The administration of foot and mouth disease vaccine with oil adjuvant and its influence on the diagnosis of bovine tuberculosis

A. NADER *, B. LÓPEZ **, L. LÁZARO ***, F. ERRICO ****, V. RITACCO *****, C.G. GROTTER * and I.N. DE KANTOR **

Summary: The possible influence of vaccination with oil adjuvanted foot and mouth disease vaccines on the tuberculin response was investigated in 32 normal guinea pigs and 190 non tuberculous bovines. Circulating anti-Mycobacterium bovis IgG antibodies were analysed by an enzyme-linked immunosorbent assay (ELISA), in order to determine the effect of the vaccination on the humoral response against mycobacteria in cattle. Control animals were either non vaccinated or injected with aluminium hydroxide adjuvanted vaccine.

Administration of foot and mouth disease vaccine had no apparent influence on the tuberculin responses of either guinea pigs or cattle, nor did it influence the level of anti M. bovis antibodies in cattle.

KEYWORDS: Foot and mouth disease vaccine - Oil adjuvant Tuberculin test.

INTRODUCTION

For the past 50 years, aluminium hydroxide has been used as an adjuvant for foot and mouth disease vaccines. In 1968, the Pan American Foot and Mouth Disease Center initiated trials employing an oil adjuvant vaccine (FMV OA). These studies demonstrated that this vaccine produced higher and more stable antibody titres and it was thus incorporated into the regular vaccination programmes in a number of countries (3, 4, 5).

The FMV OA is a stable inactivated virus suspension in Freund's incomplete adjuvant (FIA). The latter contains both a mineral oil and an emulsifier (3, 4). FIA stimulates the proliferation of T lymphocytes, the production of lymphokines and a humoral response to antigen. Granuloma formation may occur at the site of antigen injection and consequently a non specific stimulation of the delayed allergic response may appear (1, 11).
It has been demonstrated that foot and mouth disease vaccine with or without oil adjuvant does not interfere with the tuberculin response in guinea pigs and cattle previously inoculated with *Mycobacterium bovis* (9). However, its effect on the response to mycobacterial antigens in animals not sensitised by *M. bovis* has not yet been investigated.

In 1988, in Argentina, some livestock owners and veterinarians expressed their concern to the National Service of Animal Health that, in apparently tuberculosis free herds, some animals which had received FMV-OA gave a positive tuberculin reaction to the caudal fold test. The comparative cervical tuberculin test subsequently revealed that the animals were non-specific reactors.

If a relationship existed between vaccination with FMV OA and the occurrence of non-specific tuberculin reactions, this would pose a serious problem to tuberculosis control programmes which employ the tuberculin test and where cattle are vaccinated with FMV OA (2, 12).

The present study was designed to determine if FMV-OA vaccination could interfere with the tuberculin test. To this end, non tuberculous guinea pigs and cattle were used. Furthermore, an enzyme-linked immunosorbent assay (ELISA) for detection of anti-*M. bovis* IgG antibodies, which is being used under experimental conditions as a diagnostic tool in bovine tuberculosis (13, 14) was used to analyse the possible influence of FMV-OA vaccination on the level of anti mycobacterial antibodies in cattle.

**MATERIALS AND METHODS**

**Foot and mouth disease vaccine and adjuvants**

The following vaccines and adjuvants were used in this study:

- (A) FMV-OA, batch 005 (Med. Vet.)
- (B) FMV-OA, batch 542 (Summun)
- (C) FMV-OA, batch 004 (Cooper)
- (D) FMV OA, batch 003 (Aftolane)
- (E) FMV-OA, batch 006 (Med. Vet.)
- (F) Freund's incomplete adjuvant (FIA)
- (G) foot and mouth disease vaccine with aluminium hydroxide and saponin (San Jorge Bagó).

FIA contained nine parts of mineral oil (Marcole 52, Exxon Corp., USA) and one part of an emulsifier: deanhydromanitol mono-oleate (Montanide 80, Seppic, Paris).

**Experiment in guinea pigs**

Thirty two albino male guinea pigs (Cpz Hart) weighing 350-550 grams were distributed at random in six groups of four guinea pigs each and one group of eight animals. Groups A, B, C, D and E were administered 0.25 ml of vaccines A, B, C,
D and E, respectively, by the intramuscular (IM) route at weeks 0, 4, 10 and 14. An equal dose of FIA was administered to animals in group F at the same time intervals. The eight remaining animals served as unvaccinated controls. Each guinea pig was tuberculin tested with 0.1 ml of 0.001 mg/ml \textit{M. bovis} purified protein derivative (PPD) tuberculin by the intradermal (ID) route. The diameters of erythema were measured 24 hours later. In this species, reactions equal to or smaller than 10 mm are considered negative or non-specific.

**Experiment in cattle**

One hundred and ninety tuberculin-negative Holstein cows from a herd without recent history of tuberculosis, although located in an endemic area of Argentina (Province of Buenos Aires), were employed in this study.

Cattle were distributed at random in six groups of 30 animals each and two control groups of five animals. Groups 1 and 2 were administered vaccine G, groups 3 and 4 vaccine A and groups 5 and 6 vaccine B, at the initiation of the experiment and 14 weeks later. Animals in groups 7 and 8 were not vaccinated. Doses of 5 ml FMV OA were inoculated in the neck, by the IM route; equivalent doses of aluminium hydroxide vaccine were inoculated by the subcutaneous route.

Animals in groups 1, 3, 5 and 7 were tested by the double comparative cervical tuberculin test at the beginning of the trial and 4 weeks later. A simple caudal fold test was conducted at week 20. Animals in groups 2, 4, 6 and 8 were tested, following the same method, at the initiation of the experiment and at weeks 8 and 22. In order to determine antibody levels in the sera, blood samples were collected from each animal at the time of tuberculin testing.

**Tuberculin tests**

The comparative tuberculin test was carried out by inoculating ID 0.1 mg of bovine PPD and 0.05 mg of avian PPD; the caudal fold test used an ID inoculation of 0.1 mg of bovine PPD. Reactions were read 72 hours after inoculation, by the measurement of increase in skin fold thickness.

The criterion employed to interpret the results of the comparative test was that adopted by the European Economic Community (6).

In the case of the caudal test, any increase in skin fold thickness equal to or larger than 5 mm was considered positive, reactions of 3-5 mm as inconclusive and those smaller than 3 mm regarded as negative.

**Enzyme-linked immunosorbent assay (ELISA)**

Blood samples were obtained by puncture of the coccygeal vein. Sera were separated and kept at 20°C until processing. The assay was performed as described previously (13, 14).

Results were registered on a micro ELISA reader at 405 nm and expressed as optical density (OD) values. OD values equal to or higher than 0.180 were considered positive.
RESULTS

Experiment in guinea pigs

Tuberculin responses in every group of animals (vaccinated and controls), fell within the range considered to be negative or non-specific: 0-9 mm.

Experiment in cattle

Tuberculin tests using bovine or avian PPD were, in general, negative. There was only one exception: an animal belonging to group 6 which, at the beginning of the trial and at week 22, showed a positive response (data not presented). Post mortem examination of this animal revealed minimal lesions in a bronchial lymph node. Since no acid-resistant bacilli were observed by microscopic examination and \textit{M. bovis} could not be isolated by culture, this bovine was classified as non tuberculous and the tuberculin reaction considered non-specific.

Table I shows the number of animals positive for antimycobacterial antibodies by ELISA before vaccination (time 0) and after various intervals post vaccination. Mean OD values obtained with the ELISA were negative in the eight groups of animals under study. Nevertheless, six animals (from groups 3, 5, 6 and 7) gave low positive results by ELISA on one or more occasions during the post vaccination surveys. These cattle were all tuberculin negative; in fact, the only tuberculin-positive animal in the herd was negative when tested by the ELISA. There was no significant statistical difference in the incidence of ELISA-positive, tuberculin negative animals between vaccinated and control groups (10).

\textbf{TABLE I}

\textit{Results of an ELISA for detection of anti-Mycobacterium bovis IgG antibody at various time intervals after administration of foot and mouth disease vaccines in 190 cattle from a herd free of tuberculous infection}

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of cattle</th>
<th>Vaccine</th>
<th>Number of positive animals/Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>G (AH)</td>
<td>0/30</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>G (AH)</td>
<td>0/30</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>A (OA)</td>
<td>0/30</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>A (OA)</td>
<td>0/30</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>B (OA)</td>
<td>1/30</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>B (OA)</td>
<td>0/30</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>A (OA)</td>
<td>1/5</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>A (OA)</td>
<td>0/5</td>
</tr>
</tbody>
</table>

\*IMD vaccine administered to groups 1, 2, 3, 4, 5, and 6 at weeks 0 and 14.
\*AH aluminium hydroxide adjuvant vaccine.
\*OA oil adjuvant vaccine.
CONCLUSIONS

From the above results, it can be concluded that foot and mouth disease vaccination does not interfere with the response to tuberculin tests in non tuberculous guinea pigs or cattle. Furthermore, anti-\textit{M. bovis} IgG antibody levels determined by ELISA did not show significant variation attributable to foot and mouth disease vaccination in cattle.

One of the 190 animals tested showed a positive reaction to the tuberculin test. This animal had to be slaughtered in accordance with the regulations of the tuberculosis control programme in Argentina. Since tuberculosis was not confirmed, the reaction was regarded as non-specific or a "false positive". Such a rate of false positive results (1/190 or 0.5%) coincides with the specificity of the simple caudal and comparative cervical tests demonstrated in previous studies (7, 8, 15). Since the herd was regarded as tuberculosis-free for the last 10 years, the positive ELISA results in six tuberculin negative animals were also interpreted as false positive reactions. These false positive ELISA results (representing a rate of 3%) correspond to the expected specificity of this test for the diagnosis of tuberculosis (13, 14).

This study did not confirm previous assumptions of livestock owners and veterinarians concerning false positive tuberculin results in bovines after FMV-OA vaccination. In addition, the specificity of an ELISA for the diagnosis of tuberculosis in cattle was not affected after this vaccination. Therefore, no valid objection exists for the application of diagnostic tests for tuberculosis either tuberculin or ELISA after foot and mouth disease immunisation with oil adjuvant vaccine.

REFERENCES


