demonstration of an antibody rise, or on the fact that clinical and epidemiological history also strongly suggested psittacosis.

In two selected groups of human sera tested, the percentage of serologically positive specimens was somewhat

Table 1—Survey of Cattle and Sheep Sera for Antibody against Psittacosis Virus

Source of Sera	Number of Sera Tested	Per cent of Sera Showing Complement-fixation Titer in These Ranges:		
		1:8–1:16	1:24–1:64	Above 1:64
Cattle, western Montana	151	16	6	0
Cattle, southern California	302	30	18	3
Cattle, Idaho	53	40	17	2
Cattle, Kansas *	199	26	21	17
Cattle, Colorado	117	10	10	0
Sheep, Idaho	473	7	7	2

* Being studied for bovine encephalomyelitis

higher. Among 339 workers in turkey processing plants in Utah, 25 or 7.4 per cent showed psittacosis antibody titers of 1:16 or greater. In a group of 42 sera from sheepherders in Idaho, seven or 16.7 per cent were positive.

The majority of the cattle and sheep sera tested were collected in connection with Q fever and tularemia antibody surveys and not because there was clinical evidence of infection with a psittacosis-like virus in the herds. The results of complement-fixation tests on these sera, shown in Table 1, indicated that low to moderate levels of psittacosis antibody are also widely scattered among sheep and cattle. In a few individual animals antibody titers reached a presumably diagnostic level. The fact that of approximately 200 Kansas cattle being studied for bovine encephalomyelitis 17 per cent gave titers higher than 1:64 is of particular interest and substantiates the value of the complement-fixation test in reflecting the occurrence of this disease in cattle.

Isolation Studies

The discovery of this widespread distribution of antibodies against the psittacosis group logically raised the question concerning the nature of the stimuli giving rise to them. There is no reason to believe that all the sources of psittacosis-like viruses in nature have now been revealed. Indeed, it has been stated by two well known workers in this field, Drs. K. F. Meyer and B. Eddie,[6] that "Any laboratory worker who is willing to carry out a series of autopsies, and inoculate through blind passage in mice the spleen, liver, kidneys, and cloacal content of different birds or the lungs of mammals may in time discover previously unknown hosts of a psittacosis-like agent."

In our own investigations, isolation attempts are continually being made to extend the knowledge of reservoirs of these viruses. Two groups of pigeons were first studied. One of them comprised 20 birds collected in the Snake River Canyon near Twin Falls, Ida., where pigeons occur in large numbers. Six of the 20 birds were found serologically positive in complement-fixation tests. No strains were isolated, possibly due to conditions during shipment of the tissue specimens to the laboratory. The second group of 20 pigeons was collected in western Montana. Six of these were likewise serologically positive. These six, together with two that had been negative for antibody, were available for isolation studies. One of them, with a complement-fixation titer of 1:128, yielded a strain of ornithosis virus. Five years ago, three other

strains were isolated from pigeons in that area by other workers using similar methods.[7]

It is known that pheasants existing under the artificial environment of a pheasant farm can harbor and disseminate ornithosis virus.[8] Data on the occurrence of the virus in pheasants living in their natural habitat are almost completely lacking in the literature. In the current study, a survey was made of pheasants inhabiting the same area of western Montana where ornithosis virus is readily obtainable from pigeons. Liver, spleen, and cloacal contents of 103 pheasants were tested, but the virus was not obtained from any of them.

Explanation of the presence of psittacosis-group antibody in cattle must take into consideration the recently reported viruses of this group which occur in cattle. The disease, called bovine encephalomyelitis, has been studied extensively in cattle by Wenner and co-workers.[9] In our laboratory cooperative work with him on the etiological agent has shown conclusively that it cross-reacts completely with psittacosis virus in complement-fixation tests and is thus a member of the psittacosis-lymphogranuloma group. In 1951, York and Baker [10] characterized an apparently nonpathogenic agent which they had isolated from the intestinal tract of calves in New York State. It also was found to be antigenically related to psittacosis.

In our own investigations two strains similar to the York and Baker strain have been isolated from feces of calves in a western Montana herd, thereby furnishing evidence that this agent is probably also of widespread occurrence.

Whereas the white mouse has for years been the laboratory animal universally used for making isolations of viruses in the psittacosis group, it should be understood by the laboratory worker that the mouse is of little value in isolating the two agents from cattle. They produce little or no response in mice following any route of inoculation but readily infect guinea pigs by the intraperitoneal route.

The clinical and gross pathological picture observed in guinea pigs infected with the "L" strain of bovine encephalomyelitis virus may be described as follows: Following inoculation of infected yolk sac, there was a sharp, febrile response beginning on the first day. When infected animal tissue was used, the febrile response was always delayed until the second or third day after inoculation, usually the third. The earlier response to yolk-sac inoculum was perhaps due to a toxin produced in embryonated eggs but not in animal tissues. With either inoculum, illness in guinea pigs was always prolonged, with 10–12 days of continuous fever being common. Emaciation and weight loss were extreme, but no other marked external symptoms were apparent. Death, when it occurred, usually took place shortly after the fever subsided, on an average of about 14 days after inoculation.

Necropsy revealed a variety of gross pathological changes centered predominantly in the abdominal cavity. Most noticeable was the large amount of fibrinous exudate which coated the liver and spleen and sometimes extended to also cover the stomach and omentum. This exudate persisted for at least 20 days after inoculation. During this interval, the spleen remained normal in size or became only slightly enlarged. Small amounts of fluid were present in both the peritoneal and pleural cavities.

The clinical and pathological picture in guinea pigs infected with any of the calf-intestinal strains studied thus far was similar to that produced by bovine encephalomyelitis virus. Experience to date would, however, indicate that the intestinal strains are less virulent for guinea pigs since illness was always less severe and deaths did not occur.

Discussion and Summary

Evidence has been presented that antibody to viruses of the psittacosis-lymphogranuloma group is frequently encountered in complement-fixation tests on human, cattle, and sheep sera collected in the northwestern states. Lymphogranuloma venereum is practically nonexistent in this area; similarly, the presence of antibody to these viruses is only occasionally explained by a recent illness suggestive of psittacosis. Whether it results from subclinical infections with some member of this group or from constant exposure to a virus present in the environment but not capable of producing illness is not known.

The existence of at least two natural reservoirs in the area for the dissemination of psittacosis-like viruses has been demonstrated by the isolation of ornithosis virus from pigeons in western Montana and by isolation of a related virus from the intestinal tract of calves in the same region.

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