

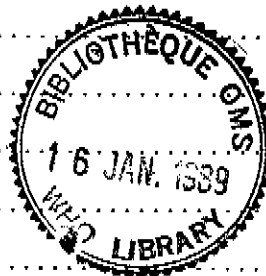


REPORT OF THE FAO/WHO INFORMAL MEETING ON  
ORAL/CONJUNCTIVAL BRUCELLOSIS VACCINE

Rome, Italy, 6-7 SEPTEMBER 1988\*

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\* Food and Agriculture Organization of the United Nations, Rome, Italy

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Dr R. J. Olds, Animal Health Officer, Food and Agriculture Organization of the United Nations, Rome, welcomed all the participants. He stressed the importance of the meeting with respect to productive livestock development and health.

Professor A. Mantovani, Istituto Superiore di Sanità, Rome (WHO Collaborating Centre for Research and Training on Veterinary Public Health), also welcomed the participants on behalf of the Collaborating Centre.

Dr T. Fujikura thanked FAO and Istituto Superiore di Sanità for hosting the meeting and recalled the aims of the meeting, as follows:

- (1) To review the working group activities on oral/conjunctival brucellosis strain 2 vaccine and the results of the collaborative studies conducted since the meeting held in Weybridge in September 1987<sup>1</sup>.
- (2) To discuss further collaborative research and plans of work for the next term.
- (3) To discuss conditions and regulations at international level, taking into account the biosafety and precautionary measures involved in introducing live attenuated brucella vaccine strain.

Dr T. Fujikura and Dr R. J. Olds opened the meeting on behalf of the Directors-General of the World Health Organization and the Food and Agriculture Organization of the United Nations.

Professor A. Mantovani was elected Chairman; Dr A. A. Mustafa served as Rapporteur.

#### Introduction

The meeting commenced with the presentation of papers on the collaborative studies carried out by the FAO/WHO Collaborating Centre for Reference and Research on Brucellosis, Central Veterinary Laboratory, Weybridge, UK, the Institut national de recherche agronomique, Nouzilly, France, the Pan American Zoonoses Centre, Buenos Aires, Argentina, and the FAO Project, Tripoli, Libya.<sup>2</sup>

1. Results of collaborative studies on Brucella suis strain 2 and the vaccines
  - 1.1 Differentiation of Brucella suis strain 2 from field strains

Brucella suis strain 2 originated in China where it has been used as an oral vaccine. The World Health Organization expressed interest in this strain and initiated a research programme for its evaluation following the recommendations of the Joint FAO/WHO Expert Committee on Brucellosis, Geneva, November 1985.<sup>3</sup> As part of this programme, a working group has searched for markers which will differentiate the

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<sup>1</sup> Report on collaborative studies on safety and potency of oral brucellosis strain 2 vaccine, Weybridge, United Kingdom (1987). WHO Restricted document.

<sup>2</sup> UTFN/LIB/025, FAO Project, c/o UNDP Office, P.O. Box 358, Tripoli, Libya.

<sup>3</sup> Joint FAO/WHO Expert Committee on Brucellosis. Sixth Report. Technical Report Series No. 740, WHO Geneva, 1986.

vaccine strain from field strains. Strain 2 and the B. suis biovar 1 strain 1330 were compared using antimicrobial agents and tests for pre-formed enzymes. Discriminatory tests were repeated with 12 field strains of divergent geographical origin.

Antibiotic assays were carried out on serum dextrose agar using Oxoid sensitivity discs. The antimicrobial compounds tested comprised penicillins, aminoglycosides, peptides, cephalosporins, macrolides, nitrofurans, lincosamides, nitroimidazoles, tetracyclines and unclassified chemicals. Preformed enzyme tests comprised chromogenic substrates for carbohydrate fermentation, aminopeptidases, lipases, glycosidases and esterases.

Ampicillin, cefamandole, cefoperazone, ceftriaxone, cephalixin, latamoxef and polymixin B differentiated between strain 2 and the type strain but could not differentiate strain 2 from the field strains. Similarly, the preformed enzyme tests for L-arabinose, sorbitol, fructose, galactose, mannose and tributyrin could differentiate between strain 2 and 1330 but not between strain 2 and the field strains.

## 1.2 Virulence, immunogenicity, and duration of immunity

### 1.2.1 In mice:

It was shown in a preliminary report<sup>1</sup> that:

(1) vaccinal strain B. suis strain 2 has a lower residual virulence than vaccinal strain B. abortus strain 19 and B. melitensis strain Rev 1;

(2) strain 2 induces immunity against the reference strain B. abortus 544 equal to that induced by strains 19 and Rev 1.

Because strain 2 is intended for use by the oral or conjunctival route, which implies colonization of the regional lymph nodes before extension to other lymphoid organs, a comparative study in mice was made of colonization of the popliteal node after a subcutaneous footpad injection, with strains 2, 19 and Rev 1. In contrast with strains 19 and Rev 1, strain 2 did not consistently colonize the lymph node. However, it colonized the spleen, and immunity induced by this footpad vaccination against B. abortus strain 544 was equal to that of strain 19 and Rev 1 when tested 100 days after vaccination.

Immunity induced in mice by the three vaccine strains at 45 and 150 days after vaccination was tested with three challenge strains: B. abortus 544, B. melitensis H 38 and B. suis 1330. Whereas Rev 1 vaccine strain induced good, long-lasting immunity against the three challenges, strain 19 induced good, persisting immunity against B. abortus and B. suis and lower, but persisting, immunity against B. melitensis. In contrast, strain 2 induced good immunity against the three challenges at day 45, but immunity against B. melitensis waned with time.

These differences may result from:

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<sup>1</sup> Bosseray N. and Plommet, M.: Souche vaccinale Brucella suis S2, virulence, immunogénicité, caractères marqueurs. December 1987. (Document BRUC.VACC./WP/88.4.1) (See Annex II).

(a) serotype specificity, since strains 19, 544, 2 and 1330 are of the A-type, whereas strains Rev 1 and H 38 are of the M-type;

(b) higher residual virulence (persistency) of strains Rev 1 and 19 compared to strain 2, implying better immunogenic stimulation.

The next goat and sheep experiments should give high priority to lymph node colonization and persistence of immunity.

#### 1.2.2 In guinea pigs:

##### 1.2.2.1 Virulence

Results obtained from virulence tests in guinea pigs vaccinated by the subcutaneous route showed that ten days after vaccination, sacrificed guinea pigs did not present lesions. The vaccinal strain was isolated from the animals that were administered  $10^9$ ,  $10^7$  and  $10^5$  cells. These animals were serologically positive.

Guinea pigs sacrificed at day 49, although serologically positive, did not show visible lesions and the vaccinal strain could not be isolated from any of them.

When vaccination was performed by the oral route (1 dose), 14 days after vaccination, sacrificed guinea pigs did not show lesions although they were serologically and bacteriologically positive. The two guinea pigs that were sacrificed at day 41 were positive to serological tests only.

When 2 doses of vaccine were administered by the oral route, the vaccinal strain could be recovered 17 days later in one of the two sacrificed guinea pigs. Both animals were serologically positive and showed no lesions. Thirty days after vaccination, another two animals were sacrificed; these were serologically and bacteriologically positive and one of them showed brucellosis-typical lesions.

##### 1.2.2.2 Immunity

Results obtained in this study indicated that 25 days after challenge all vaccinated guinea pigs were serologically positive but did not show lesions; the challenge strain could not be isolated from any of them. Control animals had brucellosis-typical lesions, were serologically positive and the challenge strain of B. abortus biovar 1 strain 2308 was isolated from all of them and further confirmed by bacteriological studies. Fifty days after challenge, control animals showed lesions and were serologically and bacteriologically positive. All guinea pigs vaccinated were serologically positive and none showed lesions. The strain could only be isolated from two guinea pigs vaccinated with  $10^5$  cells and challenged with  $10^4$  cells.

In conclusion, results obtained in these studies indicated that the pathogenicity of the vaccinal B. suis strain 2 is very low when administered to guinea pigs by the subcutaneous or oral routes.

The results obtained in guinea pigs vaccinated by the subcutaneous route confirm those obtained by Chinese investigators.<sup>1</sup> The immunity conferred by the vaccine is acceptable when challenged with B. abortus strain 2308.

Results obtained from these preliminary studies are sufficiently promising to justify the continuation of the experiments.

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<sup>1</sup> Xie Xin, Orally administrable brucellosis vaccine B. suis strain 2 vaccine. Vaccine, 4 212-215 (1986)

### 1.3 Results of strain 2 vaccine field trial in Libya<sup>1</sup>

B. suis strain 2 brucellosis vaccine was tried orally on a flock of sheep and goats belonging to the Sheep and Camel Raising Project, Charian, Libya, composed of 446 adult ewes, 50 young lamb ewes and 20 adult goats, including an unvaccinated control group composed of 50 adult ewes, 10 lamb ewes and 5 goats. Observation of safety and antigenicity of the vaccine, persistence of antibodies, and immunogenicity in challenge tests, was made for one year.

#### 1.3.1 Safety

With respect to safety of the vaccine, the results of the trial confirmed the findings of the Chinese scientists in that the vaccine was safe even when twice the recommended dose was administered. No untoward reactions were observed in the vaccinated animals, and none of the pregnant ewes and goats aborted irrespective of whether they were vaccinated before or at different stages of pregnancy. The lambing record of the flock that year was excellent compared to the other flocks in the area. This is very important for the prevailing mode of husbandry, where breeding rams and billy goats are continuously with the flock and breeding is not controlled. The results also indicated that there was no shedding of brucella in the milk or the vaginal discharges of vaccinated ewes and goats at lambing, since no bacteria could be isolated from the samples taken, and none of the in-contact, non-vaccinated control ewes and breeding ewes developed a significant serological titre to brucellosis.

#### 1.3.2 Antigenicity of the vaccine

The results of serological tests indicated that the vaccine elicited a good antibody response which rapidly disappeared. In adults, after one year, a small proportion (2.25%) showed persistent antibody level. In lambs of about 6 months, antibodies completely disappeared after one year. This also confirmed the results of the Chinese scientists that vaccination with this vaccine does not present problems for future serological testing of animals. This is of special significance, since it allows freedom of action if it is decided, at a later stage, to adopt a combined approach of vaccination and test and slaughter for eradication of brucellosis.

#### 1.3.3 Protective capabilities of the vaccine

Protection rate in the goats was 71.4%, while the average for the ewes was 53.3%. The results are summarized in Table 1 (page 7). The relatively low protection rate results, in comparison to the 82% rate reported by the Chinese workers, could be attributed to several factors: a major factor is the drastic challenge with 2 virulent field strains given by a route of maximum absorption such as the conjunctival mucous membrane.

It is known that cell mediated immunity is of significance in the immune response of the sheep to bacterial infections such as brucella, pasteurilla and listeria, rather than humoral immunity, which is important in toxin neutralization in the process of the immune response to clostridial infections such as enterotoxaemia. This type of immunity is perhaps more prone to penetration by massive doses of infection than the humoral type of immunity.

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<sup>1</sup> UTFN/LIB/025, FAO Project, c/o UNDP Office, P.O. Box 358, Tripoli, Libya

A probable factor causing variations in the rate of protection could be the different stages of pregnancy of the individual animals tested, which was beyond the experimental control. Since it is certain that, in nature, animals are usually exposed to a much smaller dose of infection, it seems justified to conclude that the results indicate that B. suis strain 2 does indeed stand up to the claim of the originators in that it is a safe, easy-to-apply vaccine, which could be of value in situations where vaccination to control the disease in infected flocks is indicated.

Vaccine Dose Group C.F.U.*	Total No. Tested	No. Aborted	No. Non-Pregnant Infected	Total No. Infected	Total No. Protected
3.75 X 10 <sup>9</sup>	15	6	1**	7	8
5 X 10 <sup>9</sup>	17	9	0	9	8
7.5 X 10 <sup>9</sup>	14	4	1**	5	9 (53.3%)
1 X 10 <sup>10</sup>	14	6	1**	7	7
Goats***	7	2	0	2	5 (71.4%)
Control	10	7	3 <sup>+</sup>	10	0

Average protection rate: 55.22%, while the infection rate in the non-vaccinated control group was 100%.

+ One ewe died and B. melitensis isolated from internal organs.

+ One lambed normally one day after first challenge dose, later developed bursitis. At slaughter, B. melitensis was isolated from internal organs.

+ One was empty and B. melitensis isolated from internal organs at slaughter.

\* Colony-forming unit.

\*\* B. melitensis isolated from organs at slaughter.

\*\*\* Comprised of animals vaccinated with doses of 5 X 10<sup>9</sup>, 7.5 X 10<sup>9</sup> and 1 X 10<sup>10</sup>.

## 2. Protocols for collaborative studies on strain 2 vaccine production, standardization and control, and the controlled experiments in sheep and goats

### 2.1 Biological characteristics of strain 2

Since little specific information was available,<sup>1</sup> strain 2 (obtained from WHO through the Central Veterinary Laboratory, Weybridge, UK) and the vaccine were kept, controlled and standardized by procedures similar to those used for strain 19 and Rev 1 described by Alton et. al.,<sup>2</sup> and indicated in reports of WHO expert committees.<sup>3-4</sup>

Whilst strain 2 is a typical B. suis biovar 1 strain, indistinguishable from other B. suis biovar 1 strains (except for minor characteristics and low virulence in mice), it does have specific growth characteristics, in particular a higher growth rate than B. abortus and B. melitensis, the ability to grow in an extended temperature range (15°C to 44°C) and a minimal nutrient requirement. Consequently, in animal experiments, the vaccine strain may be differentiated from challenge strain either by specific tests, i.e. B. suis versus B. melitensis (growth characteristics on isolation plates followed, if required, by other tests), or, in the case of B. suis challenge, by special differentiation tests with reference strain 1330, incubation of Blood Agar Base (BAB) plate at 20°C can be used. Strain 1330 developed normal colonies in 12 days, in the same quantity as it would develop in 3 days at 37°C, while strain 2 formed small colonies after 14 days at 20°C. Alternatively, antibiotic sensitivity can be used.

As with other Brucella strains, genetic stability is an important feature in particular for a vaccine strain. Hence, great care should be taken to avoid smooth to rough variation in conservation of the strain and in vaccine production, as described for strain 19 (from short experience, strain 2 looks stable since such variation has not been observed). In addition, genetic variations not related to smooth and rough colonial morphology may affect virulence and immunogenicity. To avoid such inapparent modifications, control of both traits should be performed on each original seed strain as indicated below.

### 2.2 Control of strain 2

It should be ensured that the ampoules contain original strain stocks and/or seed lots. Before being distributed or used in pilot experiments, the vaccine strain should be tested for:

- (1) residual virulence - it should not exhibit a significant difference in virulence than the original strain. This may be tested by measuring the time taken for mice to recover from a standardized immunization;
- (2) immunogenicity - the strain should protect mice against the standard B. abortus strain 544 challenge.

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<sup>1</sup> Report on collaborative studies on safety and potency of oral brucellosis strain 2 vaccine, Weybridge, United Kingdom (1987). WHO Restricted document.

<sup>2</sup> Alton, G.G., Jones, L.M. and Pietz, D.E.: Laboratory techniques in brucellosis. Second edition. WHO Monograph series No. 55.

<sup>3</sup> WHO Expert Committee on Biological Standardization: Twenty-Second Report, 58-70, WHO Technical Report Series, No. 444, 1970.

<sup>4</sup> WHO Expert Committee on Biological Standardization: Twenty-Eighth Report, 85-97, WHO Technical Report Series, No. 610, 1977.

### 2.2.1 Virulence:

Two tests can be performed: determination of the 50% recovery time (RT50) in 4 points or a simplified, alternative test in 1 point. However, determination of RT50 is more accurate and is highly recommended.

Recovery time: Group of 32, 5-6 week old female Swiss (CD-1) mice are subcutaneously injected with  $10^8$  live strain 2 bacteria, which are photometrically estimated on a fresh suspension, then controlled by CFU plate count. After 3, 6, 9 and 12 weeks, total spleen individual cultures are performed, by spreading each spleen homogenate onto at least 3 trypticase soy agar (TSA) or BAB plates.

The RT50 is calculated by a probit transformation test of Bonnet-Maury *et al*<sup>1</sup> and must not be significantly different from the RT50 of reference strain 2 ( $5.6 \pm 1.3$  week).

Alternative test: Group of 24, 5-6 week old female Swiss (CD-1) mice are subcutaneously injected with  $10^8$  live strain 2 bacteria, as above. After 8 weeks, spleens are removed, individually ground in 10 volumes of diluent. Cultures are performed by spreading two fractions of 0.4 ml of the spleen homogenate onto TSA or BAB plates. This allows a level of detection as low as 5 organisms per spleen.

Three mice or less may be found infected at autopsy.

### 2.2.2 Immunogenicity:

Control of immunogenicity of strain 2 should be performed as previously described for other Brucella vaccines<sup>2-3</sup> and supported by a report on strain 2.<sup>4</sup>

One group of 24, 5-6 week old female CD-1 mice is inoculated subcutaneously with strain 2 to be tested ( $1 \times 10^5$  CFU), and 1 group of 12 mice with the reference (killed) vaccine (20 units) or with strain 19, taken as reference (subcutaneously,  $10^5$  CFU). One control group of 12 mice (placebo) is injected with saline.

Forty five days after inoculation, the mice are intraperitoneally challenged with B. abortus reference strain 544 ( $2 \times 10^5$  CFU/0.2 ml). Spleen counts performed 15 days after challenge give accurate indication of protection afforded by the inoculation.

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<sup>1</sup> Bonnet-Maury, P., Jude, A., Servant, P.: La mesure statistique de la virulence et de l'immunité. Application à l'étude de la virulence du bacille typhique et à la mesure du pouvoir immunisant des vaccins antityphoïdiques. Rev. Immunol., 18, 21-49, 1954.

<sup>2</sup> Bosseray, N., Plommet, A.M, Plommet, M.: Theoretical, practical and statistical basis for a general control method of activity for antibrucella vaccines. Develop. Biol. Standard., 56, 257-270, 1983.

<sup>3</sup> Plommet, M., Bosseray, N.: Propositions pour une méthode générale de contrôle d'activité des vaccins antibrucelliques. Develop. Biol. Standard., 56, 247-255, 1983.

<sup>4</sup> Plommet, M., Bosseray, N.: La souche vaccinale Brucella suis S2. Virulence, immunogénicité, caractères marqueurs. Rapport No. 2. (Document BRUC.VACC./WP/88.4, September 1988 - see Annex II)

Exact statistical analyses should be performed on transformed data as indicated in the documents mentioned in footnotes 2 and 4 given below.

### 2.3 Lyophilization of strain 2

The strain was first checked for the smooth phase, then seeded on BAB medium slants and incubated at 37°C for 24 hours. The strain was harvested in the following solution for lyophilization:<sup>1</sup> bovine albumine, 5 g; saccharose, 7.5 g; sodium glutamate, 1 g; distilled water, 100 ml; sterilization by filtration on millipore membrane 0.22 µm. The suspension was distributed (2 drops) in sterile tubes or ampoules and lyophilized with a Speedivac Edwards 30 P1/637 apparatus or any other of similar capacity, by a three step process: freezing by centrifugation under vacuum, 1 hour; for main dessication, 20-22 hours; for last dessication on manifold before sealing tubes under vacuum, 5 hours. About 70% of bacteria should survive the process. Sealed tubes and ampoules should be stored at 4° C.

### 2.4 Conservation of strain 2

As for other live vaccines, one laboratory, or a group of international laboratories, should be designated to keep the strain with its original properties, and to distribute it to other laboratories. For this, all batches of the lyophilized strain (original seed) must be tested in laboratory animals (mice and/or guinea pigs) and in large animals (relevant procedure has not yet been defined) before distribution.

Before setting up the responsible laboratory/ies, strain 2 must be kept lyophilized (using a process such as the one given in section 2.3), by the FAO/WHO Collaborating Centre for Reference and Research on Brucellosis at the Central Veterinary Laboratory, Weybridge, United Kingdom. It should be possible to keep the strain for a long time without any change occurring provided that careful attention is paid to obtaining only smooth colonies on BAB or TSA medium, and that survival rate after lyophilization in glass sealed ampoules is of 50% or more.

For day-to-day laboratory use, strain 2 should be propagated every two months on BAB or TSA slants after large seeding, incubation for 24h at 37°C and conservation at 4°C.

### 2.5 Production of strain 2 vaccine for pilot experiments

Strain 2 vaccine can be prepared for laboratory and field experiments in the same way as strain 19 and Rev 1, except that a shorter incubation time is required. Whilst large-scale production may be performed in liquid medium fermenter (growth characteristics and genetic stability of strain 2 warrant success for this production), for pilot projects, small-scale production should be easily performed on agar medium either in tubes or in Roux flasks. TSA or BAB medium may be used.

Tubes are seeded from a fresh agar culture by streaking, and are incubated for 24 hours at 37°C. The harvest is washed once in buffered saline by centrifugation, standardized at appropriate dilution by photometric determination (with strain 2, 24 hours on BAB, optical density at 600 nm, 1cm is 0.165 for  $4.5 \times 10^8$  colony forming units (CFU)/ml).

Roux flasks are seeded with a fresh suspension of the strain in 5 ml (buffered saline (BS). After flooding the entire agar surface, the excess of liquid is removed using a pipette (about 3 ml). Incubation for 24 hours. Harvest by flooding with two successive volumes of 10-15 ml of BS and gentle agitation. Washing and standardization as above.

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<sup>1</sup> Greaves, R.I.N.: Progrès récents en lyophilisation, 167-179, Herman, Paris, 1962.

About  $0.5$  to  $1 \times 10^{11}$  live bacteria are to be obtained from one tube and  $1.2 \times 10^{12}$  from one Roux flask. The buffered saline suspension should be kept at  $4^{\circ}\text{C}$  and used within a few hours.

It is desirable to lyophilize strain 2 vaccine (see footnote 2, page 8).

For vaccination studies, the suggested dose in mice is  $1 \times 10^5$  viable bacteria and in goats, by the conjunctival route  $1 \times 10^8$ .

Pilot vaccines should be examined for safety, purity, potency, and related biological characteristics, in line with the Report on Collaborative Studies on Safety and Potency of Oral Brucellosis Strain 2 Vaccine<sup>1</sup>.

#### 2.6 Experiment on vaccinal doses in sheep and goats

Oral vaccination in drinking water is an easy way to administer the vaccine to large populations, but this route is not an experimentally accurate one (uncertain dosage) nor cheap (large doses required). In addition, environmental and human contamination may occur. Thus, conjunctival administration would be a good and simpler alternative since it is known from strains 19 and Rev 1 data that vaccination by this route is: (a) very efficient; (b) induces shorter serological responses than subcutaneous vaccination; and (c) does not provoke abortion when administered during pregnancy.

Hence, the second step of the research, after completion of mice experiments, would be to define the right dose of strain 2 to administer in sheep or goats that: (1) regularly induces colonization of lymph nodes of the head; (2) gives the smallest serological response.

Lambs and/or kids have to be vaccinated at different doses and killed after 1-6 weeks to enumerate the strain in nodes. Concomitantly, blood tests will be performed from the time of vaccination to slaughter.

The above experiment can be carried out at the Institut national de la recherche agronomique, Nouzilly, France, in 1988.

#### 2.7 Experiment on protection in sheep and goats

Protection conferred to sheep or goats by strain 2 has to be determined in comparison with that given by *B. melitensis* Rev 1 strain vaccine, taken as reference. Both should be administered by the conjunctival route, known to induce good immunity in ewes with strain Rev. 1.

From results of mice experiments, it seems likely that immunity induced by strain 2 vaccine would decrease with time. Consequently, a booster dose has to be considered after the first pregnancy to ensure life-long protection.

The following plan should be considered:

- (1) Female lambs to be vaccinated at 4-6 months with strain Rev 1, strain 2, or not vaccinated;
- (2) observations: clinical, serological and microbiological (research of the vaccine strain in genital excretions and milk during the first pregnancy and at birth) to be made to give evidence of the safety of this vaccine and the serological responses.

- (3) after the first pregnancy a subgroup of strain 2 vaccinated ewes to be administered a booster vaccination;
- (4) a challenge with virulent B. melitensis strain H 38 to be administered during the second pregnancy to those remaining animals, which happen to be pregnant at that time. Infection to be followed by serological, microbiological and clinical tests. Protection to be considered by infection out of total challenged ewes in control, Rev 1 and strain 2 groups.

The total number of ewes needed will be about 130-140 at the beginning to obtain at least 15 pregnant ewes per experimental group during the second pregnancy.

### 2.8 Field experiments

The objective of field experiments is to extend laboratory observations to different, more "natural" conditions, for safety and efficiency of strain 2 vaccine.

Requirements are:

- (a) high prevalence of the disease in the country;
- (b) good teams of trained scientists and technicians;
- (c) proper laboratory facilities and training;
- (d) sufficient number of herds or flocks on which accurate observations can be made. For example, five flocks of 100-200 ewes, or three flocks of 300-500 ewes would be convenient.

In addition, the field experiments may be considered as field demonstrations or as establishing "common knowledge", thus government or university experimental flocks may be preferred.

### 3. Conditions and regulations on the use of B. suis strain 2 and the vaccine

The progress of collaborative studies so far tend to confirm that strain 2 vaccine has the following advantages:

- (a) it is safe to use in pregnant animals;
- (b) it is not excreted in milk;
- (c) it lends itself to easier handling in the laboratory, grows fast on simple media, and is relatively more stable than brucella strain 19 and Rev 1 vaccine strains are so far known to be;
- (d) vaccinal antibodies disappear rapidly within one year after vaccination;
- (e) confers a reasonable serviceable immunity.

Future collaborative work should concentrate on confirmatory evidence of these advantages along the following lines:

### 3.1 Control of the strain 2 vaccine

Similar techniques to those used with strain 19 and Rev 1 vaccines can be used for the production and control of strain 2 vaccine (extensively described in scientific literature - see footnote on page 8). Some small adaptation for growth in agar medium, and possibly in liquid medium in fermenter is required, but from information available, a laboratory with adequate facilities and well trained microbiologists, can produce strain 2 vaccine.

Great care should be taken to avoid genetic shift of the strain. The FAO/WHO Collaborating Centre for Reference and Research on Brucellosis at the Central Veterinary Laboratory, Weybridge, United Kingdom, should be in charge of the strain for its control and distribution to national or interregional producer laboratories.

Suitable biological marker(s) differentiating vaccinal strain from field strains of B. suis have not yet been obtained. However, classical reference challenge strain B. suis 1330 and strain 2 can easily be differentiated.

### 3.2 Conditions and regulations on use of strain 2 vaccine

It is essential to initiate every batch of vaccine from the original seed/or seed lot which is available from the FAO/WHO Collaborating Centre for Reference and Research in Brucellosis, Weybridge, United Kingdom, or from any other laboratory which has established a seed lot of the strain 2 vaccine.

Before importing an original strain 2, the seed lot, and/or pilot vaccine products, institutions should ensure that the laws and regulations pertaining to importation of such materials in their country are adhered to, i.e. an import permit should be obtained through the appropriate government channels of the country beforehand. Special attention needs to be paid in the case of countries where B. suis is not known.

Prior to embarking on vaccine production and field trials of the vaccine, it is essential that the agreement of the relevant authorities in the country/ies concerned be obtained (detailed plans for approval should be submitted well in advance, taking into account all related laws and regulations).

## 4. Field trials and their evaluation

Field trials should preferably be designed and conducted in such a way as to make the results statistically valid. They should be designed to answer one question at a time. The following models could be followed:

- (a) Two flocks of a minimum size of 300 animals each could be separated and marked. One flock would be vaccinated with strain 2 vaccine, and the other with Rev 1 vaccine. The immunity of strain 2 would then be compared to that of Rev 1 under natural field conditions where there are high incidences of brucellosis.
- (b) Mass vaccination of large numbers of flocks in an infected area, where some infected flocks would be vaccinated and the other unvaccinated: flocks could be compared for herd immunity by using increased abortion rate and increased number of reactors as parameters.

The experiments at station level now being carried out in France will provide valuable information on the immunity level and duration of strain 2 vaccine. More work at this level is needed.

5. Conclusions and recommendations

- (1) As a result of the collaborative studies on Brucella suis strain 2 vaccine carried out by the working group in 1987 and 1988, the group found strain 2 to be suitable for the production of a live attenuated brucellosis vaccine.
- (2) Results obtained from the controlled field trials in sheep and goats indicated the safety and potency of the strain 2 vaccine in protecting pregnant animals against high-dose challenge of virulent field strains (B. melitensis). No excretion of the vaccine strain from the vaccinated animals was demonstrated.

No cases of human infection associated with vaccine strain 2 have so far been observed, but should such cases arise during strain 2 vaccine trials, these should be reported and evaluated. Every effort should be made to isolate the strain.

- (3) Those institutions and countries which are interested in strain 2 vaccine production by importing the vaccine strain and applying it in various field conditions, should observe strictly regulations, laws and biosafety measures. When strain 2 is introduced into countries, the original strain and/or seed lot strain should be requested from FAO/WHO Collaborating Centre for Reference and Research on Brucellosis, Central Veterinary Laboratory, Weybridge, United Kingdom. Pilot production and field application should be based on the project plans agreed upon and approved by the government authorities. Technical assistance should be sought from FAO and/or WHO whenever necessary.
- (4) Information exchange on strain 2 vaccine and epidemiology of the strain should continue as one of the important activities of the working group.
- (5) International and regional cooperation should be strengthened to prevent and control brucellosis in humans and animals, through the application of strain 2 vaccine to various field conditions. Reduction of the incidence of human brucellosis should also be investigated carefully together with that amongst the animal population.
- (6) Further collaborative research will be carried out in 1988 to 1989 at laboratory and field levels, as follows:
  - (a) studies on genetic and biological markers of B. suis strain 2;
  - (b) controlled experiments on the efficiency of vaccination routes, doses, mode of immune response, duration of immunity with target animals (sheep, goats, cattle) require further study at laboratory level. L'Institut national de la recherche agronomique, France, is interested in conducting a series of experiments between 1988 and 1989. Joint FAO/WHO field trials of strain 2 vaccine are envisaged in Libya and possibly one or two other interested countries in 1989.
- (7) Interest in collaborative work on the field evaluation trials of strain 2 vaccine has been expressed by the Middle and Near East Animal Production and Health Project (MINEAD/FAO),<sup>1</sup> and a centre for such collaborative work will be selected by that project group.

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<sup>1</sup> Please refer to Animal Health Service, Animal Production and Health Division, Food and Agriculture Organization of the United Nations, Rome, Italy.

The possibility of using strain 2 vaccine in nomadic and other difficult farming situations should be further investigated. In this respect, the experiences of the Chinese scientists should be better analysed.

- (8) FAO and WHO are urged to continue their joint efforts. Several meetings on brucellosis control are being planned by FAO and/or WHO within the next few years, which, to be of maximum benefit, will require considerable coordination in planning and organization by both organizations.
- (9) A high level of coordination is needed between the national veterinary and medical authorities in the planning and implementation of human and animal brucellosis control measures, with the understanding that human brucellosis can only be effectively controlled, and ultimately eradicated, through effective animal brucellosis control.

The next working group meeting is envisaged in France during the period 7-9 February 1989.

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ANNEX I

LIST OF PARTICIPANTS

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Secretariat

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Dr T. Fujikura, Veterinary Public Health, Division of Communicable Diseases, World Health Organization, Geneva, Switzerland (Co-Secretary)

Dr A. A. Mustafa, Bacteriologist, UTFN/LIB/025, FAO Project, c/o UNDP Office, P.O. Box 358, Tripoli, Libya (Rapporteur)

Dr R. J. Olds, Animal Health Service, Animal Production and Health Division, Food and Agriculture Organization of the United Nations, Rome, Italy (Co-Secretary)

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\* Invited, but unable to attend.

ANNEX II

LIST OF WORKING PAPERS

- BRUC.VACC./WP/88.1 Draft agenda
- BRUC.VACC./WP/88.2 Proposed list of participants
- BRUC.VACC./WP/88.3 Differentiation of Brucella suis strain 2 from field strains by FAO/WHO Collaborating Centre for Reference and Research on Brucellosis, Weybridge, United Kingdom
- BRUC.VACC./WP/88.4 Souche vaccinale Brucella suis 2, virulence, immunogénicité, caractères marqueurs, by Drs N. Bosseray and M. Plommet, September 1988
- BRUC.VACC./WP/88.4.1 Souche vaccinale Brucella suis 2, virulence, immunogénicité, caractères marqueurs, by Drs N. Bosseray and M. Plommet, December 1987
- BRUC.VACC./WP/88.5 Brucella suis strain 2 vaccine. First preliminary report, by Pan American Zoonoses Centre (CEPANZO), Buenos Aires
- BRUC.VACC./WP/88.6 Production and control of the pilot oral/conjunctival Brucella suis strain 2 vaccine, by Drs M. Plommet and N. Bosseray
- BRUC.VACC./WP/88.7 Field-oriented trial of the Chinese Brucella suis strain 2 vaccine in Libya, by Dr A. A. Mustafa
- BRUC.VACC./WP/88.8 Controlled field trials on oral/conjunctival immunization with Brucella suis strain 2 vaccine, by Dr J.Kolar
- BRUC.VACC./WP/88.9 Conditions and regulations of field trial of live attenuated oral/conjunctival brucella vaccine (strain 2), by Dr T. Fujikura