IMMUNIZATION AGAINST HEMORRHAGIC SEPTICEMIA.*

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The term hemorrhagic septicemia was first applied by Hueppe, in 1886, as a collective name for all those diseases which were caused by the ovoid bacterium, the *Bacillus bipolaris septicus*. The designation of Hueppe included chicken cholera, rabbit septicemia, hemorrhagic septicemia of cattle and swine plague as the original group. Later investigation proved that there are a number of other infectious diseases in animals, which, directly or indirectly, are etiologically identified with the *Bacillus bipolaris septicus*. Thus, this organism was found to be responsible for the buffalo disease (so-called Barbone), infectious pneumo-enteritis of sheep and the infectious pleuro-pneumonia of calves. This enumeration of diseases does not embrace all affections in which this germ is involved, as there are other infectious maladies, such as influenza of horses, catarrhal pneumonia of calves and distemper of dogs, in which the ovoid bacterium is held to be an important factor. Its association with the latter diseases, however, has not yet been satisfactorily established; nevertheless it is known that some of the pathological changes observed are caused by this micro-organism, and at least it must be considered in these instances as a secondary invader.

All species of domestic animals are susceptible to the infection, although the pathogenic action of the organism for a certain species is usually higher than for other species, and under certain conditions may even be absent; nevertheless it is known that under appropriate conditions the organism may gain in virulence and become a typical pathogenic agent for any of the species. Thus it has been observed that after an outbreak of hemorrhagic septicemia in cattle, hogs have become affected with swine plague

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on the same premises, and likewise the disease in sheep has developed subsequent to an infection of hogs.

It is therefore apparent that the group of these diseases is caused by the same germ, which possesses only a variance in virulence for the different species of animals. This fact is also substantiated by the morphological and biological characteristics of the germ.

These ovoid bacteria live as saprophytes, but under the influence of certain conditions they become parasitic, in which state they sometimes attain a very high virulence. After some generations they gradually lose their parasitic nature and return to their original saprophytic state. As parasites, in their passage through the animal body, they show certain characteristics, as a result of which they are known as a variety of the *Bacillus bipolaris septicus*. Thus, as a rule, the diseases of the different species of animals are caused by these specific varieties, viz., the hemorrhagic septicemia of cattle by the *B. boviscpticus,* swine plague by *B. suiscpticus,* chicken cholera by *B. aviscpticus,* the disease in sheep by *B. oviscpticus,* etc.

These varieties, however, have a common saprophytic origin, which justifies their being grouped into one family, and all diseases caused by micro-organisms with the following characteristics should be included in this group: Ovoid bacteria without motility, gram negative, polymorphous with involution forms. They do not liquefy gelatine and do not coagulate milk, nor change its reaction. The bouillon cultures have a peculiar odor. On acid potatoes they form no visible growth, and in pancreatic bouillon no indol is formed; they are usually erobic, but may grow anerobically. They produce no spores and have no flagellæ. They possess a greatly varying virulence, which is usually very high. These specific characteristics are invariable, and the absence of any of those enumerated would exclude the organism from the hemorrhagic septicemia group.

The group relation of the organisms of this family is also substantiated by the following observations: Chickens may be immunized against chicken cholera with cultures of the rabbit septi-

cemia organism with the same satisfaction as with the attenuated cultures of chicken cholera (Kitt). Likewise Jensen immunized chickens against cholera with the bacteria of calf septicemia. Mayr and Kitt immunized rabbits against swine plague and chicken cholera with sera of the latter diseases. Perroncito produced a fatal septicemia in calves with inoculations of the swine plague organism. Galtier found swine plague bacteria infectious for sheep, goats, calves and horses. Voges has even succeeded in producing a disease as fatal as cholera in chickens by feeding them swine plague bacterja. Further it has been proven that, in spontaneous outbreaks, hogs may become affected with the virus of chicken cholera. Finally, Lignieres, in his exhaustive experiments, proved the virulence of the hemorrhagic septicemia organism for all domestic animals, in which the most varied clinical picture may result from the infection.

These, together with the findings and observations of other investigators, have established the close relationship of the different varieties of organism of this group. Moreover, through this knowledge it has been possible to prepare a polyvalent vaccine from the different varieties, which gave satisfactory results in the combatting of outbreaks and which is still being recommended and employed in various localities.

Immunization against various forms of hemorrhagic septicemia has engaged the attention of numerous investigators since the time the causative factor was identified. Pasteur was the first to work out a method for immunization against chicken cholera, which, however, failed to come up to the desired expectations. He employed for the immunization an attenuated culture of the chicken cholera organism. The attenuation was accomplished by exposing the cultures to atmospheric conditions for a certain length of time. He found that cultures subjected to these conditions lost their virulence to a certain degree if they were then cultivated at body temperature. The material obtained from this attenuation was then used for the immunization against chicken cholera. The failure of this method in practice can be attributed chiefly to the fact that cultures exposed to this method of attenua-

tion will not in all instances produce a uniform product, and therefore it can be readily understood why in some instances great losses were sustained from the use of such vaccine.

Later, other investigators prepared vaccines for the immunization of the different varieties of these diseases, and while the results were encouraging, they have not in all instances succeeded in their purpose. Lignieres' method appears to have been the most satisfactory, and its utilization in practice has also been probably more extensively adopted than any of the other methods. The method of attenuation he employed consists of growing the cultures of the respective organism at 42 to 43 degrees C. and preparing from the cultures grown at this temperature two different strengths of vaccines. The weaker vaccine is grown for five days at this temperature, whereas the stronger vaccine for the second injection is grown for only two days.

Kitt was the first to establish that the attenuated vaccine prepared from one of the varieties of the germ may also prove effective against other varieties. The proof of this fact is of very great importance in the control of the disease, since it may not make material difference whether the vaccine used originates from the bacteria of the particular variety it is desired to immunize against or another variety. Thus it is possible to immunize chickens against cholera with the vaccine prepared from the organism producing rabbit septicemia.

On the other hand, it must be recognized that immunization against a disease of this group cannot invariably and uniformly be successful with vaccines from another variety of the disease, and it should therefore be considered that the best results can only be expected when the vaccine is prepared from the organism of the same variety. Hence, in all cases where it is possible to employ an autogenic vaccine, such should be used. The preparation of the polyvalent vaccine is highly recommended by Lignieres, and according to his experience it may be used with satisfactory results in practice. The polyvalent vaccine is prepared from a mixed culture of the hemorrhagic septicemia organisms, originating from sheep, cattle, dogs, horses, hogs and

chickens. The culture is grown under the attenuating influences of a high temperature, as described above. The practical application of the polyvalent vaccine is at the present time receiving the recognition of certain workers in the control of the disease among various species of animals.

The serum immunization against these diseases has also been investigated quite extensively. Potent sera can be prepared which will have an immunizing effect against the respective disease, but the application of this method of immunization in practice has not proven practical, since a serum inoculation produces only a passive immunity, which conveys to the animals a resistance that remains for only a relatively short period.

In consideration of the laborious task of preparing a horse to furnish the potent immunizing serum and also the length of time which this preparation requires, one can readily see the advantage which would be derived from a vaccine in preference to an immune serum. This feature was particularly emphasized in a recent experience, where it was necessary to immunize animals within the shortest possible time, in order to prevent further losses from the disease in a buffalo herd.

In the following report our experience with vaccine immunization against hemorrhagic septicemia is described, and in consideration of its success, further applications of this method in outbreaks of hemorrhagic septicemia among other species seem advisable.

Hemorrhagic Septicemia of Buffalo (Barbone).

During the month of December, 1911, the Department of Agriculture received information from the Department of the Interior of the existence of a fatal disease in the buffalo herd in the Yellowstone National Park, with the request that an expert be sent to make an investigation of the disease.

Dr. E. J. Cary, veterinary inspector of the Bureau of Animal Industry, was detailed to carry out the investigation at the park. In all, twenty-two animals died between December 3 and December 15, young animals especially being victims of the disease. The symptoms, and particularly the post-mortem findings, were

confusing, and it was therefore deemed advisable to forward some of the tissues for diagnosis to the Pathological Division. The bacteriological examination as well as test inoculations proved an infection with hemorrhagic septicemia as the specific micro-organism (*Bacillus bipolaris bubalisepticus*) was isolated from all tissues, and test animals which were inoculated with material from the specimens died of typical hemorrhagic septicemia, the specific organism being also recovered from the blood of these animals.

This disease of buffalo, known also as barbone, was first recognized in Italy, in 1886, while three years later its presence was established in Hungary. No previous outbreak of barbone has been recorded in this country. In Russia, Egypt, Indo-China and the Dutch West Indies the disease occurs frequently in enzootic form, and in the latter place over 11,000 buffaloes succumbed between 1888 and 1891. It usually appears as a disease of the soil in marshy pastures where large numbers of buffalo are kept. Its appearance in such a remote and isolated place as the Yellowstone Park, however, is difficult of explanation, although the bacilli are known to be widely spread in nature and to occur not infrequently in the digestive tract and air passages of healthy animals. As a result of certain unknown conditions, which might include those influences that weaken the resistance of the tissues, as exposure, starvation, anemia, etc., the bacilli become virulent and produce characteristic lesions. It is not an uncommon experience with hemorrhagic septicemia to have it appear periodically in certain localities, without any apparent connection to which the introduction could be traced. The appearance of the disease in sheep reported by Ward in Minnesota might have some bearing on the disease in Wyoming, but if so it would probably be through birds of prey such as buzzards and hawks.

The authorities in charge of the buffalo herd at the Yellowstone Park were immediately notified of the nature and cause of the infection among the animals and preventive measures were recommended for controlling the spread of the disease. At the

same time it was deemed advisable to undertake the vaccination of the entire herd with bacterial vaccines prepared from the recovered organism. For this purpose two vaccines were prepared of different strength. The vaccine for the first inoculation was prepared by growing the organism five days at 42.5° C., while the vaccine for the second injection was cultivated in the same temperature for only two days.

For the preparation of vaccine Erlenmeyer flasks of pepton bouillon media were inoculated with the organism after it had been cultivated for several generations on agar, and the bouillon cultures were then placed under temperature conditions stated above. The straight attenuated culture after thorough shaking was used for vaccinations in some of the animals, while others received the same vaccine to which one-half of one per cent. of carbolic acid had been added. This was undertaken in order to determine whether the preserved vaccine possesses the same immunizing qualities as the unpreserved material.

Two varieties of the hemorrhagic septicemia organisms were utilized for the preparation of vaccine, the one strain representing the germ isolated from the buffalo disease in the Welloystone National Park, while the other was a variety of hemorrhagic septicemia of cattle isolated from animals which died of that disease in Colorado. The vaccines prepared from these two varieties were tested for their potency on laboratory animals and also on sheep, a comparison of the action of the two different vaccines being carefully made.

The virus isolated from the buffalo disease was especially virulent for rabbits. Inoculations of these animals with 1 c.c. of a suspension of salt solution containing only one-fifteenth of a loopful of bouillon culture killed the animals in from 12 to 18 hours, while one-twentieth of a drop of blood from rabbits dead from the disease was fatal to other rabbits in less than 24 hours on subcutaneous inoculations. The virus of the cattle variety was not as virulent, although test animals succumbed to subcutaneous inoculations on the third day, showing on post-mortem examination the characteristic manifestations of the disease.

Both strains of vaccines were employed in parallel tests on a group of rabbits and also at the same time on sheep. For immunizing purposes subcutaneous injections of the vaccines were given to the animals at ten-day intervals. For the first vaccination the more attenuated, and for the second vaccination the less attenuated vaccine was injected. The injections invariably were made subcutaneously on the inside of the thigh. The dose for the rabbits was .2 c.c. per injection, while the sheep were given .7 c.c. of each vaccine. Likewise another series of animals was tested, using the same amount of a vaccine which was preserved with 0.5 per cent. carbolic acid.

On the sixth day following the second inoculation the immunized animals were given a subcutaneous injection of the pure culture of the organism. Those which were immunized with the buffalo variety were injected with the virulent culture of this organism, while the others received the cattle variety. At the same time check animals which were not immunized were employed for each group and these were injected with the same quantity of virulent culture as given to the immunized animals. The immunized rabbits failed to show any indication of disease from the injection of the virulent culture, while the control animals succumbed in the usual time. The same results were noted in the sheep, although one of the immunized animals showed a slight elevation in temperature which, however, subsided after one day. On the other hand, the control animals of this group succumbed to the infection with typical symptoms and lesion of the disease. The fact that the animals immunized with the carbolized vaccine showed the same immunity as those immunized with the straight attenuated cultures is an interesting feature of this experiment, and while this condition appears at first hand to indicate the advantage from the use of the preserved vaccine, subsequent complement fixation tests undertaken on these immunized animals showed that those animals which were immunized with the straight vaccine gave a partial fixation of the complement for a much longer period than those which were immunized with the carbolized vaccine.

The results of these tests further substantiate the view that the vaccines of one of the varieties of the organism are potent against diseases produced by the other varieties of the germ. Thus rabbits and sheep were successfully immunized with the vaccines prepared from the *Bacillus bubalisepticus and the Bacillus bovisepticus.*

After obtaining these favorable results the vaccine was sent to the veterinarian entrusted with the vaccination of the buffaloes, and instructed to vaccinate all animals of the herd by the same procedure at ten-day intervals. One cubic centimeter of the vaccine constituted a dose for each animal.

Following vaccination, the herd was carefully observed and no immediate effects were noticed from the vaccination, and up to the present time there has been no indication of the recurrence of the disease among the buffaloes.

In the progress of the preparation of the vaccine experiments were also conducted in the laboratory to determine whether the complement fixation test could be applied for the diagnosis of the disease, and also for the purpose of determining the relative degree of immunity conferred upon the vaccinated animals in artificial immunizations. An antigen was prepared from the original organism recovered from the outbreak among the buffalo in the form of a shake extract. The hemolytic system consisted of sensitized rabbit serum (amboceptor), guinea-pig serum (complement), and washed sheep corpuscles. The test was employed with sheep serum and rabbit serum of artificially infected animals, and the results proved entirely satisfactory. A complete fixation was obtained in all instances when applied to 0.1 c.c. of serum of infected animals, while the controls showed no fixation whatsoever.

After the vaccination of the sheep and rabbits, blood serum was obtained from these animals and tested with the complement fixation test. The results in these instances also showed a fixation of the complement, although not as complete as in the infected animals, nevertheless showing that the animals responded after vaccination with the production of immune bodies. This

reaction has been noted even three months after the vaccination, and the testing of the blood will be continued from time to time in order to determine the length of the period in which the animals possess immune bodies subsequent to vaccination.

The utilization of the complement fixation test in the diagnosis of hemorrhagic septicemia, and also its value in determining the relative immunity established by vaccination, is of great importance, not alone in this disease, but also in the possiblity of its utilization for other diseases.

PHYSICIANS AND VETERINARIANS A UNIT IN PREVENTIVE MEDICINE.—The following letter from past-President De Vine of the United States Live Stock Sanitary Association, expressing approval of and perfect accord with the sentiments expressed in the address of his successor, Dr. Ravenel, seems to so clearly demonstrate the views that each personally advocates, the one being a veterinarian and the other a physician, that we have taken the liberty of reproducing it. Dr. De Vine says:

"President Ravenel's address was extemporaneous and his remarks were chiefly concerning the relation of bovine to human tuberculosis and the great importance of bovine tuberculosis as a public health problem. He cited the findings of the Royal Commission in which it was clearly shown that bovine tuberculosis is transmitted to mankind, even the pulmonary form in exceptional cases; also the very valuable report of Dr. Park on the same subject. In fact his remarks up to this point were practically identical with the subject as I gave it in my annual address this year at our State meeting (New York). He further pointed out the necessity of the union of the medical and veterinary professions on health problems and stated that at the last meeting of the National Society of prevention of tuberculosis, of which Dr. Ravenel was President, that resolutions were passed inviting members of the veterinary profession to join the society and take part in the program; he also advocated the union of the professions under one Federal head to deal with national health problems.

"Following Dr. Ravenel, Dr. Geo. B. Young made a short and sincere address of welcome showing plainly his knowledge and sympathy of the work we are engaged in. Dr. Bahnson the Southerner, who can make more faces telling a story and do it right than any man I ever saw, responded in his usual way by putting facts so that they sounded as pleasing as fiction; until he reached the statement which to most Northerners was startling when speaking of Southern cattle fever; he said the cattle tick cost the Southern States $15,000,000 by death and approximately $100,000,000 by loss of commerce with other states annually, and that with our present knowledge of the life and methods of destruction of the tick, that eradication could be made possible and positive for the expenditure of a quarter of the amount of the annual loss, if war against the ticks were waged intelligently and persistently for a few years. He says that the presence of the ticks is entirely due to the indifference of the people and the lack of application of regular dipping of the cattle which is now the key of solution."