Inflammation is frequent at the site of injection in turkeys vaccinated with various commercial fowl cholera bacterins. Seven commercial and three laboratory bacterins were compared in 12-week-old turkeys as to extent and severity of irritation with 3 routes of injection: subcutaneous, intradermal, and intramuscular. Commercial fowl cholera bacterins, particularly those with paraffin oil as the adjuvant, varied greatly in the irritation produced. The amount appeared consistent with the bacterin or adjuvant and somewhat independent of route of injection. The reaction to selected bacterins was comparable in 4-week-old turkeys to that in 12-week-olds. The reaction was usually less severe at 15 days postinjection than at 5 days. The intradermal route gave the most consistent results, probably because less bacterin diffused from the injection site, yet the amount of reaction was still not sufficiently consistent to be used quantitatively. Reaction to a given bacterin or adjuvant varied the most with the subcutaneous route.

INTRODUCTION

Inflammation at the site of injection (usually the neck) is frequent in turkeys vaccinated with various commercial fowl cholera bacterins. These reactions have often been the cause of extensive condemnations in turkey processing plants (4). Some manufacturers of bacterins credit this severe reaction to faulty procedures in injection and not to irritating substances in the bacterin. They also claim, however, that a reaction is necessary for the development of sufficient immunity (5).
This investigation was instigated after observing in a field visit that approximately 2% of 80,000 turkeys had a severe reaction at the site of injection in the neck (field case no. 1). These turkeys were depressed and their heads were retracted (Fig. 1). The bacterin they received was from the same manufacturer as commercial bacterin no. 1 evaluated in this study. This study compared inflammatory responses induced in 12-week-old turkeys at the site of injection with 7 commercial and 3 laboratory fowl cholera bacterins. Several commercial bacterins were also evaluated in 4-week-old turkeys to determine whether the inflammatory response varied with age.

Observed since this study was completed was another case (field case no. 2), involving 70,000 turkeys, in which approximately 1% had a severe reaction in the neck after injection of fowl cholera bacterin. The manufacturer of that bacterin had also produced, in a previous lot, commercial bacterin no. 3 evaluated in this study.

**MATERIALS AND METHODS**

**Turkeys.** Used for this study were 56 twelve-week-old, 34 ten-week-old, and 32 four-week-old broad-breasted turkeys not previously vaccinated for fowl cholera.

**Evaluation of commercial and laboratory bacterins.** Seven commercial and 3 laboratory fowl cholera bacterins were compared as to the irritation induced in turkeys. The laboratory bacterins were produced by mixing killed bacterial suspension (density 10 × 1 McFarland unit) of *Pasteurella multocida* (isolant 8954) 1:1 with one of the three following adjuvants: Freund's Incomplete Adjuvant (2) (Difco Laboratories, Detroit, Michigan); 6% sodium alginate; and 4% sodium alginate with 6% calcium gluconate (8) (Colab Laboratories, Chicago, Illinois). The procedure for preparing the bacterial suspension has been described (6).

Each bacterin or adjuvant was inoculated into four 12-week-old turkeys. Each was injected with 0.5 ml of bacterin or adjuvant as follows: subcutaneously in the neck; intradermally in the neck; intramuscularly in the breast; or intramuscularly in the thigh muscle. Two inoculated turkeys were killed at 5 days postinoculation (PI), and 2 at 15 days PI. Tissues were fixed in 10% formalin. Sections were cut 6 μ thick and stained with hematoxylin and eosin (1). Sections of muscle were stained for calcium by Kossa's method (1). For control purposes, the 3 adjuvants and the killed bacterial suspension from the laboratory bacterins were also in-
Table 1. Macroscopic comparison of tissue irritation induced in 12-week-old turkeys by various commercial and laboratory bacterins and adjuvants injected by three routes.\(^a\)

<table>
<thead>
<tr>
<th>Test group no.(^b)</th>
<th>Bacterin &amp; adjuvant(^c)</th>
<th>5 days PI</th>
<th>15 days PI</th>
<th>Irritation rating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SC</td>
<td>ID</td>
<td>IM</td>
<td>SC</td>
</tr>
<tr>
<td>1</td>
<td>OBI</td>
<td>(PO)</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>CB2</td>
<td>(AHA)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>CB3</td>
<td>(PO)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>CB4</td>
<td>(PO)</td>
<td>+</td>
<td>+</td>
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<tr>
<td>5</td>
<td>CB5</td>
<td>(PO)</td>
<td>+</td>
<td>+</td>
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<tr>
<td>6</td>
<td>CB6</td>
<td>(PO)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>CB7</td>
<td>(O)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>LB1</td>
<td>(PO)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>LB2</td>
<td>(SACG)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>LE3</td>
<td>(SA)</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>LE4</td>
<td>(O)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>O</td>
<td>(FIPO)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>O</td>
<td>(SACG)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>O</td>
<td>(SA)</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

\(^a\)SC = subcutaneous; ID = intradermal; IM = intramuscular.

\(^b\)With each group, 4 turkeys were injected with each bacterin; 2 turkeys were killed 5 days postinjection (PI), and 1 turkey 15 days PI.

\(^c\)CB = commercial bacterin; LB = laboratory bacterin; PO = paraffin oil; AHA = aluminum hydroxide absorbed; O = no bacterin or no adjuvant; FIPO = Freund's incomplete (paraffin oil); SACG = 4% sodium alginate & 0.6% calcium gluconate; SA = 6% sodium alginate.

\(^d\)++ = slight; + + = moderate; + + + = intense; + + + + = severe.
Tissue irritation from fowl cholera bacterins

occulated into the same number of 12-week-old turkeys at the same sites.

Commercial bacterins 1, 2, 6, and 7 were evaluated also in 32 four-week-old turkeys by injecting 0.5 ml of bacterin either subcutaneously in the neck or intramuscularly in the thigh, but with only one injection per turkey. Turkeys were killed for study by the same schedule followed with the 12-week-old turkeys.

**Evaluation of bacterin in field case 2.** Ten 10-week-old turkeys were inoculated subcutaneously with bacterin that had been heated at 70 C for 10 minutes, from the same lot as that used in field case 2, and 24 ten-week-old turkeys were inoculated subcutaneously with the same lot of unheated bacterin. The latter group was kept until marketed, at 20 weeks old.

**Criteria for irritation.** The criteria for evaluating and comparing the irritation produced by the various bacterins and adjuvants were the clinical signs and amount and extent of caseous inflammation observed grossly at necropsy. Macroscopically, lesions were

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Fig. 1. Retraction of the neck in turkeys (field case 1) injected with an irritating bacterin (commercial bacterin 1). Photo taken 5 days after injection.
rated as slight, moderate, intense, and severe. Differences were confirmed by microscopic examination.

RESULTS

Clinical signs. Commercial bacterins 1 and 2 were the 2 most irritating bacterins evaluated. When injected subcutaneously in the neck at 5 days PI, each appeared to cause both the 12-week-old and 4-week-old turkeys to retract their necks toward the back similarly to turkeys observed in field case 1 (Fig. 1). Intramuscular injection of the same bacterins in the thigh made the turkeys lame. The remaining commercial and laboratory bacterins and adjuvants caused no clinical signs in turkeys of either age when injected either subcutaneously or in the thigh. None of the bacterins or adjuvants injected in breast muscle or intradermally in the neck appeared to affect turkeys clinically in either age group. One of the 24 turkeys injected with the unheated commercial bacterin used in field case 2 died 3 days PI, and 6 turkeys had a retraction of their necks for a few days PI.

Macroscopic lesions. All of the commercial and laboratory bacterins and adjuvants produced some degree of macroscopic irritation at all sites of inoculation 5 days PI (Table 1). Commercial bacterin 1 plus paraffin oil produced the most severe irritation macroscopically at 5 days PI. Next in severity was commercial bacterin 2 with aluminum hydroxide. This bacterin produced an intense reaction at 5 days PI that subsided markedly by 15 days PI.

Moderate irritation at 5 days PI (Table 1) resulted from remaining commercial bacterins plus paraffin oil and from the laboratory bacterins with Freund's adjuvant, 4% sodium alginate and 0.6% calcium alginate, and 6% sodium alginate. Tissue reactions induced by the 3 adjuvants evaluated separately were similar to those from the adjuvants mixed with killed bacterial suspension. The commercial water-soluble bacterin 7 produced little irritation at either 5 or 15 days PI. The amount of irritation appeared consistent for a bacterin or adjuvant, regardless of route of injection.

With injection subcutaneously in the neck, the macroscopic lesions at 5 days PI varied markedly—from an extensive yellow caseous necrosis (Fig. 2), produced by the most irritating commercial bacterins (nos. 1 and 2), to a minimal necrosis, produced by the water-soluble bacterins (commercial bacterin 4 and laboratory bacterin 1). In general, the lesions were more extended and diffuse with the subcutaneous route.
With the intradermal site, irritation at 5 days PI was a single enlargement and more consistent in extent. The enlargements measured approximately 1.5 × 4 × 4 cm from commercial bacterin 1, 0.5 × 1 × 1 cm from commercial bacterin 6, and 0.25 × 0.5 × 0.5 from commercial bacterin 7. The size of the yellowish caseous necrotic core at the intradermal site, when sectioned, depended on the irritating properties of the bacterin or adjuvant injected.

With intramuscular injection, bacterin or adjuvant was diffused in the fascia between the muscles, resulting in a diffuse yellow caseous necrosis of a severity depending on the irritating properties of the bacterin or adjuvant. There was a gritty feeling when these areas were cut with a knife at 15 days PI.

Generally, macroscopic lesions at all sites of injection were less severe at 15 days PI than at 5 days. This reduction in reaction was probably more marked with the subcutaneous injections, although there were exceptions.

At 5 days PI the enlargements averaged approximately 1 × 4 × 4 cm at the injection sites of either the unheated or heated bacterin used in field case 2 (Fig. 3). Heating did not impair the

Fig. 2. Irritation 5 days postinoculation in 12-week-old turkey given commercial bacterin 1 subcutaneously. Notice the caseous necrosis extending into the subcutaneous fascia (arrow).
bacterin’s ability to produce a severe inflammatory reaction. Sixteen of the 23 remaining turkeys injected with unheated bacterin used for field case 2 had granulomas at 15 days PI comparable to that shown (Figs. 3, 4). At 3 weeks PI the granulomas on 2 of the turkeys had burst and were draining. The death of one turkey that received unheated bacterin appeared to be caused by severe irritation around the vagus nerves resulting from diffusion of the bacterin into the area. Enlargements comparable to that in Fig. 4 remained until slaughter, when they were observed to contain a hard caseous core surrounded by fluid.

Microscopic lesions. The basic microscopic lesion at all injection sites at 5 days PI was a marked heterophilic infiltration and an early reticuloendothelial proliferation. The numbers of these cells depended on the amount of irritation induced by the bacterin or adjuvant. Also, at 5 days PI multinuclear giant cells surrounded masses of necrotic heterophils in a palisade arrangement. When oil adjuvants accompanied the bacterin or were injected independently, there were round vacuoles in the eosinophilic necrotic mass and the surrounding tissue, including the multinuclear cells.

Fig. 3. Irritation (arrow) induced experimentally in 10-week-old turkey by subcutaneous injection of unheated bacterin used in field case 2. Photo taken 15 days postinoculation.
When alginate was the adjuvant there were fewer vacuoles. At the injection site, at this time, there was also a diffuse infiltration of lymphocytes and macrophages, the development of immature lymphoid follicles, and an increased vascularization.

At 15 days PI, there was an increased number of multinuclear giant cells, macrophages, and plasma cells, but fewer heterophils and less vacuoles when paraffin oil adjuvants had been injected. At that time also, the lymphoid follicles were more mature and the proliferation of fibroblasts was evident (Figs. 5, 6). In many injection sites at 15 days PI, it appeared that the eosinophilic mass was being removed by the multinuclear giant cells and the lesions were being resolved.

When microscopic lesions were compared at the various injection sites, the inflammatory reaction was most diffuse at the subcutaneous site. With intramuscular injection, there was a fragmentation of the muscle fibers surrounding the septal fascia, and calcium granules were present in the muscle at 15 days PI with the more irritating commercial bacterins (Fig. 7). With intradermal injections, an acanthosis of the epidermis resulted from the more irritating bacterins and adjuvants.

Fig. 4. Irritation (arrow) induced in 10-week-old turkey; the same as in Fig. 3 but remaining until slaughter, when it was found to contain a hard caseous core surrounded by fluid. Photo taken 15 days postinoculation.
There were no oil vacuoles at injection sites with either the water-soluble bacterins or aluminum-hydroxide-absorbed bacterin. With the alginate adjuvants, there were a few vacuoles.

Microscopic lesions were as severe in turkeys injected subcutaneously with the commercial bacterin from field case 2 as in turkeys vaccinated with commercial bacterin 1.

**DISCUSSION**

The lesions observed after subcutaneous injection of commercial bacterin 1, and a subsequent log of commercial bacterin 3, were similar to the severe reactions observed in field cases 1 and

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**Fig. 5.** Microscopic view 15 days postinjection of an intradermal injection of a 12-week-old turkey with commercial bacterin 1. Notice the eosinophilic necrotic debris (A), paraffin vacuoles (B), lymphoid follicles (C), numerous vessels (D), and epidermis (E). H&E, ×28.
2. Irritation varied among the various commercial bacterins with paraffin oil as the adjuvant, as also observed by field servicemen, poultry meat inspectors, and veterinary diagnosticians. The irritating component was thought to be the oil adjuvant. The best explanation we have been able to obtain is that different lots of paraffin oil vary in refinement, and consequently in content of irritating substances. Turkey age did not appear to influence irritation.

Adjuvants are substances which, when mixed with antigens, improve antibody production, presumably by prolonging the release of antigen to the reticuloendothelial system (2). A certain amount of local irritation by the bacterin having an adjuvant has been

Fig. 6. Magnification of rectangle in Fig. 3 showing lymphoid follicles (A) surrounding blood vessel (B). H&E, x320.
thought necessary for high antibody production (5,7). How much irritation is necessary, however, is a question that has never been answered. Some think that part of the irritation in fowl cholera bacterins, in addition to the paraffin oil, is due to the endotoxins (7). The molecular basis of the mechanism for an adjuvant is not well understood. A variety of substances are capable of enhancing the immune response to an unrelated antigen. Most adjuvants produce a hyperplasia of the reticuloendothelial system and a proliferation of immunocompetent cells (3).

Irritating fowl cholera bacterins injected subcutaneously in the neck often result in condemnation of the necks at slaughter (4). Occasionally, the bacterin will diffuse ventrally in the neck and encircle the vagus nerve, resulting in inappetence because of the dysfunction of the nerve (4). Freund's adjuvant has been observed to diffuse from the site of injection into the adjacent tissues (9). Intramuscular injection of water-soluble or aluminum-hydroxide-absorbed bacterins, even though recommended, or of bacterins with paraffin oil can result in a fibrosis in the breast or leg muscle, ultimately downgrading carcass quality.

Fig. 7. Microscopic view 15 days postinjection of an intramuscular injection of a 12-week-old turkey with commercial bacterin 1. Notice the necrotic debris in the fascial septum (A) and the fibrous connective tissue (B) and muscle fragments (C) which surround the necrosis. H&E, ×40.
Tissue irritation from fowl cholera bacterins

Manufacturer recommendations should be followed in vaccinating turkeys for fowl cholera, and the bacterin should be injected into the correct site. Even so, our results cast some doubts on faulty injection as a cause of vaccination reactions, since the same bacterin appeared to produce similar reactions regardless of injection route. Producers of commercial biologics should test various batches of paraffin oil for their irritating properties before marketing them.

Four sites of inoculation were used so that a more precise index would be obtained of the irritating qualities of the various bacterins being evaluated. The intradermal avenue gave the most consistent irritation for each bacterin or adjuvant. Diffusion of injected material appeared less with this route than with subcutaneous or intramuscular routes. Even so, the diameter of the intradermal site 5 days PI appeared to vary sufficiently to question use of the diameter of reaction as a quantitative characterization of the irritating properties.

REFERENCES

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