Brucellosis—Adult Vaccination Program Research

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The people who are experts in public speaking say that you should never start your program with an apology about your public speaking capability or anything else. First of all, if you are a poor public speaker they will find out about it anyway. I have two apologies this morning, one having to do with my not particularly good public speaking ability. The second one is that some of the material that I had hoped to present this morning will not be presented because it has not received prior approval from my supervisors. So, I do hope this matter can be clarified before the *Proceedings* comes out and so that, hopefully, this material will appear in the *Proceedings.** I hope that we can, however, present an interesting program to you on the work that we have been doing in Florida in our adult vaccination work. And so, much of the material that I will present this morning was in fact presented at the Miami meeting of the Animal Health Association in 1976. I will update you on some of the work that we are currently doing.

The control of brucellosis and other diseases in large cattle populations presents some very serious problems, and these are complicated if these susceptible replacements are introduced from several sources. In Florida we have approximately 400 dairy herds which average about 500 cows each. Approximately a third of these replacement cattle are brought in every year and most of them are purchased from other states. When these areas from where we purchase these animals de-emphasize vaccination, and most of them have, then of course this has a very serious effect upon our population in the recipient areas. This, of course, is true in Florida, so that the percentage of protection or vaccination in these herds has decreased over the years. We estimate now that only about 20% of the animals that we import as replacements from other areas of the country are vaccinated. So that, again, depending on a particular herd, up to 80, and in some cases more, percent of the herds are unvaccinated.

In addition to this, because we are importing animals from other areas which are not free of brucellosis, some of these animals are incubating brucellosis and so if these are introduced into these highly susceptible populations, we have some very serious problems.

The kind of problem we face in Florida is what I call "a sea of cows." Then if you have this kind of event occur in these cows, it does not take a very clever person to understand what we talk about when we epidemiologists talk of exposure potential.

Now, in Florida we have found that the probability of having brucellosis infection is completely dependent upon the size of the herd. As the herds become larger, the percentage of those herds having brucellosis increases. In fact, I will quote you a rather startling statistic and that is that in 1972 in our dairy herds we had approximately 4400 reactors. In 1976, this number had increased to almost 8400 reactors. Puerto Rico, another area where I frequently travel, has a somewhat similar situation although the herds are not as large there. Nevertheless, they do import a large number of animals from the states and again most of these are of course coming down unvaccinated as calves. In Puerto Rico in 1972 in the dairy herds there were 738 reactors. In 1976 this number had increased to 2400. So you can see what has happened then in recent years in Florida and Puerto Rico, and again we believe it is largely related to the increasing herd size and, of course, the fact that most of the animals coming in are fully susceptible to the disease.

In 1973 and again in 1974 the concerned dairymen and animal health officials petitioned for the use of adult cattle vaccination to control the spread of brucellosis in large dairy herds. This was denied by the committee, but in 1974 the committee did agree to sponsor adult cattle vaccination studies on selected dairy herds and these began in May of 1975.

Five experimental herds were selected and different dosages of Strain 19 and different methods of its administration were evaluated. Included in this were studies on several serologic tests. We, in fact, included five serologic tests in these studies. These included the tube test, the ethynol test, the card test, the rivinol test and complement fixation. And then these were correlated with exhaustive bacteriological studies in the evaluation of these tests to determine their efficacy sensitivity and specificity in differentiating vaccinal titers from those from which we could prove that the animals were infected. These findings were, as I have already suggested, reported at the 1976 meeting of the Animal Health Association. I would like to tell you then, very briefly, about some of the different approaches that we took in these herds.

The first herd which we studied was approximately

*The results will appear in the 1978 *Bovine Practitioner.*

a 900-cow herd. We decided to vaccinate all of the cows with a standard dosage of Strain 19, the 5 cc calf dosage. Then to determine what could be done in terms of reducing brucellosis and as I have already suggested, the evaluation of the various seriologic tests. In this particular herd we did not have bacteriological capabilities. We began by doing a three-month post-vaccinal test, and six months and so forth, so that most of the testing had been done on a three-month schedule, even to this day. But at the three-month test date we did not have bacteriological capabilities so we did not remove any of the serological reactors. At the six-month date we then began to remove these animals which we could prove were infectious. At that time, of the 900-cow herd, there were approximately 27 that were removed. Since that time the number of animals that have been removed on each of the tests has dramatically dropped and, in fact, up until very recently the last infected animal that was taken out of the herd was in December, 1976. However, in April and May of this year, our old story again–he imported replacement cows and in each of these two loads of cows was an infected animal. So, while we are not in the same situation that we were before, we do have somewhat of an increase in brucellosis again back in the herd. At the last test, we took out approximately eight cows that were proven to be infected.

The second herd we decided to evaluate against the intradermal inoculation of Strain 19. Of course this is not new. Many of you in this room know of the work that was done with intradermal inoculation. We used 0.2 cc intradermally in half of the cattle, approximately 200 head, and our control method was to use the other 200 head using the 5 cc dosage. Approximately the same testing schedules as before, in terms of the numbers of animals removed from the herd and, hopefully then, the protection afforded by these two different methods. We saw essentially no differences in these two. We did see considerable difference in serologic response in that the intradermal inoculation did not give nearly the large serologic responses that we did get from the standard 5 cc dosage. But again there seemed to be very little difference in protection. Same situation in this herd. The herd came along very well and in the early part this year he bought 75 replacement heifers from his father-in-law who had a very badly-infected herd and here we go again, more infection! Again, not a serious problem in terms of numbers of animals, but again reintroduction of brucellosis. These herds are coming along well, but nevertheless, this is our story in many herds in Florida.

The third herd that was included in the studies, as far as I know, is the largest dairy herd in the world. This herd has approximately 8,000 cows. In this herd the approach was somewhat different in that the entire herd was not tested and the reactors removed. The herd was tested on a somewhat piecemeal basis. The animals were tested at the end of their lactation and their serologic-positive animals and card test-positive animals were removed and the negative animals were vaccinated and then essentially not tested again until the end of their next lactation. This procedure has continued to this day. Of course, by now the entire herd has been vaccinated, but we continue to vaccinate the replacement animals that come into the herd, approximately 200 cows every other week, and test the cows that are drying off. We have been able to very dramatically reduce the amount of brucellosis in this herd, but we have not completely eliminated the disease from this herd. It is somewhat difficult to evaluate the efficacy of the vaccine in these cases in terms of protection and particularly since only one vaccination method was used.

In herd four, this was a herd again of approximately 900 cows and in this case we decided to try a reduced dosage of Strain 19. Again, this was not new. Reduced dosages of Strain 19 had been tried some 20-25 years ago and at that time it was found that the reduced dosage seemed to be just as protective as the larger dosage. When I talk in terms of reduced dosage, I am talking in terms of approximately 1/20 of the standard calf dosage. In this case we divided the herd again 50-50. That is approximately 400 remaining in each of the two groups (after we had removed 100 reactors), given the standard dosage and the reduced dosage. Over a period of time, in trying to evaluate the efficacy of these two methods against each other, it was our conclusion that the reduced dosage gave just as good protection as the standard dosage. And as you might expect, there was a considerable difference in the serologic responses of the reduced dosage versus the full dosage. That herd has continued to this day. We are still removing a few reactors from the herd on each three-month test, but there are very few, approximately averaging one cow per month out of the 800-cow dairy.

On herd five we took a different approach. We had been criticized because we had not left unvaccinated controls in these herds and in herd five we did in fact leave 20% of the cattle unvaccinated. We vaccinated 40% of this herd with the standard 5 cc dosage and the remaining 40% of the animals received a conjunctival inoculation. This was based upon some work that has been published from France in which Strain 19 is inoculated into the conjunctival sac. In France they vaccinated these cattle or inoculated them at six-month intervals. That is, they gave them two inoculations at two-month intervals. In their studies they challenged these animals and found that the conjunctival inoculations seemed to be just as effective as the calf vaccination in their challenged animals. So we decided to try this procedure in our herd, in this experimental herd. We have found in comparing the 40-40-20 that in the two 40% groups, that is, those that received the standard dosage and those that received the conjunctival inoculation, there seemed to be no difference in the protection that has been afforded by these. In the 20% we had considerably more infection in the controlled animals than we did in the others. With the conjunctival in-

oculation there is a rare animal that will respond to a card test, but essentially you see no serologic response in any test. The principle behind this inoculation method is that the Strain 19 are filtered by the regional lymph nodes and do not become an aseptic environment as you would have with the subcutaneous inoculation of Strain 19 and that there is some mediated immunity produced by the filtration of Strain 19 by the regional lymph nodes.

In 1976 the Animal Health Association adopted adult vaccination as part of the national program and the recommendation was to adopt the reduced dosage into the program so that the accepted method now for adult vaccination in the national program is to give 1/25 of the standard calf dosage, or approximately 3 x 10^9, subcutaneously. There are certain restrictions on the herd. The herd must be infected and there must be a permanent identification of these animals and a permanent quarantine, but this is now adopted as part of the national program. In Florida we have now vaccinated approximately 70 of our dairy herds, adult vaccination. In Puerto Rico approximately the same number, so that we now have a large number of animals inoculated by this method. We have continued to evaluate each of these serologic tests and in fact have dropped some of the tests–ethynol test and standard tube test–which we had found did not seem to provide any usefulness in diagnosis. And so these have been dropped from our work. We continue then to use the card test and the screening tests in these cattle using the rivinol test, and then the complement fixation test largely as the final diagnostic method.

In the experimental herds, in less than 1% of the cattle that were inoculated have we been successful in finding Strain 19 in the milk. In the subsequent studies in the vaccinated herds in Florida and Puerto Rico, this has even been considerably less than that.

One of the difficulties of the entire adult vaccination program is the fact that you are going into infected herds and you are inoculating quite clearly some animals that are incubating the disease. We certainly have no evidence whatsoever that the vaccine will change the course of the disease in these animals. And so then it is on the first post-vaccinal test that you identify a considerable number of infected animals. Again we believe that this is not a failure on the part of the vaccine to protect. This is simply those animals that you would have identified had no vaccine been used at all. On the second post-vaccinal test–this has held true in all of our experimental herds and certainly in our herds that we are now starting to re-test as part of our program in Florida and Puerto Rico–that is when you see the dramatic drop in the numbers of animals that would be removed from the herd.

We have continued as part of the study to do a cost-benefit analysis in the four herds. We hope to expand this now into some of our other herds in terms of comparing the costs to the producers in a before-and-after story. I do not know another way to do this other than how many reactors had the man lost before the vaccine was administered and how many has he lost since that time? We have had some very dramatic reductions in the cost to the producers and subsequently, of course, to the governments in terms of indemnification and cost of testing animals in these herds, up to as much as 80% reduction of costs to both the producers and to the governmental agencies. In comparing the numbers of animals removed from these herds, in a before-and-after story, if one compared the several months prior to this kind of inoculation and several months post-vaccination, we have had in most of our herds well over a 90% reduction in the numbers of animals that have been removed from the herd.

In 1966 some Australian workers published results of a new serologic test for bovine brucellosis. This was called the indirect hemolytic test. This particular test uses soluble antigen which is coated on the bovine red blood cells and in the presence of complement and specific antibodies, these then become litics for the bovine red cells.

We are doing this test in microtiter. The serums must be inactivated as in the complement fixation test. We began the test with a 1:4 1:8 and serially diluted these up to a 1:128 test. So that in the first row, looking up and down, you can see that in the first there is hemolysis there. This then would be a 1:4 titer in this particular test. On the next one, of course, having unhemolyzed cells all the way up, this then would be a negative test, and so forth. You can read across the different kinds of reactions. This microtiter plate, after a 1-hour incubation, is then centrifuged and the unhemolyzed cells go to the bottom. For those of you who are familiar with reading the complement fixation test, it takes some degree of mental readjustment to read this test because you are reading precisely the opposite to what you do with a complement fixation test.

In any case, we are continuing to evaluate this test in our laboratory, comparing it using serums from a variety of animals that have been inoculated in various ways and comparing this then with bacteriologic results, largely milk samples, but in some cases tissue samples from those animals. We also hope to continue to do some work on improved bacteriological diagnoses.

So, if I could very briefly summarize what we have found in our herds. The large unvaccinated populations, the concentrated populations, cause great difficulties in terms of eliminating brucellosis through test and slaughter programs. Adult vaccination has been a great assistance to us in these herds in reducing the amount of brucellosis in these herds. It is certainly more likely to be a problem in these herds if you are importing susceptible animals and in some cases we have clearly shown that we are importing animals that are incubating the disease, that is, these animals that are negative at the time they are tested. As a matter of fact, they undergo a number of tests from the time they leave herds, for example, in

Wisconsin and finally end up in Florida and then subsequently break out with brucellosis when they finally reach our susceptible herds in Florida. Studies in Florida, using Strain 19 in a variety of vaccinal doses and methods, have not shown largely any differences in protection from Strain 19 regardless whether the dosage was the full dosage or by the method of inoculation that we have used. In all cases we seemed to have found excellent protection by inoculating Strain 19. Finally, the reduced dosage, 1/25 of the standard dosage, given subcutaneously is now adopted into the national program. We have done and continue to do cost-benefit studies and we hope to continue these studies in the herds, comparing pre- and post-vaccination studies.