Patented Dec. 5, 1944 2,364,579

UNITED STATES PATENT OFFICE

2,364,579

HOG CHOLERA VACCINE

Ralph W. G. Wyckoff, Suffern, N. Y., assignor to Lederle Laboratories, Inc., New York, N. Y., a corporation of Delaware

No Drawing. Application November 12, 1941, Serial No. 418,839

4 Claims. (Cl. 167—80)

This invention relates to the production of a hog cholera virus vaccine and is more particularly concerned with such a vaccine associated with a finely divided adsorbing agent.

This application is in part a continuation of my prior application Serial No. 332,503 filed April 30, 1940.

In the standard mode of vaccination of hogs against hog cholera, hyperimmune serum and virus are administered at the same time, there thus being produced a low-grade and controlled infection which gives rise to a powerful and lasting immunity. For successful vaccination by this method, appropriate amounts of serum and virus must be administered; if too much virus in relation to serum is given, disease may result; if too little, adequate protection will not develop. In practical use it is difficult to be assured of a proper balance between these two agents because the virus vaccine now employed progressively loses potency on standing. At present one seeks to circumvent this difficulty by giving the virus vaccine a short dating, but this procedure is at best only partially satisfactory and it is at the same time wasteful and costly.

It is one of the objects of the present invention to produce a cheaper, more stable and more effective virus vaccine for the active immunization of hogs against hog cholera. It is a further object to put the vaccine in such a form (1) that a less critical balance against serum will result, (2) that the minimal amount of serum will be required to counterbalance the virus necessary for successful vaccination, and (3) that the highest possible degree of immunity will be established through vaccination by the gradual and prolonged liberation of virus within the body of the hog being immunized.

These and other objects are obtained by a vaccine containing aluminum hydroxide or other suitable finely divided adsorbing agent, preferably colloidal, in such an amount that part, but not all, of the virus from infected tissue or blood is adsorbed thereon. When such a partially-adsorbed virus vaccine is injected into a susceptible hog, the free virus it contains is immediately available to initiate the immunizing process while the gradual liberation of that part of the infectious principle which is adsorbed insures a continued low-grade infection resulting in a high degree of resultant immunity.

An illustrative vaccine is made by the following procedure. A highly infectious spleen of a hog fully diseased with hog cholera is taken with suitable sterile precautions, finely ground in a colloid mill or by some other suitable device and made to a 5% tissue suspension with sterile distilled water. After standing for several hours in a refrigerator, this spleen suspension is cleared of gross particles by low-speed centrifugation or in some other suitable fashion. Enough freshly drawn, highly infectious virus blood is then added to make the suspension 10% with respect to this blood. To the fluid thus obtained a 10% potash alum solution is slowly added with constant shaking until the pH of the final mixture has reached a value of 5.2, as determined by measurement with a glass electrode or other pH measuring device of sufficient accuracy. A brown precipitate begins to form in the liquid shortly after addition of the potash alum solution starts and continues as long as this addition proceeds. After the proper pH has been reached, the suspension is allowed to stand for at least a day in the refrigerator before further use. A better vaccine will result if the mixture is shaken continuously during the first 24 hours. During the manufacture of the vaccine enough phenol is added to make it 0.5% with respect to this chemical. After one or more days' ageing, and after appropriate tests to insure bacterial sterility, the vaccine is ready to use.

This adsorbed-virus vaccine could be made in a number of other ways. Other virus-containing organs of the hog, such as the liver, lymph nodes, blood, lungs, etc., could be used. Grinding could be carried out in different ways to provide a finer or a coarser suspension. Different proportions of tissue extract and of virus blood could be employed. Another adsorbent than hydrous aluminum oxide could be added or formed in the solution. The suspension could be made up with physiological salt solution or with buffered salt solution in place of distilled water.

The hydrous oxide precipitate could be made using aluminum salts other than potash alum. In the typical example cited above, alum solution was added to the amount of approximately 0.2% in the virus suspension; satisfactory vaccine could be made with other amounts of alum especially if appropriate amounts of acid or alkali were added to give a final pH of 5.2. As stated above, other adsorbents than hydrous aluminum oxide could be formed in the virus solution or introduced into it for the production of satisfactory vaccine. Suitable adsorbents include calcium phosphate, silica gel, adsorbent carbon, kieselguhr and similar materials which have no deleterious effect when injected with the vaccine. The ratio of adsorbed virus to free virus is not especially critical

although significant amounts of each are required.

It is a very important advantage of a vaccine compounded as outlined above that it is much more stable on storage than is the usual virus blood vaccine and that in consequence it has a much lengthened useful life. In a work published several years ago, McBryde and his coworkers of the United States Bureau of Animal Industry demonstrated that the optimum stability of the virus of hog cholera occurred not at the neutral pH of the blood but in an acid medium of pH 5.2. In reporting this work, these authors pointed out the advantages that would accrue from keeping and using virus at this acid pH, but they concluded that it was not practical since their blood vaccine gelled and solidified on being acidified. The preparation of my partially adsorbed-virus vaccine at a pH of about 5.2 for the first time makes it possible to take advantage of the greatly enhanced stability of hog cholera virus at its point of maximum stability, since at this pH adsorption of the virus occurs under optimal conditions.

It is a further advantage of my partially adsorbed-virus vaccine that less than the usually recommended amount of serum need be used with it for the entirely safe and highly effective immunization of pigs. Safe vaccination can be obtained with a minimal dose of serum using my vaccine since the virus which is immediately liberated and must be counteracted by the added serum constitutes only a fraction of the total that is present in the injected vaccine.

A typical use of adsorbed-virus vaccine for the active immunization of hogs is the following: 60 pound, susceptible hogs are injected subcutaneously with 2 cc. of my partially adsorbed-virus vaccine. At the same time 14 cc. of hyperimmune hog serum is injected subcutaneously into the hog at another point. During the next two weeks vaccinated pigs may be expected to acquire an active immunity to hog cholera without exhibiting any fever or other clinical manifestations of the disease. This immunity may be demonstrated three weeks or more after injection by the subcutaneous injection of 1 cc. of highly virulent hog cholera virus. It will be found that the immunity is sufficiently great so that no symptoms of hog cholera result from the administration of this test dose of virus. Two vaccinated control hogs receiving such a test dose of virus will develop fever and other symptoms of hog cholera in from 5 to 8 days after injection and will usually succumb to the disease within two weeks.

At the present time more than 1000 hogs have been immunized successfully with my vaccine using the scheme outlined above. In vaccinating these animals there have been no untoward reactions and no cases of hog cholera have developed in a vaccinated animal either during the vaccination or at the subsequent time. Some of the vaccinated animals have been from hog cholera infested regions while others were taken from regions in which hog cholera is not prevalent.

In the foregoing examples of successful vaccination the amount of serum used was ½ that prescribed for the existing virus-serum vaccination procedure. This amount is believed to be about the optimum for safe and effective vaccination with partially adsorbed-virus vaccine. Successful vaccination would, however, result from the administration of a larger amount of serum and smaller amounts also could be used. If very small volumes of serum are employed, many pigs will be successfully vaccinated but a certain percentage may exhibit more or less severe symptoms of an active hog cholera infection. On account of the gradual liberation of the infectious principle from my adsorbed-virus vaccine, a wider latitude is permissible in the amount of serum to be administered for safe and solid immunization.

For the purposes of sterilizing the vaccine, I may use other proportions of phenol than that specifically described. Likewise, any other antiseptic may be employed for all, or a part, of the phenol.

It will be obvious that other changes and variations may be made in preparing my vaccine and the invention is not restricted to the specific details described except as set forth in the claims.

I claim:

1. A hog cholera vaccine comprising hog cholera virus associated with hydrous aluminum oxide and an aqueous fluid in such an amount that part of the hog cholera virus is adsorbed onto the aluminum hydroxide, the rest being free in the liquid.

2. A hog cholera vaccine comprising hog cholera virus associated with hydrous aluminum oxide and an aqueous fluid in such an amount that part of the virus is adsorbed onto the aluminum hydroxide, the rest being free in the fluid and the entire mixture being at a pH of approximately 5.2.

3. The process of preparing a hog cholera vaccine which comprises adding potash alum to aqueous infectious hog cholera virus whereby there is precipitated colloidal aluminum hydroxide in an amount such that the resulting suspension contains part of the virus adsorbed to the precipitated aluminum hydroxide and the rest of the virus is free in the aqueous fluid.

4. A process of preparing a hog cholera vaccine which comprises forming an aqueous suspension of infectious hog cholera virus, adding to the suspension an aqueous solution of potash alum until the mixture has a pH of about 5.2 whereby colloidal aluminum hydroxide is precipitated, the amount of aluminum hydroxide being such as to adsorb most but not all of the virus.

RALPH W. G. WYCKOFF.