

## A comparison of the oral application and injection routes using the Onderstepoort Biological Products Fowl Typhoid vaccine, its safety, efficacy and duration of protection in commercial laying hens

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### ABSTRACT

This study was undertaken to establish whether the Onderstepoort Biological Products Fowl Typhoid (OBPft) vaccine registered as an injectable vaccine was effective and safe when administered orally to commercial layers. Its efficacy and duration of protection were compared with application by intramuscular injection. Commercial brown layer hens were used as they were found to be highly susceptible to *Salmonella gallinarum* infections. In the vaccine safety trial birds were euthanased at timed intervals spanning 4 weeks post-vaccination. Necropsies were performed and samples were taken and tested. No clinical signs or mortalities could be attributed to the OBPft vaccine nor could active shedding of the vaccine strain be detected. Slight pathological changes were noted with both routes of vaccination; however, these changes were transient, returning to normal within the observation period. The injected groups showed a better serological response with the rapid serum plate agglutination (RSPA) test than the orally vaccinated groups. In the duration of protection trial, birds were challenged at 3–8-week intervals post-vaccination. All unvaccinated birds died. Protection 8 and 16 weeks after vaccination was above 60 %, by 24 weeks after challenge, the vaccine protection was below 30 %. It was found that there was no significant difference ( $P < 0.05$ ) in the protection offered by either the oral or injected route of vaccination with the OBPft vaccine.

**Key words:** efficacy, OBP fowl typhoid vaccine, safety, *Salmonella gallinarum*.

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### INTRODUCTION

*Salmonella enterica* subsp. *enterica* biovar *gallinarum* (SG) is the causative agent of fowl typhoid, a septicaemic disease. In the peracute form, birds may die without showing any noticeable clinical signs. In grower and adult birds, a watery to mucoid yellowish diarrhoea is the most characteristic clinical sign in the acute phase of the disease<sup>8</sup>. In the chronic form of the disease, severe anaemia is the predominant sign<sup>1</sup>. In addition to the anaemia, progressive loss of body weight, reduced feed consumption and egg

production, ruffled feathers, shrunken pale combs and wattles are characteristic signs<sup>12</sup>. The carcasses of acute cases are septicaemic with enlargement of the liver and spleen with necrotic foci and the liver often turns a shiny bronze colour. The lungs may develop a characteristic grey discoloration. Lesions including oophoritis, salpingitis, orchitis, peritonitis and perihepatitis are also described in mature fowl<sup>8</sup>.

Fowl typhoid has been largely eradicated from North America, Western Europe and Australia but in parts of South America, Asia and Africa the disease is still responsible for significant losses<sup>2,4,7,8</sup>. In South Africa, there have been ongoing outbreaks of the disease in layer flocks (S P R Bisschop, unpubl. data, 2004). Fowl typhoid has also increased greatly in parts of Latin America<sup>14</sup>. In areas where this disease is endemic, it has become one of the most important diseases of chickens<sup>6,14</sup>, and occurs in both backyard chickens and in commercial poultry<sup>10,13</sup>.

Disease eradication can be achieved by implementing good hygiene and management practices together with routine serological tests and a slaughter-out policy<sup>3</sup>. Where eradication techniques cannot be fully implemented, vaccination and treatment of the disease with antibiotics is carried out. Inactivated vaccines are of limited value in SG control, as they fail to control intestinal colonisation<sup>15</sup>. Live, attenuated vaccines are thus an important component of SG control in developing countries.

At present 2 live fowl typhoid vaccines are registered for use in South Africa: Nobilis SG9R<sup>11</sup>, containing a live attenuated strain of SG (SG9R) and the Onderstepoort Biological Products Fowl Typhoid (OBPft) vaccine which contains the 5503 rough attenuated strain of SG<sup>9</sup>. Both are registered for administration by intramuscular or subcutaneous injection; while only the Nobilis SG9R is registered for oral administration. This study was carried out with the principle aim of determining the efficacy of the OBPft vaccine when applied orally.

### MATERIALS AND METHODS

#### Chickens

A total of 133 Hyline brown commercial layer chicks from a single *Salmonella*-free parent stock were allocated to the trials as shown in Table 1. Birds were given commercial starter, grower and laying rations (Epol, Rustenburg) and municipal water *ad libitum*. Experimental and control birds were housed in separate rooms and all cleaning and feeding was planned to minimise the risk of cross-contamination.

#### Vaccine and challenge strain

The Onderstepoort Biological Products Fowl Typhoid vaccine (OBP, South Africa) was used in this trial. The challenge strain used was the 318/03 *S. gallinarum* strain isolated from a fowl typhoid outbreak in South Africa. A challenge dose of  $1 \times 10^9$  cfu was initially administered orally to each bird, but this was later changed to  $1 \times 10^8$  cfu per bird. It was stored at  $-86^\circ\text{C}$

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in a brain heart infusion broth (Difco) containing 30 % glycerol. Subcultures were made from the frozen stock; densities were adjusted to an optical density of 0.5 at a wavelength of 540 nm, using a spectrophotometer (Ultrospec II, LKB Biochrome) and diluted 1:5 or 1:15 to obtain the required bacterial concentration. Viable bacterial counts were done to confirm the spectrophotometric readings.

#### Vaccination and challenge procedures

The injected groups were vaccinated by the certified OBP vaccine method according to instructions injecting 1 ml of the vaccine subcutaneously under the skin of the breast. With the oral group the same certified method was used but 1 ml of vaccine was given *via* oral gavage directly into the crop. This was done to ensure that under experimental conditions all birds got the same dose.

#### Safety trial

To determine the safety of the vaccine, 10 birds were allocated to each of the 4 treatment groups as shown in Table 1 with 2 birds from each of the treatment groups euthanased and autopsied at intervals 2, 7, 14, 21 and 28 days post-vaccination, after the method of Silva and colleagues<sup>15</sup>. Three birds were used as unvaccinated controls.

#### Duration of protection trial

For the duration of protection trial, 90 birds were allocated to treatment groups as indicated in Table 2. Ten birds from each treatment group were challenged at each of 8, 16 and 24 weeks after the last vaccine was administered. Birds were monitored twice daily for signs of disease. The time to death was recorded (mean death time – MDT) and any surviving birds were euthanased 2 weeks after each challenge. Necropsies were done on all birds.

#### Laboratory analyses

Faecal swabs were collected at 2, 5, 8 and 12 days post-challenge. Intestinal and crop scrapings, liver, lung and spleen biopsies were taken from dead and euthanased birds. The faeces, intestinal and crop scrapings were placed in 10 ml of buffered peptone, then incubated in air overnight at 37 °C and plated on MacConkey agar (Oxoid Biological Products, Basingstoke, UK). These plates were incubated at 37 °C overnight and any non-lactose fermenting colonies were purified by culture onto 7 % horse blood enriched Columbia blood agar (Oxoid Biological Products, Basingstoke, UK). All Gram-negative, catalase positive, oxidase negative colonies were identified using a bio-

Table 1: Treatment groups.

Group	Number of birds		10-week-old vaccination	14-week-old vaccination
	Safety trial	Duration of protection trial		
1	10	30	Injected	Injected
2	10	30	Oral	Oral
3	10		–	Injected
4	10		–	Oral
Negative control	3	30	–	–

Table 2: Challenge groups for duration of protection trial.

Group	8 weeks post-challenge	16 weeks post-challenge	24 weeks post-challenge
Oral	10	10	10
Injected	10	10	10
Negative control	10	10	10

chemical test kit (API 10S, Biomerieux, France). The other organs specimens were streaked directly onto the MacConkey and blood enriched Columbia agars and incubated in air at 37 °C overnight. A representative of each colony type was purified by culture and identified using the aforementioned morphological and biochemical criteria.

Serum samples were collected from all birds immediately prior to euthanasia during the safety trial. In the duration of protection trial, serum was collected 0, 7 and 14 days after challenge. Serum samples were tested for circulating antibodies to SG using the Bacillary White Diarrhoea (BWD) plate agglutination test. Agglutination reactions were scored on the 0 to +++ interpretation scale provided by the product supplier<sup>11</sup>. Weak serological responses (+) are difficult to read consistently and must be interpreted with caution as false positives do occur.

#### Statistical analysis

For the duration of protection trial, the MDTs were compared among treatment groups using a 2-way ANOVA;  $P < 0.05$  was considered significant (SAS 8.02).

## RESULTS

#### Safety trial

Spleno-megally was the only macroscopic pathology in all vaccinated birds. It could be seen from the 2nd day after vaccination, peaked at 7 days post-vaccination and gradually returned to normal by 28 days post-vaccination. Only 1 bird that had received a single vaccination by injection still had a discoloured and speckled spleen 28 days post-vaccination. The birds in the groups vaccinated once, showed slightly more marked pathologi-

cal changes than those vaccinated twice.

Vaccine could only be re-isolated from the spleens of birds in both the groups receiving a single vaccination. Where the vaccine was applied by injection re-isolation was successful at 7, 14 and 21 days while in the group vaccinated orally, re-isolation was successful only on day 21.

Circulating antibody responses to vaccination peaked at between ++ and +++ in groups 1–3 at 21 days after vaccination, while the group receiving a single oral vaccination showed no noticeable antibody response by the end of the trial (Fig. 1). The small number of serum samples at each bleed makes further interpretation of results difficult.

#### Duration of protection trial

All the unvaccinated birds died between 5 and 9 days post-challenge. Mortality in the vaccinated groups occurred 5–12 days after challenge. Both vaccinated groups at each challenge had significantly greater ( $P < 0.05$ ) MDTs than the control group throughout the trial. There was no significant difference in MDT at any point between the vaccinated groups (Fig. 2).

Figure 3 shows the percentage protection offered from mortality by the vaccine.

Survival rates among vaccinated birds at 8, 16 and 24 weeks after vaccination were significantly ( $P < 0.05$ ) better than the unvaccinated birds where all birds died acutely (Table 3). Eight weeks after challenge 13/20 vaccinated birds (65 %) survived challenge, while 16 weeks post-challenge 14/20 vaccinated birds (70 %) survived. Twenty-four weeks after vaccination, survival rates in both groups of vaccinated birds were significantly ( $P > 0.01$ ) lower than in the earlier challenges, with only 5/20 (25 %) surviving. No statistically significant differences in survival

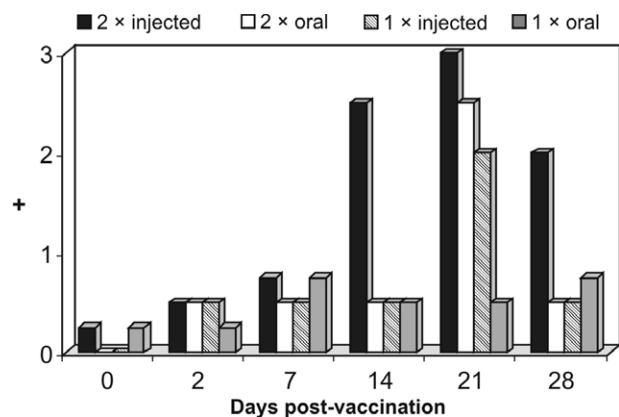


Fig. 1: Antibody response to vaccination with OBPft vaccine.

Table 3: Survival rates post-challenge for each of the challenge groups.

Group	8 weeks post-challenge	16 weeks post-challenge	24 weeks post-challenge
Oral	6/10	7/10	3/10
Injected	7/10	7/10	2/10
Negative control	0/10	0/10	0/10

rates ( $P > 0.01$ ) could be detected between the 2 vaccine application routes at any time.

As can be seen in Fig. 4, the agglutinating antibody titres were very low in all birds prior to challenge. The serological response was strongest in vaccinated birds challenged 8 weeks after vaccination. The response could be detected from 7 days post-challenge and rose further to 14 days when the birds were euthanased. A progressively more muted response was noted in vaccinated birds challenged 16 and 24 weeks after vaccination. Serological responses at 7 days were similar

for both vaccinated groups, while by 14 days after challenge the response in the injected group was stronger. Serological responses in unvaccinated birds could not be evaluated because they died acutely after challenge.

Pathological changes typical of fowl typhoid were observed among the unvaccinated birds, except where peracute death occurred. The most common changes noted were, enlarged liver (bronze in colour), spleen with necrotic foci and grey lungs often with necrotic foci. Septicaemia with egg peritonitis and or airsacculitis usually due to a secondary

*Escherichia coli* infection occurred.

All faecal swabs collected 2, 5 and 12 days post-challenge were negative for SG.

Positive faecal SG results occurred in both injected and orally vaccinated groups in the challenge trial 8 weeks post-vaccination, where faecal swabs were collected 8 days post-challenge. The control birds could not be tested as they all died acutely before the 8 day faecal swab collection.

## DISCUSSION

### Challenge dose

The challenge strain used was selected from *Salmonella gallinarum* isolates in the collection of the Poultry Reference Centre (Faculty of Veterinary Science, University of Pretoria), because it proved to be the most pathogenic of the available strains. Pilot studies in layer pullets further indicated that 100 % mortality could consistently be achieved at challenge doses of  $1 \times 10^9$  cfu/bird. This was broadly consistent with the results from the literature that gave an  $LD_{50}$  of  $2 \times 10^8$  cfu/bird for a South American isolate of the SG strain<sup>2</sup>. During the course of the main trial it became apparent that hens in lay were more susceptible to challenge than the pullets used in the pilot study and the challenge dose was consequently decreased for subsequent challenges.

### Safety trial

The oral application of OBP fowl typhoid vaccine is as safe as the injected method when vaccinated at 10 and 14 weeks of age in commercial layer pullets.

There were no clinical signs of disease or mortality in response to vaccination and there was no evidence of shedding of the vaccine strain in the faeces or from the intestines during the monitoring period of the safety trial. These data are similar to the findings by Young and colleagues who tested the safety of the SG9R vaccine administered by injection<sup>16</sup>.

The transient splenomegaly observed in all vaccinated birds, even those previously vaccinated, indicated that the product is not entirely innocuous and served as a clear indicator of exposure to viable live vaccine. The fact that viable vaccinal bacteria could be isolated from the spleen of birds that received a single dose of vaccine indicates that booster vaccination results in improved clearance of the bacteria, most probably due to an improved immune response. Under the stresses associated with commercial poultry production it is possible that vaccine application could have an appreciable impact on growth in rearing and an

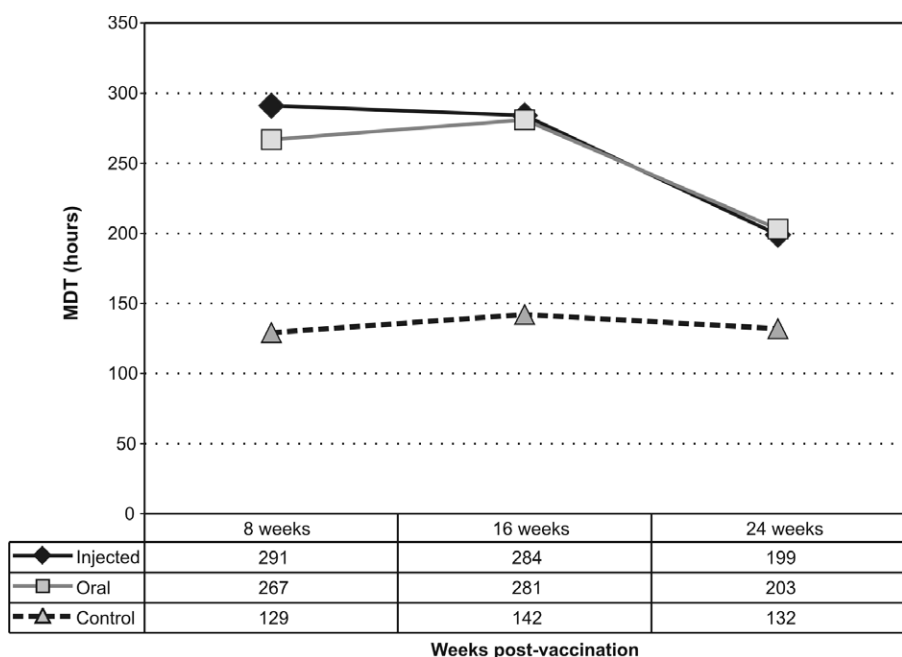


Fig. 2: Mean death time (MDT) in hours after challenge with a virulent strain of *Salmonella gallinarum* at intervals after vaccination with OBPft vaccine by oral or injected routes.

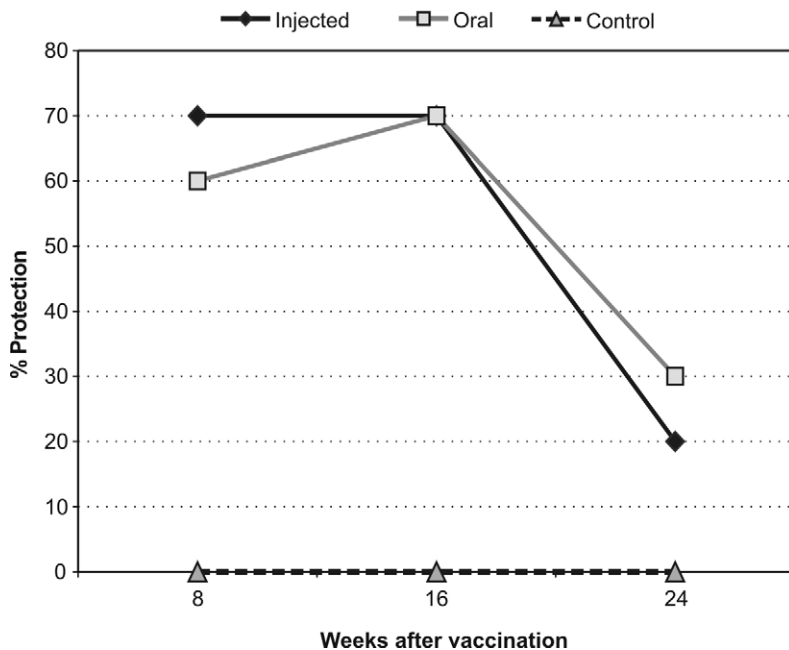


Fig. 3: Percentage protection after challenge with a virulent strain of *Salmonella gallinarum* at intervals after vaccination with OBPft vaccine by oral or injected routes.

even more significant effect if applied to hens in lay<sup>2,5</sup>.

The serum antibody response to vaccination, rose earliest, was strongest and lasted longest in the group receiving 2 vaccinations by injection, with substantial but more muted responses from the group receiving 2 oral vaccinations as well as the group receiving a single injection. Of interest was the transient nature of the serological response to vaccination with titres in all groups, with the exception of the group receiving a double injection, returning to low levels by 28 days after vaccination and almost disappearing altogether by 8 weeks after vaccination as seen in the duration of protection trial. It is possible that serological responses in the cases of salmonellosis are only indicative of exposure and not necessarily immunity, as salmonella is a facultative intracellular pathogen that

elicits predominantly a cell mediated immune response. Stronger serological responses were observed after challenge in all groups between 7 and 14 days after challenge.

#### Duration of protection

In the challenge 8 weeks after the 2nd vaccination the group vaccinated orally had a survival rate of 60 % and the injected group a survival rate of 70 %, using a challenge dose of  $1 \times 10^9$  cfus per dose. This compares unfavourably with the results obtained by Cameron and Buys when they applied the vaccine only by injection<sup>9</sup>. They obtained protection of between 80 % and 100 % 8 weeks after vaccination using  $2-5 \times 10^8$  cfu of SG challenge strain 1007<sup>9</sup>. The difference in the results may be accounted for by the higher challenge dose, possible differences in the virulence of the challenge strains

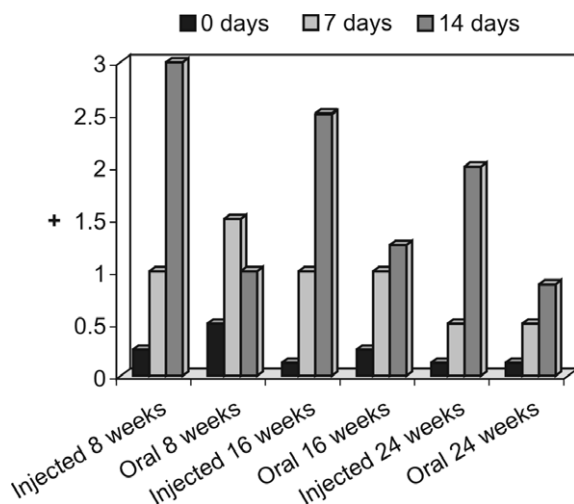


Fig. 4: Mean serological titres at different intervals after *Salmonella gallinarum* challenge.

used as well as possible differences in the genetic susceptibility of the birds (Cameron and Buys used New Hampshires) to the disease in the 27 years between the 2 studies.

Because the initial challenge dose caused peracute mortalities in all unvaccinated birds the challenge dose for the remaining challenges at 16 and 24 weeks post-vaccination was reduced to  $1 \times 10^8$  cfus. At 16 weeks after vaccination, the injected and oral groups both showed 70 % protection from mortality. When Cameron and Buys challenged birds 16 weeks post-vaccination they found only 50 % protection<sup>9</sup>. In this case the protection observed in the current trial was in fact superior and suggests that if birds had been challenged with the lower dose at 8 weeks post-vaccination the level of protection may have been comparable to the results obtained by Cameron and Buys<sup>9</sup>.

Twenty-four weeks after vaccination protection in the orally vaccinated group (30 %) was slightly superior to the injected group (20 %) but still essentially closely similar, indicating that vaccine protection by this stage had declined greatly from the levels obtained at 16 weeks. Given the limited number of challenges that could be accommodated in a trial of this nature, it was not possible to determine exactly when protection began to decline or when it disappeared completely.

Calculation of the MDT for each treatment group yielded similar results to the level of protection. Unvaccinated controls died 129 to 141 hours after challenge, with the shortest MDT after the 1st challenge with the higher challenge dose and the longest after the 2nd challenge. At all challenges, the MDTs of vaccinated groups were significantly better than those of the vaccinated groups and did not differ significantly from each other.

The spleen, liver and lungs showed the most marked pathological changes in this trial.

SG was also recovered from these organs, in birds that had died. Of the surviving vaccinated birds, 15.5 % had egg peritonitis. *Escherichia coli* but not SG was cultured from the peritoneum of some of these birds. This ubiquitous bacterium is a common cause of opportunistic infections in birds suffering from other diseases<sup>13</sup>.

The OBPft vaccine protected birds well against challenge with virulent SG for at least 16 weeks after vaccination. By 24 weeks post-vaccination the level of protection had declined to 30 %. Other live SG vaccines claim good protection up to 12 weeks post-vaccination<sup>16</sup>. Thus the OBPft vaccine shows good protection for



at least 4 weeks longer whether given orally or via injection.

## CONCLUSION

In this study it was shown that the oral administration of the OBP fowl typhoid vaccine is as safe, the protection offered is as good and the duration of protection is as long as the injected route of vaccination, using the same vaccine.

## RECOMMENDATIONS

The data from these trials indicate that birds can be safely and adequately vaccinated using either the injected or oral route at 10 and 14 weeks of age during pullet rearing. However, protection beyond 16 weeks after the last of 2 vaccinations given 4 weeks apart cannot be guaranteed. Thus further booster vaccinations through the drinking water (this method is far more practical and less likely to cause production losses in layer hens than vaccination by injection) should be considered to possibly provide lifelong protection. Further trial work to determine the ideal intervals for subsequent vaccinations as well as the impact of vaccination of hens in lay in commercial systems should be considered.

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