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FIELD AND LABORATORY METHODS FOR THE STUDY OF INFECTIOUS DISEASES

R. P. Hanson and L. H. Karstad

The record of research on diseases of man and domestic animals is impressive. Tools and procedures have been developed that probe into the subtle balance between the animal and its parasites. All sorts of abnormalities are measured and their causes fathomed. Thousands of men have devoted their time and have used millions of dollars in ferreting out the secrets of disease.

In contrast we know little of the diseases of wild animals. We have only limited resources, men and money to devote to such research. The situation may not be as hopeless as it first appears. The tools and procedure developed for medical and veterinary research can be applied to the study of diseases of wildlife. Distemper virus is isolated from raccoon in the same way that it is from dogs. Antibodies for encephalitis are detected in the blood of crows, just as they are detected in the blood of man.

My first purpose is to discuss some of the tools and procedures that can be used in the study of wildlife diseases. As modifications may be required when a tool is used at a new task, my second purpose is to discuss some of these adaptations. As the results obtained in laboratories are meaningless without interpretation, my third purpose is to discuss the meaning of laboratory findings on wildlife disease.

Let us suppose that some deer are found dying in Florence County in Wisconsin. What are the procedures and tools available in the laboratory?

1. First are the pathological techniques. A trained pathologist would carefully examine all organs for gross abnormalities. A histopathologist would take little pieces of tissue from selected parts of the animal, fix those tissues in a type of embalming fluid, and after further preparation, cut slices finer than thinest tissue paper. These slices would be stained and examined in the microscope. Tissues that look normal to the naked eye may reveal abnormalities under the microscope. A clinical pathologist would take samples of blood and determine if the proper number and kinds of blood cells were present or if poisons were present in the blood.

2. Second are the cultural techniques. The bacteriologist and the virologist would also take tissues for observation. They would grind these tissues and inoculate the material onto agar where bacteria will grow or into animals where viruses will grow. In this way the infecting agents of disease, are isolated and identified.

3. Third are the serological procedures. In the blood serum of an animal is found a substance that keeps a record of all the diseases that infect that animal. We call the substance antibody. Antibody can be detected by ~~a series of~~ several tests. If a pheasant has had pullorum disease, its serum will cause the bacteria of pullorum disease to form clumps on a glass plate. This clumping is easily seen and makes a simple laboratory test which we call an agglutination test. If one of those little Japanese quail ( ) has had Newcastle disease and managed to survive (most of them don't) its serum will prevent the virus of Newcastle

disease from clumping red blood cells. This is called hemagglutination-inhibition test, a rather frightening name for a test that is easily run.

Some antibodies are much harder to detect than those for pullorum disease and Newcastle disease. Then the man in the laboratory tries a complement-fixation test, or a neutralization test. If antibody to encephalitis is present, it will prevent several other reagents from reacting with each other and the sheep red blood cells used as an indicator in the complement-fixation (or CF) test will not break down or lyse. The lab man says that the complement is fixed. If antibody isn't present, the sheep red cells break down or are lysed. In the neutralization test, the serum is mixed with the virus and then the mixture is inoculated into a chicken embryo or a mouse. The lab man watches the embryo or mouse. The right kind of antibody will stop the virus or neutralize it. The wrong kind will allow the virus to kill the embryo or mouse.

In the situation of the dead deer from Florence County, several of the tests might be used. In some situations all tests might be tried. The determining factors are the importance of the problem, the kinds of laboratory facilities available (few laboratories could do all tests) and the cost of the tests.

Let's take some specific examples which will illustrate the use of tests, the necessary modification of the tests and the interpretation of results. In our laboratory, we are particularly interested in two diseases of man, livestock and wild animals--vesicular stomatitis and eastern equine encephalomyelitis. Both are caused by viruses. Both of these diseases appear at intervals in Wisconsin and Georgia and we have studied them in both regions.

Wild animals rarely come around to the door of the laboratory when they are sick and suggest that a test be run to find out what the difficulty is. It is also unfortunately true that many times when you get some funds to study a disease and a place to work, no more specimens can be obtained. Without sick animals, one cannot study pathology or make isolations, but one can still do serology.

Many of you had the flu during the past 3 months and have since recovered. If I took a throat washing from you now, it is doubtful that I could isolate flu virus. While you were sick, the virus could have been isolated by inoculating chicken embryos. Although I cannot isolate the virus now, I could take your blood and demonstrate antibodies in it to Asian flu. This would indicate that you did indeed have flu and not a common cold.

In our investigations of vesicular stomatitis and equine encephalomyelitis in wildlife we started with serological procedures. The first problem was the procurement of blood samples. Raccoon and bobcats were taken by trapping, deer by hunting and by the use of immobilizing darts. The latter procedure developed by Jack Crockford and Frank Hayes in Georgia is one of the most interesting developments in capturing large animals. Feral swine were taken by corral traps and by hunting with dogs. Birds were obtained by hunting and the use of the Japanese mist net. Immature birds were also readily captured by hand and bled in rookeries.

The handling of blood samples posed a problem. Use of a syringe in drawing blood requires training, especially in the case of small birds. Syringes must be kept at hand and kept clean. Blood stored in tubes should be kept cold or it will spoil. The tubes must be handled carefully

or they break. Shipment through the mail is expensive. To avoid these problems the paper disk method of handling serum or blood was developed. The paper disks used are 17 mm in diameter, consist of absorbent filter paper and are available from Schleicher and Schuell Company. Blood may be drawn by syringe, allowed to clot and the disk dipped into the serum. The serum is dried on the paper. The disk may be dipped into whole blood and dried. Blood may be obtained by puncturing a vein (as the nurse would obtain it from your ear lobe) and the disk allowed to adsorb the blood that wells up. Blood may also be obtained from small birds and road kills by opening the pericardial sac and placing the disks in the chamber where they adsorb free blood.

The dried blood disks or serum disks properly identified are shipped through the mail to the laboratory. An ordinary envelope suffices to send enough blood from four to five deer to conduct two serological tests on each animal. The test we used was neutralization of the infectivity of the virus for chicken embryos. We found that 30% of the bobcats, 45% of the raccoon, 60% of the deer and 85% of the feral swine had antibodies for vesicular stomatitis. This was the case in Georgia. In Wisconsin, the deer and raccoon did not have antibodies.

What do these results mean? Better than 95 percent of the time the presence of neutralizing antibodies for a virus in the serum of an animal means that he was infected some time in his life with that virus. On rare occasions the neutralizing substances are not true antibodies. The reaction is non-specific. For example, ~~most~~ many anywhere in the world horses have a neutralizing substance for yellow fever although they never have been infected. Since none of the Wisconsin deer and raccoon had neutralizing antibodies for vesicular

stomatitis, it would be very unlikely that the neutralizing substances found in deer and raccoon in Georgia were non-specific. The definitive test would be the demonstration that infection of serologically negative Wisconsin raccoon and deer with vesicular stomatitis would result in positive titers for vesicular stomatitis. It did.

Thus we can conclude that many deer and raccoon in Georgia have had vesicular stomatitis infection at some time in their life. What does this mean? Is the disease of any importance to the perpetuation of deer and raccoon? Are these animals carriers of this disease and a hazard to man and livestock? Or are these animals only incidental victims of a disease of man and livestock and of ~~no~~ little importance in its perpetuation?

To answer these questions, we felt that it was necessary to reproduce the disease. Deer and raccoon were experimentally infected. We observed the animals to determine the routes by which they were susceptible, the incubation period, the signs and lesions induced, the duration of illness, the shedding of infective ~~agents~~ virus and the immune response. On the basis of these studies we ~~could~~ conclude that the deer and raccoon were probably incidental victims of a disease of man and livestock. Furthermore, it appeared that while vesicular stomatitis may occasionally be a cause of serious disease in deer, it is usually mild. It is unlikely that raccoon suffer any ill effects from the disease.

So far the popular press has not interpreted our search for the reservoir of vesicular stomatitis with its incidental finding that deer and raccoon also suffer from the disease to be evidence that deer and raccoon are a hazard to livestock. Others have not been so fortunate. The observation that rats can be infected with the agent of atrophic rhinitis

of swine was widely reported as a discovery of a reservoir of atrophic rhinitis. It, of course, is nothing of the kind. The finding that deer in several of the midwestern states have significant serological titers for leptospirosis is very interesting but until deer are experimentally infected with the organism, a disease induced and the deer are shown to be capable of shedding the organism leptospirosis cannot be reported as a problem in deer and deer cannot be considered to be a reservoir of infection.

Infectious diseases of wild animals like infectious diseases of man and livestock are seldom causes of widespread fatalities. More important is the reduction of vitality and decrease in survival chance that is the fate of countless animals. In this way the population potential of many areas for certain species is greatly reduced. Control of such infections to the benefit of wildlife is not an unattainable goal when more knowledge has been obtained by research.