THE CONTROL OF ANIMAL BRUCELLOSIS IN MALTA

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Little publicity has so far been given to the work done in Malta in recent years towards the control of animal brucellosis, especially brucellosis in goats. This article describes work carried out between September 1956, and April 1968 at the Brucellosis Laboratory, Government Farm, Ghammieri, and on farms in Malta and Gozo. It is based on a report about to be submitted to the Government of Malta by the Food and Agriculture Organisation of the United Nations. This organisation has done much to assist the work, especially in the last four years.

The Brucellosis Laboratory at Ghammieri was built about 1936 for the purpose of investigating possible ways of eliminating brucellosis from the goat herds of Malta. The laboratory functioned for about 2 years after its completion; a good deal of general information was collected and some experimentation with immunizing agents for goats was undertaken with inconclusive results. Following the resignation of the original officer in charge and the outbreak of war, the laboratory remained closed for almost twenty years until the present phase commenced.

The programme of work about to be described originated from the report of an F.A.O. Mission under the leadership of Professor Lindsay Robb. The mission carried out a general survey of Malta's Agricultural Resources in 1955 and its report contained a recommendation that a campaign be organized on a national scale to control and, if possible, eradicate brucellosis.

The principal aim of the programme was the control of *caprine brucellosis*. At the time the work began there was no proved method of immunisation against brucellosis available for goats nor was there a proved diagnostic test available for this species on which a programme of eradication by test and slaughter could be based. The first task undertaken was to determine the incidence of brucellosis in the goat population in order that realistic plans could be made for its control and also to provide a yardstick against which the progress made could be assessed. Bacteriological examination of a selection of lymph nodes and tissues taken from a sample of 139 goats slaughtered at the Civil Abattoir detected *Br. melitensis* infection in 25 (18%) of them. This rough estimate of the incidence agreed closely with the figure that had been arrived at one year earlier by the Medical and Health Department using serological methods.

In view of this high incidence of the disease it was decided that immunization would be required to reduce the rate of infection before it would be practicable to attempt more radical methods. Extensive experimentation carried out in Tunis and sponsored by WHO and FAO had suggested that despite serious side effects a killed adjuvant vaccine would provide a worthwhile immunity. About this time, however, Dr. Elberg of the University of California announced the development of a living attenuated *Br. melitensis* vaccine called Rev. 1 and in the light of the experimental data presented it was decided to concentrate the resources available in Malta on evaluating the suitability of this vaccine for use under local conditions. During 1957/8 an experiment was carried out in which both kids and adult goats were vaccinated with Rev. 1 supplied by Dr. Elberg. These animals were exposed to *Br. melitensis* infection by natural contact during their first pregnancy after vaccination and were shown to have a useful de-

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gree of immunity. A sharp serological reaction followed vaccination and there was some slight excretion of Rev. 1 in the milk of 2 goats vaccinated while lactating but otherwise no undesirable side effects were detected.

Following the successful laboratory experiment a small scale field trial was carried out in 1958 when 122 kids were vaccinated on infected farms. A limited follow-up investigation the next spring indicated that a significant immunity had been produced in these animals when comparison was made with a sample of similar non-vaccinated kids. In 1959 local production of Rev. 1 vaccine was begun in Malta and it was made available for field use. As it was by then known that Rev. 1 was capable of causing abortion if used on pregnant goats its use was confined to young goats before first mating.

The limitation that had to be placed on the use of Rev. 1 led to an experiment being carried out in goats in which Rev. 1 was compared with the killed *Br. melitensis* adjuvant vaccine, which being dead could have a wider application. Both vaccines produced immunity without there being any significant difference between them in the level of immunity produced but the adjuvant vaccine stimulated a serious local lesion and a persistent high level of serum antibodies including complement fixing antibodies. These two drawbacks, especially the latter, led to work with the adjuvant vaccine being abandoned.

Following reports from the U.S.S.R. that brucella-infected animals with a live vaccine was inadvisable, an experiment was carried out in which Rev. 1 was injected into goats that were already infected with *Br. melitensis*. Vaccination appeared to be without effect on the general health of treated goats or on the duration of the natural infection; also 6 control goats aborted as compared with none of an equal number of Rev. 1 vaccinated goats.

The vaccination of young goats in the field continued on a small scale in 1959 and 1960 and attempts were made to assess the result in the year following vaccination by examining milk samples bacteriologically. *Br. melitensis* was isolated from 1.2% of vaccinated and 6.5% of non-vaccinated goats, i.e. in milk samples from slightly more than 600 animals.

The Rev. 1 vaccine had now become accepted as providing an effective immunizing agent for use under Maltese conditions but it was necessary to determine the length of the immunity produced in order to know whether re-vaccination would be required. An extensive experiment was carried out between 1962 and 1967 in which goats that had been vaccinated as kids were divided into two groups for challenge. One group was challenged $2\frac{1}{2}$ years after vaccination by natural exposure to *Br. melitensis* infection during pregnancy. The other group was similarly challenged $4\frac{1}{2}$ years after vaccination. In both cases an equal number of controls were included. All these animals had been kept in a brucellosis-free environment between the vaccination and the exposure to infection. Both challenge groups showed a high degree of immunity and it was concluded that Rev. 1 gave effective immunity lasting for at least $4\frac{1}{2}$ years.

In collaboration with Dr. Elberg in the University of California the stability of the degree of attenuation of the Rev. 1 strain was carefully studied. A series of passages of the vaccine strain through pregnant goats was considered to be the method most likely to encourage a mutation towards greater virulence in the Rev. 1 strain. After 6 serial passages through goats had failed to alter the virulence of the strain as judged by the persistence of isolates when injected in guinea pigs, it was concluded that there would be no risk of virulent mutants arising from the field use of the Rev. 1 vaccine.

Rev. 1 vaccine was produced in Malta from 1959 onwards at first in liquid form but late as a desiccated vaccine. The only major difficulty encountered in production was the liability of Rev. 1 to dissociate, producing rough types. Eventually a somewhat complicated system was developed to overcome this danger. Studies carried out in guinea pigs suggested that when the vaccine was being used in normal field doses a high degree of dissociation could

be tolerated without loss of immunizing efficiency; nevertheless, it was considered that a non-varying product would be desirable and only those batches of vaccine that contained a low percentage of rough cells (less than 5%) were used in field vaccination.

Field vaccination of kids continued each year on a voluntary basis. The numbers vaccinated increased slowly until in 1963 a total of 1,716 kids were vaccinated. By this time it was realised that a voluntary scheme would not achieve the desired objective and in 1964 legislation was introduced making compulsory the vaccination of all female goats when they are between the ages of 3 and 6 months. Despite legal compulsion the annual number of vaccinations never rose above 2,760, a figure considered to represent about half the female kids available for vaccination. In spite of disappointment with the number of vaccinations performed there was evidence that the level of caprine brucellosis had been falling steadily.

A major requirement of the programme was to find a diagnostic test sufficiently reliable for use in a test and slaughter campaign. Three types of material were used to evaluate the efficiency of the tube agglutination test and the complement fixation test. (1) Serum from goats slaughtered at the Civil Abattoir with a collection of lymph nodes and tissues from the same goats for culture. (2) Weekly serum samples from control goats in a vaccination experiment; these goats had been exposed to *Br. melitensis* infection by natural contact and then subjected to intensive cultural examination during life and at slaughter a few months after first being exposed to infection. (3) Weekly serum samples from non-vaccinated control goats, chronically infected with *Br. melitensis* and subjected to the same bacteriological examinations as those in (2).

Both tests were frequently negative in goats that were infected with *Br. melitensis* and frequently positive, sometimes in high titre, in goats from which no *Br. melitensis* organisms could be isolated. The complement fixation test appeared to be very specific, but the agglutination test became positive sooner and thus was more effective in providing warning of impending abortion. By the time this investigation was completed, it had become known that vaccination with Rev. 1 would seriously interfere with agglutination tests but not with complement fixation tests carried out on vaccinated goats; the latter test, therefore, was the only one that could be considered for use in a test and slaughter campaign in Malta. It was thought likely that any test could be expected to perform better under field conditions than in a strictly theoretical evaluation and furthermore that the real value of a diagnostic method for an eradication scheme could only be established by its actual use in such a scheme. For these reasons, plus the fact that it was desirable to make a start on the eradication of caprine brucellosis, a voluntary eradication scheme based on the complement fixation test was initiated. The results from the first fifty herds included in the scheme did, in fact, suggest that the complement fixation test is effective under such conditions.

Under the voluntary eradication scheme positive goats had to be slaughtered by the owners without compensation but an annual per capita bonus payment was made for each goat in herds registered as brucellosis-free. Testing began in 1965 and after it had been decided to concentrate on the island of Gozo steady progress was made. By the end of January, 1967 a total of 51 herds in Gozo had been registered as brucellosis-free. At this time, however, orders were issued by the administration to stop testing on the grounds that compulsory testing and slaughter with payment of compensation by Government for goats slaughtered, would be less costly. In spite of legislation being passed in May, 1967 to provide legal backing for the compulsory scheme, up to April, 1968 the necessary Government approval to go ahead with the compulsory scheme had not been given and the facilities and personnel available for testing goats were not used for that purpose throughout most of 1967. Over that period it was not possible even to retest herds that had been declared brucellosis-free in previous years

and some had passed nearly two years since their last test. However, in January, 1968 strenuous representations to Government resulted in permission being obtained to recommence testing under the old voluntary scheme. By April, 29 herds previously certified as brucellosis-free had been re-tested and obvious re-infection had occurred in 2 of them where there had been unauthorised introduction of untested goats; re-tests were continuing.

The percentage of goats found positive in herds tested for the first time under the eradication scheme was about 4%.

Extensive studies were carried out in the Brucellosis Laboratory to determine whether a reduction in the dose of Rev. 1 cells injected would alter the response of the adult goat. These studies were undertaken to try to find a way of immunizing adult goats that might be pregnant without causing either abortion or excretion of Rev. 1 cells. In preliminary experiments in guinea pigs it was found that a dose considered to be about the minimum 100% infective dose, set up an infection that was very similar to that produced by a dose one million times greater, except that following infection with the small dose there was a delay of 2 to 3 weeks in the establishment of generalized infection and in the development of a serological response. A second experiment in guinea pigs indicated that a high degree of immunity followed vaccination with either the very low or the very high dose.

In goats it was already known that the normal vaccinal dose of Rev. 1, i.e. 10^9 cells, regularly caused abortion in pregnant animals with copious excretion of Rev. 1 cells from the vagina and also from the udder. Two lesser doses, 10^6 and 5×10^4 cells were tried in pregnant goats. It was found that a dose of 10^6 was still capable of causing both abortion and excretion of Rev. 1 cells, though in a lesser degree than the normal vaccine dose. At the lower dose (5×10^4), however, there was neither abortion nor excretion in the small group of pregnant goats used. Some time after the completion of their pregnancies the animals were challenged, along with non-vaccinated controls and it appeared that even at the lowest dose some immunity had been produced, but statistically significant differences were not obtained.

When the dose of 5×10^4 Rev. 1 cells was given to a larger group of 30 goats, most of which were at varying stages of pregnancy, there was no sign of abortion or excretion. Challenge of these goats with graded doses of *Br. melitensis* indicated that significant immunity had been produced though the level of this immunity appeared to be lower than that produced by normal full doses.

During the course of a vaccination experiment it was found that kids born into an environment heavily infected with *Br. melitensis* usually became infected but that the infection seldom persisted beyond 2 months of age. Maternal antibodies disappeared within 4-6 weeks; no antibodies were actually produced by the kids during the first two months of life, but frequently appeared later, i.e. at a stage when it was no longer possible to isolate *Br. melitensis* on autopsy. It was concluded that serological tests in immature goats have no diagnostic value.

Br. melitensis isolated from goats' milk was often found to be in the rough phase. This phenomenon poses problems in diagnosis, as these strains behave abnormally to routine serological identification. It was shown, however, that an anti-rough brucella serum could be produced and this assisted greatly in identification of rough strains.

The possibility of using a dissociated strain as a vaccine was investigated using the guinea pig as an experimental animal. All the strains of *Br. melitensis* examined appeared to be too attenuated to produce much immunity; one such strain was passaged 20 times through guinea pigs in an attempt to increase its virulence but without effect. In spite of the fact that this line of investigation failed, it is considered that a sufficiently prolonged search could be expected to find a strain that is immunogenic for goats without producing troublesome levels of antibodies.

Investigations into the possibility of using stained antigens to produce an agglutination test that could be applied to

bulk samples of goats' milk, similar to the milk ring test now commonly used with cows' milk, were continued over a number of years. Such a test would be invaluable for checking the continued freedom from infection of brucellosis-free herds, and would eliminate the necessity for periodic testing of individual animals. It was found that these antigens were over-sensitive when applied to individual animals, yet not sufficiently sensitive when applied to samples taken from cans handed in at milk collecting centres, presumably because of over-dilution resulting from the fact that one can may contain the milk of up to 30 goats. The potential benefits that could result from the development of such a method are so great that further investigation would seem to be well worth pursuing.

Several hundred *Br. melitensis* cultures isolated in the field in Malta and Gozo were subjected to a thorough typing examination. All were biotype 1, except for a few isolations from one herd of goats that were biotype 2; this herd was also infected with biotype 1 and it seems possible that a mutation had occurred.

In a search for a serological test effective in vaccinated goats but less complicated than the complement fixation test, the heat-inactivation, mercaptoethanol and rivanol agglutination tests were all investigated. It seems possible that the mercaptoethanol test may effectively replace the complement fixation test, but more study is required before such a change can be recommended.

During the course of this work the incidence of human brucellosis in Malta showed a marked and constant decline and it is considered that some of this decline must have resulted from the measures taken to control caprine brucellosis.

Bovine brucellosis: milk ring tests were carried out on can samples from all the herds on the island of Malta late in 1956, and 74 herds (23% of those tested) gave a positive result. Later cultural investigations indicated that in about two-thirds of the infected herds the infecting organism was *Br. abortus* and that in the rest it was *Br. melitensis*. A majority of the abortus strains encountered were biotype 3, the rest being biotype 1.

A scheme for the eradication of bovine brucellosis was began in 1957 to run concurrently with the eradication of bovine tuberculosis. Progress was slow and somewhat erratic, but in 1961 participation in the scheme was made compulsory, and by 1962 it was estimated that only 5 or 6 herds remained infected with *Br. abortus*. It was now realized that the majority of infected herds were infected with *Br. melitensis* presumably from contact with infected goats. It was considered uneconomic to slaughter *Br. melitensis* infected cattle until the eradication of caprine brucellosis had been carried out. Henceforth reactors in herds known to be infected with *Br. melitensis* and not with *Br. abortus* were not slaughtered. In 1964 only one animal infected with *Br. abortus* was detected and none in 1965. Hopes that *Br. abortus* had been eradicated were dashed in 1966 and 1967 when a small number of outbreaks were detected. *Br. abortus* infection was not detected on the island of Gozo during the period covered by this article.

Some studies on *Br. melitensis* infection in cattle were undertaken. This infection normally only occurs in cattle in areas where caprine brucellosis is present. Nevertheless *Br. melitensis* is capable of causing abortion in cattle and is often excreted in the milk of infected cows for long periods, e.g. for at least one year. It is capable of stimulating a serological reaction that may remain at a high level for years. Spread of infection from cow to cow does appear to occur but less readily than is the case with *Br. abortus* infection.

Investigations into *Brucellosis in other species* were confined to sheep and pigs. Serological and cultural examination of samples taken from the abattoir in 1958 indicated that about 1% of individuals were suffering slight infections with *Br. melitensis*. It was considered that these infections had resulted from contact with infected goats and that when brucellosis has been eradicated from the goat population, it will cease to be a problem in sheep and pigs.

The F.A.O. report already mentioned contains a number of recommendations which it is hoped will help to bring the campaign for the eradication of brucellosis from Malta to a successful conclusion. The achievement of this objective would be a considerable event in Malta's history.

Most of the field work described here, especially in the campaign for the control of bovine brucellosis, was carried out by the Veterinary Service. Dr. André Cassar was Principal Government Veterinary Surgeon throughout the period and his consistent support and patient co-operation was a major contribution to whatever progress was made.