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COMPARATIVE ASSESSMENT OF COMMERCIAL AND LOCAL PREPARED *SALMONELLA* VACCINES AGAINST *SALMONELLA* INFECTION IN DUCKLING

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Received: 22 July 2024; **Accepted:** 20 August 2024

ABSTRACT

This experiment was conducted to assess the efficiency of the commercial Servac trivalent inactivated *Salmonella* bacterin in comparison with the prepared inactivated *S.* Infantis oil emulsion bacterin against *Salmonella* infection. A total of 70 Muscovy ducklings (one day old) were divided into 3 groups each containing 20 ducklings, except the control group which contained 30 ducklings. Groups 1 and 2 were vaccinated with commercial Servac trivalent inactivated *Salmonella* bacterin, (consisting of *S.* Typhyimurium, *S.* Kentucky, *and S.* Enteritidis), and the locally prepared *S.* infantis inactivated bacterin respectively, at 7 days of age subcutaneously (0.5 ml/duckling) and boostered by the same dose and route at 22 days of age. Both groups were subdivided into G1a, G1b, G2a, and G2b at 37 days of age for oral challenging (1ml containing 17× 10^9 CFU) of *S.*Typhyimurium (G1a, G2a) and *S.* Infantis (G1b.G2b), while group 3, (control group) sub-grouped into G3a (infected with *S.*Typhyimurium unvaccinated*)* , G3b (infected with *S.* Infantis unvaccinated), and G3c which kept as the negative control group. Signs, postmortem lesions, mortalities and fecal shedding were evaluated to detect the protection percentages of the vaccinated groups post-challenging. Reduced fecal shedding, *Salmonellae* recovery from internal organs, absence of signs and postmortem lesions were noticed compared with control positive groups. The candidate prepared and commercial Servac bacterins could provide ducks with 100% protection against *Salmonellae* infection without any significant difference.

Key Words: Duckling – Vaccination- *S.* Typhimurium- *S.* Infantis – cross protection

INTRODUCTION

Duck industry is the second major poultry production industry after chicken in Africa, which may be able to meet the increasing demand for animal protein due to the availability of various productive duck breeds worldwide and the distinctive characteristics that differentiate them from other poultry species (Solomon *et al*., 2006). Egypt is the leading producer of duck meat in Africa with 39000 tons, representing that duck production is receiving great attention as a source of animal protein (FAO, 2009).

As one of the foodborne poisoning bacteria and, due to its associated significant economic losses in poultry, *Salmonella*

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infection has long been a matter of concern to authorities of public health and scholars globally. Ducks have attracted the attention of many researchers as the most transmissible reservoir of *Salmonellae* to humans (Yang *et al*., 2019).

Many different *Salmonella* serotypes have been recovered from ducks, most of which are of public health concern but others can cause considerable losses in ducklings, such as *S.*Gallinarum, *S.*Pullorum, *S.* Enteritidis, *S.*Anatum *and S.*Typhimurium , which is one of the most isolated serotype causing 93% of ducklings salmonellosis infection (Zeinab, 2021). Keel disease was the previous name of duck salmonellosis as the bird appeared healthy until it keeled over and died. Dehydration due to severe enteritis, emaciation, and difficulty breathing are the main signs of *salmonella* infection and deaths can occur due to Opisthotonus and a combination of other complicated factors.

Controlling salmonellosis in poultry farms was previously dependable on a combination of biosecurity and antibiotics and, with the misuse of antimicrobials, multidrug resistance was the expected result (Chruchaga *et al*., 2001), so several efforts were performed to eradicate *Salmonella*, one of them being vaccination. Despite the availability of both live attenuated and inactivated *Salmonella,* the last confers good protection with decreased or absence of residual virulence (El-Enbaawy *et al*., 2013). Thus, this study was intended to prepare an inactivated *S.* Infantis oil emulsion bacterin and estimate its efficacy against *Salmonella* infection in comparison with the commercial Servac trivalent inactivated *Salmonella* bacterin.

MATERIALS AND METHODS

Ethics approval and consent to participate

All samples were collected under the permission of the local license. All experiments were performed in rural units of the Avian and Rabbit Medicine Department, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt (Protocol number 06/24/0178) according to the standards of OIE for use of animals in research in accordance with relevant guidelines and regulations.

***Salmonella* isolates**

S. Typhimurium and *S.* Infantis isolates were serologically and molecularly identified and supplemented by Avian and Rabbit Medicine Department, Faculty of Veterinary Medicine, Assiut University. Isolates were refreshed using XLD media (Oxoid) for further use.

***S.* Infantis oil emulsion bacterin preparation (Charles *et al*., 1994)**

Cultures of *S.*infantis isolate on brain heart infusion broth (Oxoid) containing 1×10^9 CFU were pelleted by several centrifugations (5000 rpm for 30 mints), resuspended in normal saline, inactivated by 0.5% formalin (37%) and incubated at 37°C overnight with continuous shaking. Lack of viability was confirmed on nutrient agar media. One volume of Tween 80 was mixed with 5 volumes of mineral oil (1 part of white oil+ 4 part of span 80), as an adjuvant. Equal amounts of the adjuvant and the inactivated culture were mixed to obtain a stable emulsion.

Quality control of the prepared inactivated bacterin

Sterility, safety and stability tests

The freedom of the prepared bacterin from aerobic, anaerobic bacteria and fungi was tested through culturing on nutrient agar and for safety, a double dose of the bacterin was inoculated subcutaneously in 10 ducklings (2 weeks old of age) and observed for 2 weeks for signs or lesions and directions were followed according to OIE, 2016. Observation of bacterin at 4°C and 37 °C for 4 weeks in tightly screw-capped tubes was done for stability testing until the emulsion was clearly separated (Stone, 1978).

Experimental design

A total of 70 Muscovy ducklings aged one day old (El-Shams Company for animal

production), with no history of their breeder vaccination by *Salmonella* bacterin, were housed under strict hygienic conditions in units of Avian and Rabbit Medicine Department, Faculty of Veterinary Medicine, Assiut University, divided into 3groups each contain 20 ducklings, except the control group that contained 30 ducklings.

Groups 1 and 2 were vaccinated with commercial Servac trivalent inactivated *Salmonella* bacterin, (consisting of *S.*Typhyimurium, *S.*Kentucky, *and S.* Enteritidis, Veterinary Serum and Vaccine Research Institute, Abbasia, Egypt, batch no.2021), and the locally prepared *S.*Infantis inactivated bacterin, respectively, at 7 days of age subcutaneously (0.5 ml/duckling) and boostered by the same dose and route at 22 days of age.

Both groups were subdivided into G1a, G1b, G2a, and G2b at 37 days of age for oral challenging (1ml containing 17× 10^9 CFU) from each *salmonella* isolates (Paiva *et al.*, 2009) as follows:

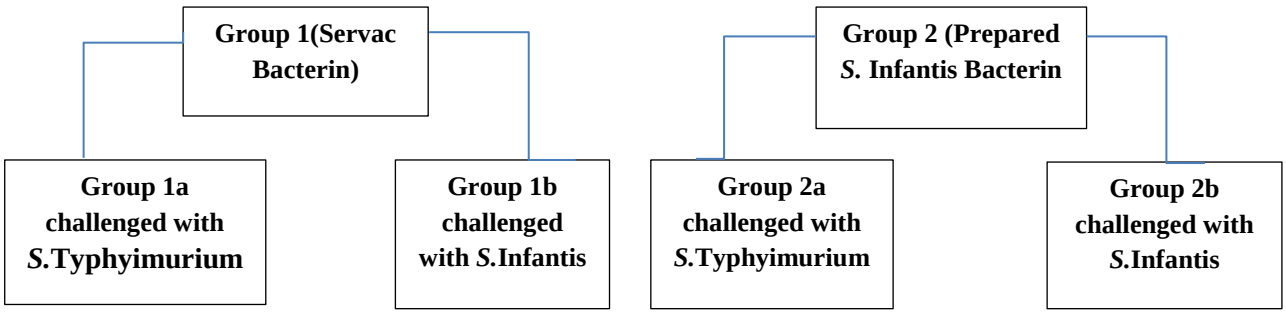


Fig1: Groups of the experimental design

Group 3 was sub-grouped into G3a, G3b, and G3c (10 ducklings /each group), both G3a and G3b were challenged at 37 days of age with *S.*Typhyimurium and *S.*Infantis respectively as positive control (unvaccinated) and G3c was kept as a negative control (unchallenged unvaccinated).

Observations of clinical signs, mortalities and postmortem lesions were reported until the end of the study.

Parameters of protection percentage assessment

Determination of fecal shedding

Cloacal swabs were collected before and after starting the experiment on 1st, 3rd, 5th, 7th, 14th, 21th and 28th, days post-challenge from each group and examined bacteriologically for *Salmonella* shedding recognition (Hofstad *et al*., 1997).

***Salmonella* recovery**

On the 4th-week post-challenge, samples of heart blood, liver, spleen, and ceca from each group were collected after culling for *Salmonella* recovery detection.

Body weight evaluation

All ducks were weighed individually at the 1st, 2nd, 3rd, and 4th weeks post-challenge.

Weight gain= weight of birds/number of birds

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) followed by the Duncans multiple range test for the detection of significance among different treatments.

$P < 0.05$ was considered statistically significant.

RESULTS

Protection assessment of *Salmonella* vaccines

It has been confirmed that the locally prepared inactivated *S.*Infantis bacterin was sterile (no bacterial growth on nutrient agar), stable and safe as neither signs nor lesions were detected after bacterin inoculation.

Concerning the *Salmonellae* fecal shedding which persisted till the end of the study in all groups with variable degrees, it was noticed that decreased intermitted in both vaccinated groups, in comparison with the positive control groups which had shedding continuity with a high percentage throughout the days of swab collection. (Table 1)

Table 1: Percentage of fecal shedding of *Salmonellae* from all groups

Groups	No. of positive ducks							% of +ve / total No. of duck samples
	1st dpc	3rd dpc	5th dpc	7th dpc	14th dpc	21st dpc	28th dpc	
1st group vaccinated with Commercial bacterin.								
G1a	4/10	2/10	0/10	2/10	8/10	4/10	6/10	26/70 **(37.1)**
G1b	6/10	2/10	0/10	0/10	2/10	0/10	2/10	12/70 **(17.1)**
Total	10/20	4/20	0/20	2/20	10/20	4/20	8/20	38/140 **(27.1)**
% G1	**50%**	**20%**	**0%**	**10%**	**50%**	**20%**	**40%**	
2nd group vaccinated with Locally Prepared bacterin.								
G2a	0/10	8/10	0/10	6/10	2/10	2/10	4/10	22/70 **(31.4)**
G2b	2/10	2/10	0/10	2/10	8/10	2/10	4/10	20/70 **(28.6)**
Total	2/20	10/10	0/20	8/20	10/20	4/20	8/20	42/140 **(30)**
% G2	**10%**	**50%**	**0%**	**40%**	**50%**	**20%**	**40%**	
3rd group challenged with ST and SI isolates.								
G3a	10/10	8/10	0/10	6/10	6/10	2/10	4/10	36/70 **(51.4)**
G3b	8/8	3/5	5/5	3/5	2/4	2/4	2/4	25/70 **(71.4)**
Total	18/18	11/15	5/15	9/15	8/14	4/14	6/14	61/140 **(58.1)**
% G3	**100%**	**73.3%**	**33.3%**	**60%**	**57.1%**	**28.6 %**	**42.9%**	

dpc= day post challenge.

Clinical signs and lesions

All ducks in the positive control groups (G3a, G3b), (unvaccinated and challenged), displayed the typical signs of *Salmonella* infection which were; loss of appetite, ruffled feathers, dullness, emaciation, huddling together, dropped wings, closed eyes (sleepy appearance), whitish diarrhea, thirsty, nervous signs (tremors of head and neck), staggering gait and lameness (Fig 2,3). The clinical picture revealed; general septicemia, air-sacculitis, severe congestion of the intestine with enlarged ceca, enlarged gall bladder, congestion and enlargement of spleen, heart**,** kidney, and liver (with hemorrhagic patches) (Fig4-7). Whereas, all the vaccinated groups were protected from clinical signs and mortalities that were detected only in the control positive group G3b infected with *S.*Infantis (40%) within 2 weeks after the challenge.

Birds in the negative control group (neither vaccinated nor challenged), did not manifest any signs of disease throughout the trial period.

Fig. (2): Infected ducks showed closed eyes (sleepy appearance).

Fig. (3): Infected ducks showed nervous signs (control positive groups).

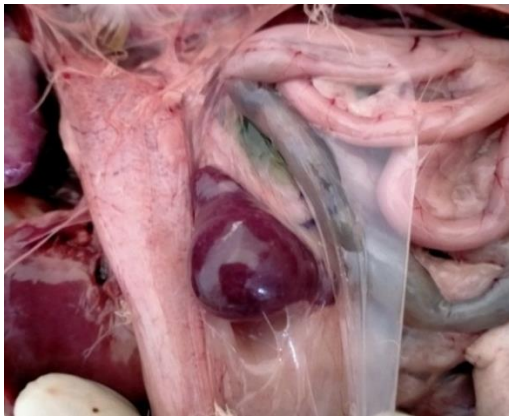


Fig. (4): Experimentally infected duck showed enlarged and congested spleen.

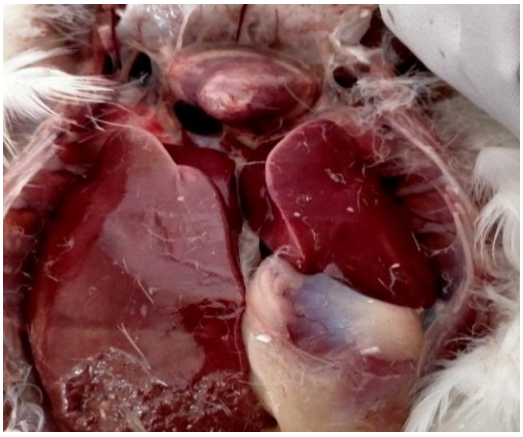


Fig. (5): Experimentally infected duck showed enlarged and congested liver.

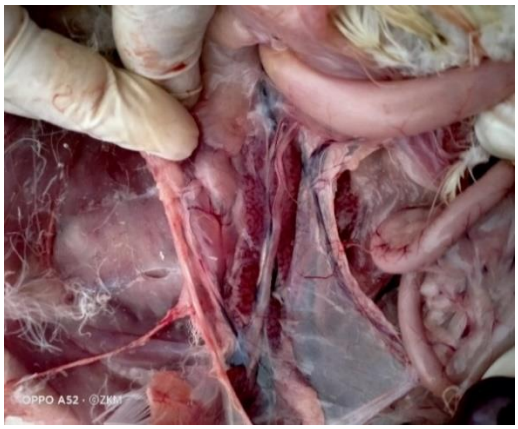


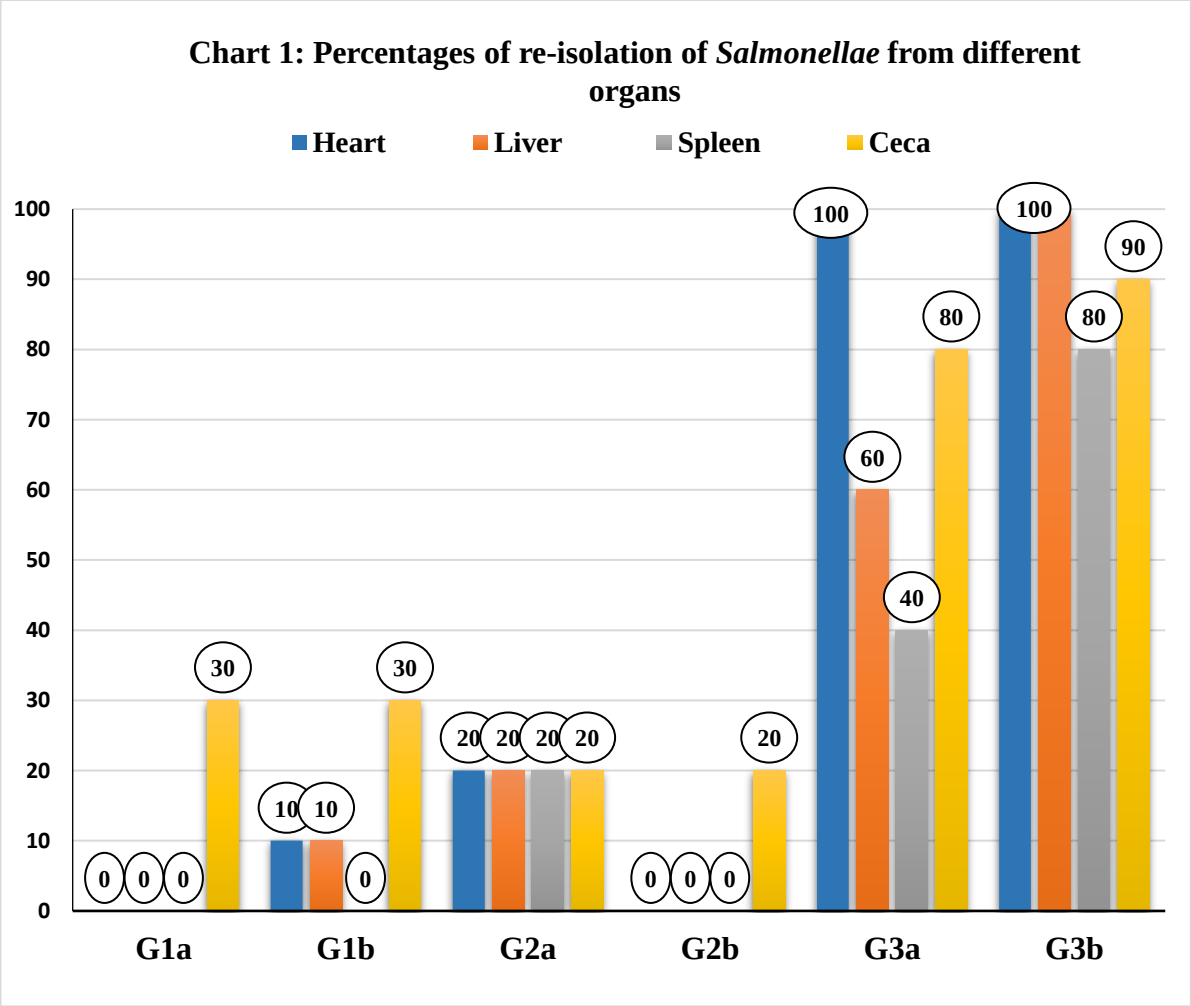
Fig. (6): Experimentally infected ducks had enlarged and congested kidneys.

Fig. (7): Experimentally infected ducks showed severe enteritis and enlarged ceci and filled with gases.

Rate of re-isolation of *Salmonella*

Total re-isolation percentages from different organs (heart blood, liver, spleen, and cecum) after 4 weeks from the challenge were varied as it was from 0% -30% in the commercial Servac bacterin group for both *S.*Typhi and *S.*Infantis, while it was 20% for *S.*Typhi and ranged from 0% -20% for *S.*Infantis in the local prepared *S.*Infantis bacterin group, however it was 80%-100% and 40%-100% from unvaccinated challenged ducks with *S.*Typhi and *S.*Infantis, respectively.

It was noticed that the highest re-isolation rate from both vaccinated groups after challenge was from cecum with 30% as shown in (**Chart 1**). On the other hand, the re-isolation ratio of *S.*Typhi **(**G3a) from the internal organs was as follows: heart (100%), cecum(80%), liver(60%), and spleen (40%) while, for *S.*Infantis **(G3b)** it was as follows: heart (100%), liver (100%), cecum (90%) and spleen (80%).



Average body weight of different groups

Table 2: Average body weight of different duck groups after challenge

Group	Weeks			
	1st	2nd	3rd	4th
G1a	$^{AC}2721\pm92.15^{b}$	$^{A}3246\pm222.48^{bc}$	$^{A}3662\pm358.98^{ac}$	$^{AD}4097\pm430.48^{a}$
G1b	$^{AC}2602\pm193.38^{b}$	$^{AC}2880\pm281.78^{b}$	$^{A}3618\pm347.47^{a}$	$^{CD}3816\pm416^{a}$
G2a	$^{BC}2029\pm118.79^{b}$	$^{BC}2335\pm77.88^{bd}$	$^{BC}2858\pm290.5^{cd}$	$^{CD}3565\pm515.84^{a}$
G2b	$^{BC}2032\pm52.10^{cd}$	$^{AC}2736\pm267.09^{bd}$	$^{AC}3380\pm308.40^{b}$	$^{AD}4220\pm391.34^{a}$
G3a	$^{BC}2055\pm135.96^{b}$	$^{BC}2304\pm116^{ab}$	$^{BC}2850\pm299.48^{a}$	$^{B}2780\pm208.33^{a}$
G3b	$^{B}1733\pm105.04^{a}$	$^{B}1848.75\pm88.61^{a}$	$^{B}2143.75\pm104.99^{a}$	$^{B}2570\pm99.50^{a}$
G3c	$^{A}3011\pm44.96^{b}$	$^{AC}2997\pm270.16^{b}$	$^{A}3761\pm277.19^{c}$	$^{A}4658\pm54.68^{a}$

a-b: Means with different superscript within the same row for each parameter differ significantly (P<0.05).
A-B: Means with different subscript within the same column for each parameter differ significantly (P<0.05)

Before challenge, there were no significant differences between all groups including the negative control group and the weekly recorded mean weights at the 1st, 2nd, 3rd and 4th weeks post-challenge (table 2, chart 2).

In 1st WPC significant differences were reported (P≤0.05) in the body weights of G3a, G3b, G2a and G2b compared with G3c. The body weights of G1a and G1b were relatively the same as those of G3c group.

In 2nd WPC, there were significant differences (P≤0.05) in body weights of G3a, G3b, and G2a in comparison with G3c, although there were no significant differences in weights of G1a, G1b and G2b and G3c.

In 3rd WPC, there was a significant decrease (P≤0.05) in body weights of G3a, G3b and G2a relative to G3c group. G1a, G1b, G2b and G3c showed non-significant decrease or increase in body weight.

In the 4th WPC, there was a significant decrease (P≤0.05) in body weights of G3a, G3b, G1b and G2a in comparison with G3c. There was a non-significant decrease in the weights of G1a, G2b and G3c.

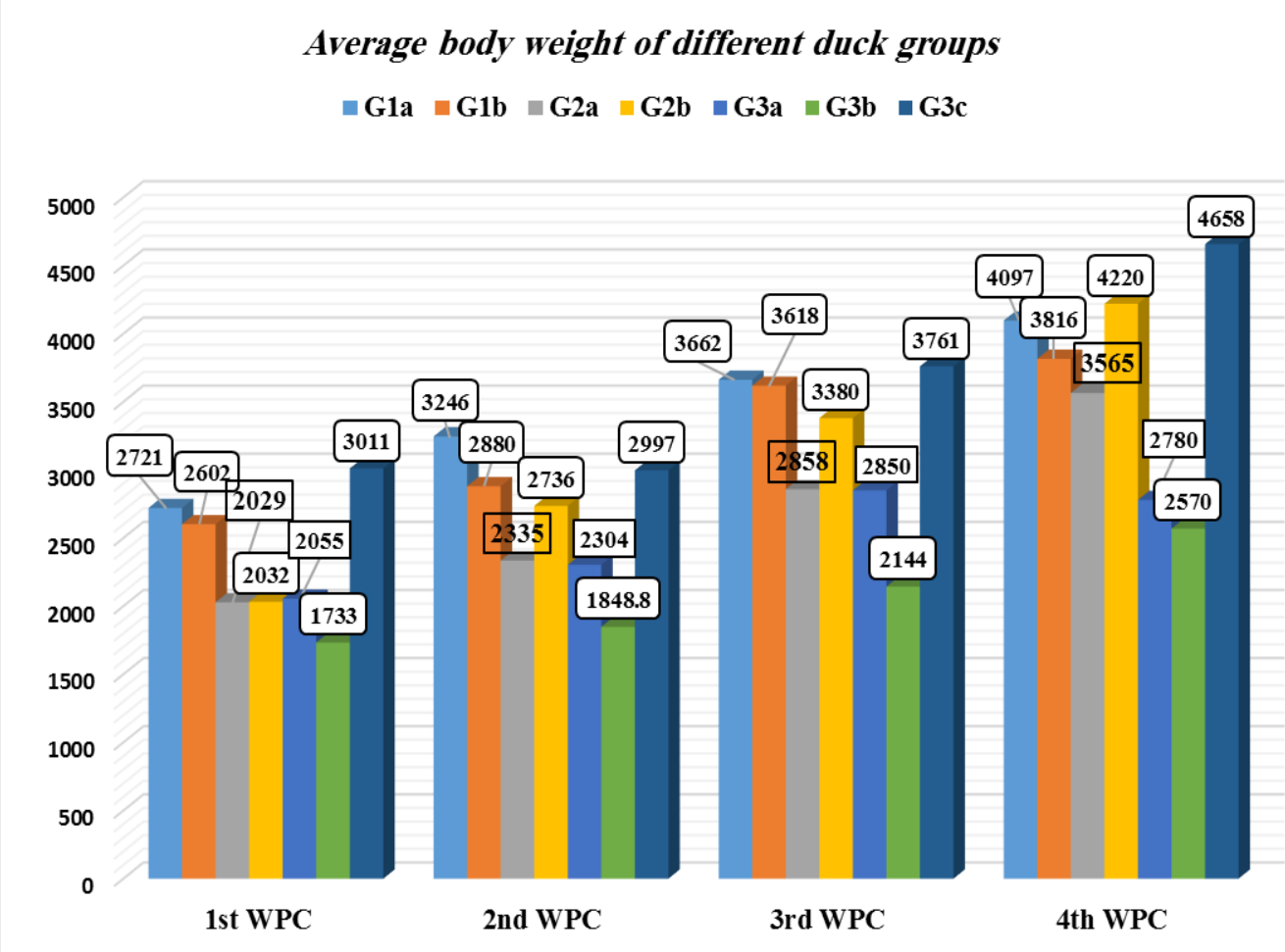


Chart (2): Average body weight in grams of vaccinated and unvaccinated challenged and unchallenged ducks.

DISCUSSION

Avian salmonellosis is a serious impediment disease to the progression of the poultry industry, especially in the developing countries of Asia and Africa. Worth efforts have been made and are continuing to eradicate the disease since there are currently no actual preventive available measures to date, only strict biosecurity (Rajagopal and Mini, 2013).

It was reported that *S.* Typhimurium is the most prevalent and predominant foodborne serotype of salmonellosis worldwide by Herikstad *et al*, 2002; this is in the same line with Niu *et al*, 2020 who revealed that *S.* Typhimurium was the most commonly isolated serotype from duck farms in China, however, *S.*Infantis considered the 4th prevalent serotype in poultry according to EFSA, 2015, thus in this study two *Salmonella* inactivated bacterins, one of them the commercial Servac trivalent containing (*S.*Typhimurium, *S.*Enteritidis and *S.*kentucky), and the other was locally prepared from field duck isolate (*S.*Infantis). The locally prepared inactivated *S.*Infantis mineral oil adjuvant bacterin passed all the quality control requirements as it was proved to be pure, sterile and has no adverse side effects on ducks. These results were accepted by the Egyptian standards for the evaluation of veterinary biologics – CLEVB (2009).

Although the shedding continuity of *Salmonellae* till the end of the experiment, the protection degree that rated 100%, in both vaccinated groups 1 and 2 post challenging without any significant difference, could be accepted and proved by the absence of mortalities, signs and even lesions after duck culling. The protection achieved by both bacterin formulations is accepted to pass the bacterin for use according to Egyptian standards for evaluation of veterinary biologics – CLEVB (2009) who stated that the inactivated *Salmonella* bacterin will be satisfactory if the protection percentage in vaccinated birds is not less than 70%. These results coincide with a previous observation, which showed that the protection rate was 90% in ducks vaccinated with a combined S.T-Duck Plaque-Duck Viral Hepatitis vaccine (Ahmed *et al.*, 2014).

Limited studies have estimated the cross-protection in-between *Salmonella* serotypes conferred by vaccines derived from specific serotypes, against strains of each other's challenge (Pavic *et al.*, 2010 and Varmuzova *et al.*, 2016).

From the fecal shedding values, results indicated that there was cross-protection between *S.* Typhimurium and *S.* Infantis serotypes, and this was attributed to the shared certain O-antigen structures (including the lipopolysaccharide core and certain surface proteins), that have a definite role in the cross-protection and reactivity (Liu *et al.*, 2016 and Kintz *et al.*, 2017).

Both vaccinated groups remained normal during the course of the experiment without signs, while ducks in the positive control groups suffered from typical clinical signs and lesions of duck salmonellosis, results that were in agreement with (Ibrahim *et al.*, 2018).

There was a significant decrease in body weights in the unvaccinated challenged groups (G3a, G3b), (G1b) and (G2a) groups in comparison with group G3c (negative control) and there was a non-significant decrease in weights in group G1a, G2b and G3c. The vaccinated groups resulted in no significant reduction in body weight compared to the negative control group, but unvaccinated challenged groups showed a significant reduction in body weight compared to the negative control group at the end of the experiment, results somewhat in agreement with those obtained by (Youn *et al.*, 2016).

CONCLUSION

Locally prepared *S.*Infantis inactivated bacterin and commercial Servac inactivated

bacterin provided ducks with a satisfactory level of protection (100%) from mortality and clinical signs as well as reduced fecal shedding and colonization of internal organs controlling *Salmonella* infection. Moreover, there was cross-protection between *S.* Infantis (sero-group C) and *S.* Typhimurium (sero-group B), and further large-scale studies are needed to assess the candidate bacterin.

ABBREVIATION

S. Typhi: *Salmonella* Typhimurium WPC: week post challenge.

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التقييم المقارن للقاحات السالمونيلا التجارية والمحضرة محليا ضد عدوى السالمونيلا في فراخ البط

زينب مصطفي خليفة ، عوض عبد الحافظ ابراهيم ، طلبة يونس عبد المطلب ، ازهارمحمد عبد العزيز ، مروة محمد صفوت

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في تجربة لتقييم قدرة لقاح السالمونيلا الميت المعد من عترة السالمونيلا إنفانتيس بإجراء أختبار التحدى باستخدام العترات الضارية لسالمونيلا إنفانتيس والتيفيميوريم ومقارنته مع اللقاح التجارى الميت المعد من عترات (سالمونيلا تيفيميوريم -سالمونيلا إنترتيديس -سالمونيلا كنتاكى) لبيان إذا كان هناك حماية أفضل ضد العدوى. وقد تم تجميع مسحات شرجية من البط المحصن والمعدى بعد أختبار التحدى ومتابعة الاعراض الأكلينيكية ونسبة النافق.

تم مقارنة اللقاح المحلى واللقاح التجارى (سيرفاك) على أداء البط من خلال تقسييم البط إلى ٣ مجموعات :

المجموعة الأولى تم تحصينها باللقاح التجارى المكون من عترات (سالمونيلا تيفيميوريم- سالمونيلا إنترتيديس- سالمونيلا كنتاكى) والمجموعة الثانية تم تحصينها باللقاح المعد من العترة المحلية (السالمونيلا إنفانتيس) والمجموعة الضابطة الغير محصنة) والتى تم تقسيمها الى مجموعتين وتم إعطاء الجرعة الاولى من كلا اللقاحين عند عمر ٧ ايام والجرعة الثانية عند عمر ٢٢ يوم وإجراء أختبار التحدى عند عمر ٣٧ يوم بكلا العترتين (السالمونيلا تيفيميوريم والسالمونيلا إنفانتيس) وقد تم تجميع مسحات شرجية من البط المحصن والمعدى بعد العدوى لتتبع نسبة إفراز الميكروب خلال فترة التجربة ومتابعة الاعراض الأكلينيكية ونسبة النافق. أشارت التجربة إلى فاعلية كلا اللقاحين وقدرتهم على توفير الحماية الكاملة ضد العدوى بكلا من العترتين (السالمونيلا تيفيميوريم والسالمونيلا إنفانتيس) حيث لم تلاحظ أى وفيات أو أعراض المرض فى أى من البط المحصن. كما أوضحت النتائج أن إستخدام كلا من اللقاحين فى هذه الدراسة قد قلل بشكل كبير إفراز الميكروب من الطيورالمحصنة ولكنه لم يمنعه وكذلك قلل إعادة عزله من الأعضاء الداخلية للطيور المحصنة مقارنة بالطيور المعدية الغير محصنة.