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# Observations on experimental inactivated vaccines for contagious bovine pleuropneumonia

By M. A. GRAY, P. SIMAM

*Veterinary Research Department, Kenya Agricultural Research Institute, PO Box 32, Kikuyu, Kenya*

and G. R. SMITH

*Nuffield Laboratories of Comparative Medicine, Institute of Zoology, Regent's Park, London NW1 4RY*

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## SUMMARY

In two trials the efficacy of inactivated vaccines against contagious bovine pleuropneumonia was tested by exposing vaccinated cattle to droplet infection provided by close contact with experimentally infected 'donors'. Complete protection was given by an extreme form of vaccination in which a heavy suspension of killed *Mycoplasma mycoides* subsp. *mycoides* emulsified with Freund's complete adjuvant was given in two large doses. 'Mouse-protective antibody' (MPA) was also produced, i.e. serum transferred to mice 2–4 h before intraperitoneal challenge prevented the development of mycoplasmaemia. However, the study did not answer the question 'Is MPA protective for cattle?'. No protection was given by a milder form of vaccination in which a lighter suspension of killed mycoplasmas emulsified with Freund's incomplete adjuvant was given in a comparatively small dose on a single occasion.

## INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) – one of the great cattle plagues – is still of considerable economic importance in Africa, where it occurs in more than 20 countries south of the Sahara.

The currently used vaccines consist exclusively of living attenuated cultures of the causative organism, *Mycoplasma mycoides* subsp. *mycoides*. Although providing a substantial immunity when used under carefully controlled conditions these vaccines have certain drawbacks (Hooker, Smith & Milligan, 1980) associated with their content of viable organisms and concerned mainly with 'shelf-life' and safety. For this reason an efficient inactivated vaccine would be a useful addition to existing prophylactic measures. The few experimental studies made on killed vaccines during the past 30 years have not been encouraging (Hooker, Smith & Milligan, 1980), but firm evidence is sparse.

Dyson & Smith (1975) found that cattle given various forms of antigenic stimulation with *M. mycoides* subsp. *mycoides* produced antibody which, when

transferred to mice, prevented development of the mycoplasmaemia that normally resulted from intraperitoneal injection of the organism. This 'mouse-protective antibody' (MPA), which was distinct from complement-fixing and precipitating antibody, persisted for many months in two cattle given an inactivated vaccine containing Freund's incomplete adjuvant (Hooker, Smith & Milligan, 1980). The true significance of MPA is a matter of some importance. Little is known about the mechanisms of immunity in CBPP and the relative importance of humoral and cell-mediated effects in producing resistance to the disease (Dyson & Smith, 1975). Moreover, the study of improved methods of vaccination would be greatly facilitated if a relation could be shown in cattle between MPA and protection.

The purpose of the present study was (1) to test the ability of inactivated vaccine to protect cattle against droplet infection from infected donors, and (2) to gain information on the possible defensive function of MPA for cattle.

## MATERIALS AND METHODS

### *Cattle*

Boran cattle (*Bos indicus*) were obtained from a CBPP-free region of Kenya for both experiments 1 and 2. Before use they were tested, with negative results, by a complement-fixation (CF) test for CBPP (see below). In experiment 1 steers aged 2·5–3 years and weighing 260–300 kg were used. In experiment 2 the animals were heifers aged *c.* 2 years, weighing 160–200 kg.

### *Strains of* M. mycoides *subsp.* mycoides

The $T_1$ (Davies & Gilbert, 1969), Blenheim and Gladysdale strains (Smith, 1968) were used. The Gladysdale strain had been imported into Kenya from Australia in 1961, passaged in cattle, and in 1964 freeze-dried as infected bovine pleural exudate.

### *$T_1$ live vaccine*

This vaccine, in the form of a broth culture (Brown, Gourlay & McLeod, 1965), was obtained from stocks maintained for field use by the Veterinary Research Department, Muguga. The vaccine used in experiments 1 and 2 contained *c.* $10^8$ and $10^{11}$ colony-forming units (cfu) per ml respectively. It was administered subcutaneously in the tail-tip in a dose of 0·5 ml.

### *Inactivated vaccines A and B*

Vaccines A and B were used in experiments 1 and 2 respectively.

*Vaccine A* was prepared by methods similar to those of Hooker, Smith & Milligan (1980). The Blenheim strain of *M. mycoides* subsp. *mycoides* (Smith, 1968) was grown for 4 days in BVF-OS medium (Turner, Campbell & Dick, 1935) and centrifuged at 24000 *g* in a 24M centrifuge (MSE Scientific Instruments, Crawley, West Sussex). The deposit was washed three times in phosphate buffered saline (PBS) and resuspended to the opacity of Brown's tube (BT) 10. This suspension, estimated by the methods of Watters (1978) to contain 4·4 mg of protein/ml, was killed by heating in a water bath at 56 °C for 30 min and emulsified just before use with an equal volume of Freund's Incomplete Adjuvant (FIA; Difco) by

means of a High Speed Homogenizer and 1-inch Tubular Mixing Unit (Silverson Machines Ltd, Chesham, Bucks). Each animal received a single dose of 5 ml subcutaneously behind the point of the scapula.

*Vaccine B* was prepared by methods similar to those of Dyson & Smith (1975). The Gladysdale strain was grown for 2 days in tryptose broth (Gourlay, 1964). The organisms were harvested and washed (as above) and resuspended to the opacity of BT25 (protein 14·5 mg/ml). After being heat-inactivated (56 °C, 30 min) the suspension was stored at −20 °C. Just before use it was thawed and emulsified (as above) with an equal volume of Freund's Complete Adjuvant (FCA; Difco). Each animal received 20 ml subcutaneously behind the point of the left scapula followed 4 weeks later by a similar dose on the opposite side.

*Challenge*

In both experiments 1 and 2 the so-called 'in-contact' (or 'natural') method of challenge (Hudson & Turner, 1963; Davies *et al.* 1968) was used. In each experiment 18 cattle (6 given $T_1$ live vaccine, 6 given inactivated vaccine, and 6 unvaccinated) were mixed with 9 'donor' animals that had been infected by endobronchial intubation (Hudson & Turner, 1963) with the Gladysdale strain 10 days (experiment 1) or 5 days (experiment 2) earlier. The animals were housed in a shed measuring 13·7 m in length and 8·5 m in width, the eaves and roof ridge being 3 and 4·9 m high respectively. Any donor that died before a CF titre was recorded in the unvaccinated control cattle was replaced by a further intubated animal. The challenge commenced 5 weeks after vaccination in experiment 1 and 3·5 weeks after the second dose of vaccine B – i.e. 7·5 weeks after initial vaccination – in experiment 2. The surviving donors were killed, together with all other survivors, 20 weeks after the initial vaccination of the experimental cattle.

*Clinical examination*

The cattle were passed through a crush and examined at the same hour each morning. Rectal temperatures were recorded together with any local reaction at the site of vaccination.

*Serological examination*

All experimental animals were bled immediately before vaccination and at weekly intervals for 20 weeks. The serum samples were stored at −20 °C until examined by the CF test (Campbell & Turner, 1953), 'slide agglutination serum test' (SAST) (Gourlay, 1964), and passive mouse-protection test (PMPT) (Smith, 1971; Hooker, Smith & Milligan, 1980).

Briefly, the PMPT consisted in pretreating groups of six mice weighing 18–20 g with undiluted bovine serum samples (0·25 ml/mouse, subcutaneously) 2–4 h before intraperitoneal challenge with $10^6$–$7{\cdot}5 \times 10^6$ cfu of the Blenheim strain. Results were assessed on the basis of mycoplasmaemia 24 h after challenge. Significant protection ($P < 0{\cdot}01$; Wilson & Miles, 1975) was indicated by the occurrence of mycoplasmaemia in (1) no more than 2 of 6 test mice when, at the same time, it occurred in no less than 11 of 12 control mice pretreated with PBS, or (2) 3/6 as compared with 12/12 controls.

*Necropsy and cultural examination*

All cattle were examined. They comprised donor and experimental animals that died or were killed. The size and type of the lung lesions were recorded. Cultures were made in the medium of Davies & Read (1969) from the nasal cavity (turbinate region), trachea, lung (each lobe), pleural exudate, mediastinal lymph node, liver, kidney, spleen, blood and urine. The identity of *M. mycoides* subsp. *mycoides* isolates was confirmed by a growth inhibition test (Davies & Read, 1968).

*Comparison of the efficacy of vaccines*

Hudson & Turner's (1963) scoring system was used. Briefly, the results of challenge were assessed on points (maximum 18/animal) as follows: clinical response (based on occurrence and duration of pyrexia of $\geqslant$ 39·5 °C, or on whether death occurred), maximum 3 points; CF reaction, maximum 3 points; post-mortem examination (based on type and extent of pulmonary lesions, and on whether *M. mycoides* subsp. *mycoides* was isolated), maximum 12 points. A 'pathology score' (Tables 1 and 3) – which took account of lesions but not serological, clinical or cultural evidence – carried a maximum of 6 points for each animal.

## RESULTS

### *Immunization of cattle with inactivated vaccine A and $T_1$ live vaccine (experiment 1)*

Steers 1–6 received vaccine A and 7–12 received $T_1$; steers 13–18 remained unvaccinated. Challenge commenced 5 weeks later. Three of the nine 'donors' died before any CF titre appeared in the unvaccinated control group and were replaced by three further intubated animals. *Post mortem*, all 12 donors showed acute lesions or sequestra that yielded *M. mycoides* subsp. *mycoides* in culture.

*Clinical and serological response to vaccination*

Vaccine A invariably produced swellings at the site of injection, reaching 5 cm in diameter within 5 days and 10 cm by days 10–14. The swellings had disappeared by the time of challenge. $T_1$ vaccine was without apparent effect.

Neither vaccine produced any CF response. Cattle vaccinated with $T_1$ invariably gave a positive SAST within 2 weeks, but the intensity of the reaction gradually waned as the time of challenge approached. Unvaccinated animals and those that received vaccine A gave almost entirely negative results in the SAST.

Serum samples taken immediately before both vaccination and challenge from steers 1–6 and 7–12 were examined by the PMPT. This showed that mouse-protective antibody (MPA) developed in one animal (no. 2) that received vaccine A and in four (7, 10, 11, 12) that received vaccine $T_1$.

*Clinical and serological response to challenge*

During the period between the commencement of challenge and the termination of the experiment (5 and 20 weeks after vaccination, respectively) four of the unvaccinated steers died (Table 1) and two became emaciated. One animal (no. 1) given vaccine A died and coughing, loss of condition, and sometimes pyrexia

Table 1. *Immunization of cattle with vaccines A and $T_1$ against CBPP produced by 'in-contact' challenge (experiment 1)*

| | | Observations after challenge | | | | | |
|---|---|---|---|---|---|---|---|
| Vaccine (single dose) | Animal no. | Maximum CF titre | Pyrexia (days duration) or death | CBPP lesions at necropsy | *M. mycoides* recovered from lung | Points scored* | |
| | | | | | | Pathology | Total |
| A | 1 | — | Died | Extensive | + | 6 | 17 |
| | 2 | 320 | — | S (20 × 30) | + | 6 | 15 |
| | 3 | 640 | Pyrexia (2) | S (20 × 30 and 15 × 20) | + | 6 | 15 |
| | 4 | 320 | Pyrexia (12) | S (15 × 20) | + | 4 | 13 |
| | 5 | 80 | — | S (3 × 3) | + | 2 | 6 |
| | 6 | 320 | Pyrexia (1) | S (4 × 7) | + | 4 | 11 |
| $T_1$ | 7–12 | —† | — | — | — | 0 | 0 |
| None | 13 | 2560 | Died | Extensive | + | 6 | 18 |
| | 14 | 640 | — | S (7 × 7) | N | 4 | 7 |
| | 15 | — | Died | Extensive | + | 6 | 15 |
| | 16 | 1280 | Died | Extensive | + | 6 | 15 |
| | 17 | 320 | — | S (7 × 7) | N | 4 | 7 |
| | 18 | 320 | Died | Extensive | + | 6 | 18 |

S = sequestrum (encapsulated); size (cm) in parentheses. Extensive = affecting whole of one or both lungs.— = none. N = not tested.

* System of Hudson & Turner (1983); see Materials and Methods.

† Animal no. 11 had a CF titre of 20 and a total score of 2 points.

Table 2. *MPA in cattle immunized with vaccines B and $T_1$ against CBPP produced by 'in-contact' challenge (experiment 2)*

| Vaccine | Animal no. | Positive blood cultures* 24 h after challenge in groups of 6 mice pretreated with 0·25 ml of serum taken from cattle at the stated times (weeks) after initial vaccination | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7† | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| B (2 doses) | 19 | 5 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 2 | 0 | 1 | 2 | 1 | 0 | 2 | 2 | 0 | N |
| | 20 | 6 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 1 | 1 | 0 | 0 | 2 | 1 | 0 | N |
| | 21 | 5 | 0 | 1 | 1 | 1 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | N |
| | 22 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 1 | 2 | 3 | N |
| | 23 | 6 | 0 | 3 | 5 | 3 | 4 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 2 | 1 | N |
| | 24 | 4 | 0 | 1 | 2 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | N |
| $T_1$ (single dose) | 25 | 4 | 1 | 3 | 5 | 6 | 6 | 4 | 6 | 5 | 5 | 5 | 6 | 5 | 5 | 5 | 6 | 6 | 4 | 0 | 1 | 0 |
| | 26 | 6 | 0 | 2 | 4 | 6 | 4 | 4 | 4 | 6 | 6 | 6 | 6 | 6 | 5 | 6 | 5 | 5 | 4 | 6 | 5 | 6 |
| | 27 | 6 | 0 | 6 | 4 | 6 | 5 | 6 | 6 | 6 | 3 | 6 | 6 | 5 | 4 | 6 | 4 | 6 | 5 | 5 | 6 | 5 |
| | 28 | 6 | 0 | 3 | 3 | 4 | 5 | 4 | 6 | 6 | 5 | 5 | 5 | 6 | 6 | 5 | 6 | 6 | 6 | 6 | 4 | 2 |
| | 29 | 6 | 0 | 1 | 5 | 6 | 5 | 6 | 4 | 6 | 5 | 5 | 6 | 5 | 5 | 6 | 6 | 5 | 5 | 4 | 5 | 5 |
| | 30 | 4 | 0 | 2 | 6 | 5 | 5 | 5 | 5 | 6 | 5 | 6 | 5 | 0 | 0 | 0 | 1 | 0 | 3 | 0 | 2 | 1 |
| None | 31 | 5 | N | N | N | N | N | N | 4 | 4 | 5 | 4 | 2 | 3 | 0 | 2 | 1 | 0 | 1 | 4 | 4 | N |
| | 32‡ | 2 | N | N | N | N | 1 | N | 0 | 2 | 3 | 2 | 2 | 2 | 0 | 2 | D | D | D | D | D | D |
| | 33 | 5 | N | N | N | N | N | N | 4 | 5 | 6 | 5 | 5 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N |
| | 34 | 4 | N | N | N | N | N | N | 5 | 5 | 6 | 5 | 5 | 6 | 3 | 0 | 5 | 0 | D | D | D | D |
| | 35 | 4 | N | N | N | N | N | N | 5 | 5 | 6 | 5 | 5 | 3 | 0 | 0 | 0 | D | D | D | D | D |
| | 36 | 5 | N | N | N | N | N | N | 5 | 5 | 6 | 5 | 6 | 5 | 1 | 1 | 0 | 0 | 1 | D | D | D |

N = not tested; D = dead.

* Each PMPT included 12 mice pretreated with PBS instead of serum; 24 h after challenge mycoplasmaemia invariably occurred in at least 11 of these animals.

† 'In-contact' challenge of cattle commenced 7·5 weeks after initial vaccination.

‡ This animal's serum possessed non-specific mouse-protective properties (Smith, 1971).

occurred in the other five. The six steers given $T_1$ vaccine remained apparently normal.

CF titres appeared 5–7 weeks after first contact with the donors in all the unvaccinated animals and those given vaccine A except steers 1 and 15, which died before CF antibody was produced (Table 1). A CF titre (20) appeared in only one animal (no. 11) that received $T_1$ vaccine. All the CF titres persisted until death or until the termination of the experiment.

The positive reactions to the SAST produced by the $T_1$ vaccine increased in intensity 7–8 weeks after first contact with the donors. Unvaccinated animals and those given vaccine A became positive to the SAST for the first time 5–10 weeks after the commencement of challenge.

Due to technical difficulties PMPT results are not available for animals exposed to challenge.

*Pathological and microbiological effects of challenge*

The results and scores (Hudson & Turner, 1963) are summarized in Table 1. $T_1$ vaccine gave virtually complete protection against challenge but vaccine A little or none. Steers 5 and 6 still had a small quantity of vaccine A left at the injection site at the conclusion of the experiment.

*Immunization of cattle with inactivated vaccine B and $T_1$ live vaccine (experiment 2)*

In view of the disappointing results with vaccine A, experiment 2 was designed to discover whether cattle could be immunized by a more extreme form of vaccination with killed organisms.

Animals 19–24 and 25–30 received vaccines B (two doses at an interval of 4 weeks) and $T_1$ (single dose) respectively. $T_1$ vaccine was administered simultaneously with the first dose of vaccine B, and 7·5 weeks later all animals, including six that remained unvaccinated (31–36), were exposed to challenge. As in experiment 1, severe CBPP developed in all nine donors, and three that died before the first appearance of CF antibody in the unvaccinated controls were replaced.

*Clinical and serological response to vaccination*

The first and second doses of vaccine B invariably produced swellings at the site of injection, reaching *c.* 10 cm in diameter within 3–5 days and persisting to the end of the experiment. Of the cattle given $T_1$ vaccine, nos 25 and 26 had slightly raised temperatures and nos 25 and 29 transient local reactions 3 days after inoculation.

Animals given vaccine B all became positive to the SAST within a week of the primary dose and remained so up to and beyond the commencement of challenge. They became positive to the CF test 4–5 weeks after the first dose of vaccine (i.e. 0–1 week after the second), with titres of $\leqslant 40$. Cattle that received $T_1$ vaccine showed, 1 week later, CF titres of $\leqslant 40$ that persisted no more than 14 days and were in general paralleled by the agglutinin response as demonstrated by the SAST. Unvaccinated cattle remained negative to the CF test and SAST.

The PMPT showed (Table 2) that the primary dose of vaccine B invariably produced MPA within a week. In all animals except no. 23 this persisted until

Table 3. *Immunization of cattle with vaccines B and $T_1$ against CBPP produced by 'in-contact' challenge (experiment 2)*

| | Observations after challenge | | | | | | |
|---|---|---|---|---|---|---|---|
| Vaccine | Animal no. | Maximum CF titre | Pyrexia (days duration) or death | CBPP lesions at necropsy | *M. mycoides* recovered from lung | Points scored* Pathology | Total |
| B (2 doses) | 19 | 40† | — | — | — | 0 | 0 |
| | 20 | —† | — | — | — | 0 | 0 |
| | 21 | 20† | — | — | — | 0 | 0 |
| | 22 | 10† | — | — | — | 0 | 0 |
| | 23 | 20† | — | — | — | 0 | 0 |
| | 24 | 40† | — | — | — | 0 | 0 |
| $T_1$ (single dose) | 25 | 320 | Pyrexia (3) | S (4 × 3) | + | 2 | 8 |
| | 26 | — | — | — | — | 0 | 0 |
| | 27 | — | — | — | — | 0 | 0 |
| | 28 | 10 | — | Small‡ | + | 1 | 4 |
| | 29 | — | — | — | — | 0 | 0 |
| | 30 | 40 | — | — | — | 0 | 2 |
| None | 31 | 40 | — | — | — | 0 | 2 |
| | 32 | 1280 | Died | Extensive | + | 6 | 18 |
| | 33 | 640 | Pyrexia (4) | S (multiple, 2 × 2) | + | 2 | 8 |
| | 34 | 1280 | Died | Extensive | + | 6 | 18 |
| | 35 | 2560 | Died | Extensive | + | 6 | 18 |
| | 36 | 2560 | Died | Extensive | + | 6 | 18 |

Footnotes as in Table 1, except for '†'.

† Titre not significantly different from that before challenge (i.e. produced by vaccination).

‡ Small = unencapsulated lesion, 2 × 3 cm.

exposure to challenge. In animal 23 the primary dose of vaccine B produced only a transient response, but within 2 weeks of a booster dose MPA reappeared and was still present at the time of challenge. It was shown with serum collected 4 and 8 weeks after initial vaccination that mouse-protective properties were lost when the samples were diluted 1 in 10. All animals given $T_1$ vaccine had demonstrable concentrations of serum MPA a week later, but these disappeared within a further 14 days. Up to the time of challenge all unvaccinated animals except one (no. 32) gave negative PMPT results. No. 32 gave positive results throughout the experiment, probably as a result of non-specific protective factors (Smith, 1971; Dyson & Smith, 1975).

*Clinical and serological response to challenge*

During the period between the commencement of challenge and the termination of the experiment (7·5 and 20 weeks after initial vaccination, respectively) 4 of the 6 unvaccinated steers died, 1 became pyrexic and 1 remained normal (Table 3). The two groups of vaccinated cattle remained clinically healthy except for one animal (no. 25), which became pyrexic despite having been given $T_1$ vaccine.

In cattle that received vaccine B the CF titres produced by vaccination were apparently affected little if at all by challenge; three animals still showed CF antibody at the conclusion of the experiment. All the unvaccinated cattle and three given $T_1$ vaccine produced CF antibody 4.5 weeks or more after first contact with the donors (Table 3). The SAST results in general paralleled those of the CF test.

The PMPT showed (Table 2) that the MPA produced as a result of injection of vaccine B persisted throughout the challenge period. All unvaccinated cattle (excluding no. 32; Table 2) and two (nos. 25 and 30) that received $T_1$ vaccine produced MPA 3·5 weeks or more after exposure to challenge.

*Pathological and microbiological effects of challenge*

The results and scores (Hudson & Turner, 1963) are summarized in Table 3. Vaccine B gave complete protection against a challenge that produced severe CBPP and usually death in all except 1 of 6 unvaccinated cattle. $T_1$ vaccine gave incomplete protection. Sterile lesions at various stages of resolution were present at the injection sites of vaccine B.

## DISCUSSION

Evidence is presented (experiment 2) to show, for the first time, that vaccination with killed *M. mycoides* subsp. *mycoides* can protect cattle completely from a severe respiratory challenge provided by close contact with donors suffering from acute CBPP. The evidence is, however, based on the use of an extreme method of vaccination in which cattle were inoculated twice with very large doses of killed mycoplasmas emulsified with a powerful experimental adjuvant (Freund's complete). In an earlier trial (experiment 1) a single injection of a much smaller dose of killed mycoplasmas emulsified with a milder adjuvant (Freund's incomplete) gave no appreciable protection. Live $T_1$ vaccine – widely used in the field – gave incomplete (experiment 2) or virtually complete (experiment 1) protection.

Vaccine B (experiment 2) produced a striking MPA response as well as complete protection against challenge. The study did not however answer the question 'Does MPA have a defensive function in cattle?' In this regard three comments should be made. (1) In experiment 1, due to technical difficulties, reliable PMPT results were obtained only from serum samples collected immediately before challenge. (2) Because of the inevitable dilution associated with injecting 0·25 ml of serum into a 20 g mouse (Dyson & Smith, 1975) a negative PMPT did not exclude the presence of MPA. (3) The simultaneous development of acute CBPP and MPA in unvaccinated animals as a result of challenge did not exclude the possibility of a protective function of MPA for cattle.

Although necessitating a good deal of organization and labour, the experiments were small in scale, and further work is needed. It is scarcely surprising that occasional discrepancies between the results and those of the earlier small trials should have emerged. For example, the mouse-protective effect of serum from cattle given vaccine B was removed by diluting 1 in 10, whereas serum produced on another occasion by similar methods (Dyson & Smith, 1975) was still protective when diluted 1 in 50. However, the principle is now established that inactivated vaccine can immunize against CBPP produced by droplet infection. Future studies should concentrate on defining the minimum protective dose – especially of vaccine containing the milder of Freund's adjuvants (incomplete) – and the role of MPA.

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