KEYNOTE ADDRESS: KOCH IS DEAD

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Source: Bulletin of the Wildlife Disease Association, 5(3) : 150-156

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-5.3.150

Proceedings of the Annual Conference
of the
Wildlife Disease Association

Ames, Iowa, U.S.A.

June 16-20, 1969

Keynote Address

KOCH IS DEAD

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When some one talks about imbalance of humors or the miasmic origin[13] of disease, I always detect among listeners the amused tolerance a parent gives to an explanation from his three year old son. A discourse on Koch's postulates, on the other hand, is treated with great solemnity. In this light, my premise that there is no more dangerous half-truth among students of disease today than the postulates of Koch may be considered sacrilegious.

The basic idea that Koch got from his mentor Henle is not bad. "If an agent is the cause of disease in one individual it should be capable of causing disease in a second individual." Unfortunately, the ritual that has developed around the postulates has obscured the natural process almost completely.

Let us examine the ritual. The host, the parasite and the investigator participate in an event according to a rather rigid protocol. The host (usually a bird or mammal), and it is considered good form to use several of them, is placed in a confining chamber. Each individual is housed separately and provided with a controlled environment.[9,20,33] This usually means constant temperature, continuous light, and the presence of food and water at all times. It is of no consequence that these conditions do not exist outside the laboratory.

Then the investigator introduces a predetermined number of the parasites with, if possible, a syringe as this adds a certain style. At times the investigator may wonder whether he is introducing the host and parasite to each other under the best of circumstances or in the proper way but he usually banishes such thoughts, as he also rejects the idea that either the host or parasite might not be entities like table salt, each spoonful of which is alike. If instead they are considered to be individualistic, the simple beauty of the protocol would be spoiled by complications.

He continues the ritual, by observing the host in a predetermined fashion, at predetermined intervals, and for an arbitrarily selected period of time. When the vigil comes to an end, whether any revelation has come to him or not, he draws conclusions. And if he is truly dedicated he publishes them.

Let me digress a moment. To get a perspective it is usually necessary to go to a distant point and look back. The carillon sounds differently in the church belfry than it does in the church yard or across the town or as it is remembered the next

day. Sometimes the new awareness gained by a new vantage point remains long afterwards. This makes seeking a new perspective worthwhile. To get a perspective on the present we must go back in time and look at an earlier generation of investigators.

Here are a few notes from D. P. Gardner who published his paper in the American Journal of Medical Science over 100 years ago. His objective was to determine the nature of the fever-inducing miasmas present in the great dismal swamp of Virginia.

His procedure was to place watch glasses on posts set at regular intervals along the transect across a section of the marsh. In each he placed a selected chemical solution capable of adsorbing a gas.

His result was a failure to detect any substances adsorbed by the solutions and he pointed out that many preparations became contaminated and therefore useless because insects, particularly mosquitoes, drowned in them.

His conclusion was that failure to detect them did not disprove his concept of miasmic origin of *(malarial)* fever.

Note that his ritual was excellent. He used chemical methods and even designed the experiment for statistical analysis. We cannot fault him for not recognizing that the contaminating mosquito and not the miasma was the agent by which the fever was transmitted. After all we accept only what appears rational to us on the basis of patterns of thoughts and prejudices which we have learned. Perhaps with more historical perspective we might better recognize our limitations and deliberately look for biases.

What are some recent examples of experiments in which a simplistic approach or *a priori* thinking could easily have led the experimenter astray? Early attempts to reproduce salit poisoning, a sometimes fatal disease of swine, were not successful. A pig was placed in an isolation unit and provided salty feed identical to swill that farmers had used whose pigs died of salt poisoning. The pig starved rather than eat the salty swill. With persistence it was learned that an essential part of the etiology of salt poisoning was a behavioral pattern of the animal. If a group of pigs were fed only once a day, hunger and competition would induce them to gulp down food that they would otherwise reject, and death from salt poisoning would result unless an ample supply of water was kept continuously available.

Shymal Sinha[34] and I learned some years ago that the signs, lesions and mortality that Newcastle disease induces in chickens can be appreciably affected by the ambient temperature of the room in which the birds are held. Temperatures of a hot midwest summer day (35 C) increased both nervous signs and mortality. At winter temperatures (5 C) fewer nervous signs were seen but respiratory signs were more severe. The chickens in the trials were hatch mates and the inoculums were aliquots. A friend in the biologics industry was not surprised at this finding. Proper manipulations of room temperature had been used within the industry, he explained, to obtain desired survival values (titers) on certain potency tests.

Later Tom Griffin[14] and I learned that control of environmental conditions during the experiment was not enough. Mice that had been conditioned at a certain ambient temperature before they were inoculated responded differently than others that had been held at another temperature although when they were inoculated all were placed under identical conditions. The infective agent used was vesicular stomatitis virus and replicable differences of 30% in mortality were observed.

The prior conditioning can also be a diurnal light cycle,[15,16] a different diet,[6,30] or manner of handling or gentling of animals.[1] J. B. Wilson has related an experience that he and Joe Berry had in attempting to reproduce each other's results in a study of bacterial endotoxins. Wilson was doing his work at the University of Wisconsin and Berry at Bryn Mawr. Exchange of endotoxin and of mice did not solve the problem. The irreproducibility factor turned out to be the stressing effect

of reduced air pressure to which mice were subjected in air transport. It induced a change in physiology that persisted for 4 or 5 days and which made an appreciable difference in the responsiveness of the mouse to the endotoxin.[3,4]

Even the use of a strain of mice inbred for homogeneity, an endotoxin of uniform activity, and a carefully defined environment does not insure the experimenter a replicable response. Frances Halberg, at the University of Minnesota,[15] has demonstrated that mice inoculated at 6:00 A.M. give a different response than mice inoculated at 6:00 P.M. The explanation is that the mice are really quite different at those times. Their physiology follows a circardian rhythm, body temperature and even liver enzymes varying from high to low on each turn of the cycle. The magnitude of circardian difference can be greater than that observed between treated and untreated groups.

One of the questions with which the editorial committee of the Handbook for Examination of Poultry Biologics wrestled was the definition of a normal chicken. Everything really hinged on this and yet the only answer was the unsatisfactory one that Alice was given by the Red Queen in Wonderland, "A word means what you say it means." The normal chicken whose blood cell values are tabulated, whose organs are weighted, whose growth rates are graphically portrayed by physiologists, have been parasitized, and in some instances fed inadequate diets. The normal animal referred to in published papers is not a disease free animal but one which has had infections from which he has recovered and often times some infections which have continued to persist such as coccidiosis[21] and RIF leukosis[27] The experimenter is expected to make a reasonable attempt to exclude unintentional exposure of his host to the agent with which he is working before and during his experiment, but he is not expected to have protected the host from all prior infections.

The assumption is that even though the experimental host may have had a prior experience with an unrelated disease or even if an infection is still retained the situation will not significantly alter the course of the disease to be studied. The demonstration that virus-infected cells elaborate a basic protein, interferon, which is capable of preventing or inhibiting the attachment or growth of unrelated viruses, should have effectively ended that assumption.[20] Recently it has been shown that interferon can be induced by protozoan parasites such as malaria as well as by viruses.[16] Other forms of parasite interaction are also known but less well studied.[29]

Most bacteria and viruses and even larger parasites induce an acute infection from which the host rapidly recovers. The parasite can be isolated during the acute stage of disease but not after convalescence occurs. There have been suspicions based on field evidence that a parasite may persist in recovered animals even when no one has been able to detect it.

Evidence is increasing that failure to isolate the parasite often lies in inadequacy of the methods used by the researcher. Foot and mouth virus was isolated from saliva of immune cattle after a procedure was developed to separate virus from the virus-antibody complex in which it exists in saliva.[34] Werner Heuschele[17] working with Barney Easterday at Wisconsin recently learned that Newcastle disease virus can also be isolated from a chicken one or two months after it had recovered from an acute infection. The method in this instance required the progagation of explants of tracheal tissue which after several weeks of *in vitro* growth yield virus. During the early stages of *in vitro* growth neutralizing substances were produced by the culture. When this production ceased, virus was released.

So far I have been describing the complexity of the environmental situation under which the host and parasite meet: the prior history of the host, his behavioral patterns, his environmental conditioning, his disease history, the circumstances of the meeting, the nature of his housing and such environmental factors as temperature and light. While most of these factors may be unimportant in many instances, they have been demonstrated to be all important in others. I hope I have now made the point that bringing a parasite and host together is not enough, the circumstances

under which this is done are equally important. We no longer call the state of the host—his balance of humors, and the state of the environment—a miasmic condition of the air, but we have provided substance to observations that underlay beliefs of the ancient philosophers who coined the terms.[11]

However, we have yet to consider the host itself and the parasite itself. Neither can be purchased as certified reagents. Yet one might think that this was possible on reading many publications.

The word, dog, conjurs up many different pictures: a Chihuahua, a St. Bernard, a dachshund, a wolfhound, a beagle, a bull terrier, a collie, a pomeranian—the list could go on. All are members of a single species yet differ in size, behavior, physical ability and metabolic capability. The differences are too apparent and too familiar for someone to set up an experiment in which dogs of diverse breeds would be used in a non-random fashion. However, among many species, the selection of subjects fails to take into consideration the genetic diversity that exists in a less apparent but no less real fashion.[2]

Several decades ago it was demonstrated that mice of certain stocks differed in their susceptibility to salmonella and encephalitis virus infections.[3,7] Investigators have selected for susceptible and resistant lines of mice and crossbred for intermediate resistance. Lurie[8,9] described differences in the response of two groups of rabbits to tuberculosis. The Aleutian mink, a genetic line selected for coat color, has a blood cell defect. These mink are uniquely susceptible to a slow virus of mink (Aleutian disease) and they are more susceptible than other mink to several bacterial diseases of mink.[4] Unless there is a clear marker, like coat color, or a deliberate attempt has been made by a geneticist to look for differences, as in mice, little effort and little consideration has been given to the heterogenicity of a host species, and of the variability that this would induce into host-parasite interactions.

Even less thought is given to the heterogenicity of the parasite although abundant evidence exists for diversity in most parasitic populations. Whenever attempts have been made to select an individual parasite and grow its progeny, the resultant clones behave in a fashion that is very different from that of the original stock.

Bacteriologists learned after growing bacteria on solid agar that a single bacterium could grow into a colony, — and that a given bacterial species could produce more than one kind of colony. Smooth, rough and mucoid colonies were described and each was found to breed true and to induce disease of differing degrees of severity.[5] Yet all three forms could be isolated from the tissues or excretions of a single infected animal.

Virologists have found the same thing. Viruses grown on monolayers induce plaques which can differ in morphology. Daniel[10] and Estupinan[12] in my laboratory learned that a single strain of Newcastle disease virus, Hickman, contained at least 6 plaque types, consisting of 3 sizes of clear plaques and 3 sizes of red plaques. The 6 derived populations exhibited the entire spectrum of virulence from a clone that was avirulent (100 million virions failing to induce disease) to one that was highly virulent (ten virions inducing death of adult chickens). The clones also differed in their ability to agglutinate cells, in their rate of growth and in other properties. Most significant was the difference in mutation rate between the clear plaque clones and the red plaque clones. The clear clones remained stable through all sorts of laboratory manipulations. Two or three passages of the red clones resulted in a major change in plaque type and other properties as well.

Now comes the decision — what should the investigator use. The wild type culture with its heterogenous population consisting of some 6 sub-populations, one of the clear plaque clones which is genetically stable, or one of the red plaque clones which is genetically instable. Perhaps, as my friend in the biologics industry said, it depends upon how you want the answer to come out. Whether we think so or not, our laboratory decisions are often determined by our wishes. The individual who harvests only embryos that are dead, the one who harvests only those

that are alive, the one who harvests all, or the one who harvests one or two embryos is carrying out a decision. Each one of these decisions involves selection and will influence the outcome.

Most of all we want clean cut results than can be readily repeated. We soon can sense irregularities and avoid them. The wild population or a red plaque clone would give results that could be replicated only with difficulty. For sake of tidiness and predictable performance a parasite is selected to satisfy the investigator's desire for ease of handling, a host picked which is docile in solitary confinement and an environment chosen that meets the investigator's sense of comfort. We should not wonder why we cannot produce the events of nature.

Perhaps for these reasons, very little headway has been made in understanding emergence of new disease, interepizootic survival, and prediction of the occurrence and duration of epidemics. On the basis of our current knowledge of the behavior of viruses, most of them should have become extinct at least a millenium ago.

So far I have spoken of hosts and parasites in the commonly accepted sense of organisms. Metzoan parasites were understood by the ancients. Procarotic cells as parasites were accepted over 100 years ago. Obligate parasites without cell walls and incapable of multiplication by fusion, the viruses, have now been accepted.

Another kind of agent with many of the characteristics of traditional parasites is now being described. It is infectious in nature, capable of propagation in a variety of hosts for an indefinite series of transfers. It can be titrated on the basis of lesions or mortality induced. However, it possesses several properties that appear inconsistent with those of an organism. It resists boiling and prolonged treatment with formalin that denatures all known viruses. More significantly, ultraviolet irradiation capable of destroying nucleic acid of true viruses has no effect upon it.[24] How does one imagine a parasite without nucleic acid? Obviously we are confronted with information that does not fit our current concepts and we are reluctant to admit that those concepts may be faulty.

So far I have not mentioned the word, wildlife. I have been leading up to this point. If we are going to understand diseases of wildlife, we must consider the genetic heterogenicity of the host population and its social structure, the heterogenicity of the parasite population and fully recognize the complexity of the environment in which both exist. Koch's postulates, in the narrow sense will help us to identify parasites but will provide us no information about diseases of wildlife.

The real objective of the study of infections of wildlife is not the development of a long list of the parasites affecting each wildlife species (some 200 kinds of parasites have been found in the Canada goose for example or 20 kinds of parasites in a single goose) but the significance of these parasitisms to the health of the individual and to the size of the population. The genetic diversity of the Canada goose is illustrated by mature weights of individuals which range from 2½ to 18 pounds. The goose breeds over a range that is 2,000 miles across and 2,000 miles deep. It migrates over many routes stopping at selected places and wintering in many different refuges. How can such a population be sampled and meaningful generalizations developed?

Hemorrhagic disease of deer was shown by Richard Shope to be nearly 100% lethal in New Jersey deer. Only with great difficulty did he obtain a survivor with antibody. Other workers in Michigan, Wisconsin, and the Dakotas made the same observation. Yet Wilhelm and Trainer[37] have found that more than half the deer in Texas have antibodies. Are the strains of virus in the two areas different, do the deer differ physiologically or behaviorally, are environmental variables like temperature and nutrition involved, or is the nature of exposure unique in each area? Current evidence points to differences in circumstance of exposure.

Bill Reeves has isolated Buttonwillow virus from the blood of wild cottontails yet he told me that he has been unable to induce a viremia in captive cottontails. What circumstance was altered: virus, host or environment?

The catastrophic die off of wild animals is most probably a rare event. Do infections also exact a toll of wildlife that is far more important but less dramatic?

Do infections and toxins induce fetal mortality, death of dependent young because of developmental defects, or failure of nursing or brooding care, loss of immature or adult animals through predisposition to predation by failure to sense or evade the predator, death losses through inability to withstand environmental insult from chilling effects of rain or wind, or by failure to breed because of inability to display or to produce viable sperm or ova?

If disease acts in these ways our methods of study must move away from the idea of the pathogen and host as fixed entities reacting solely to each other and to a concept of host and pathogen populations interacting in a varying environment. Only in this way can the real role of disease in wildlife populations be defined and understood.

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