Evaluation of safety and efficacy of newly developed inactivated vaccine against *Pasteurella multocida* in turkeys

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Introduction

Fowl cholera (FC) is a serious highly contagious disease, caused by bacteria *Pasteurella multocida* and results in major economic losses to the poultry industry through death, weight loss, and condemnations (Rimler et al., 1998; Stout and Cornwell, 1976). It's enzootic disease and can spread easily within species. All birds species are affected with FC globally (Aravinth et al., 2016; Wilkie et al., 2000). Many outbreaks of FC are reported throughout the world (Rimler and Glisson, 1997).
Therefore, vaccination is the most powerful preventive method for the control of the disease (Ahmad et al., 2014). Several inactivated vaccines with different strains are available for protection against the disease.
In the present study, safety and efficacy of the newly developed monovalent inactivated vaccine based on a local *Pasteurella multocida* strain against Fowl Cholera disease, was studied in turkeys.

Materials and Methods

STRAIN

The *Pasteurella multocida* serotype A3-4 strain was isolated by M.C.I. Santé Animale during 2021 from turkey liver showing specific lesions and symptoms of fowl cholera, in turkey farm located in the Settat region. The strain was identified and stereotyped by PCR.

VACCINE

The Avipast vaccine is an inactivated and adjuvanted bacterial vaccine against fowl cholera formulated with Pasteurella multocida A3-4 strain at a dose of 10^9 CFU/0.3 ml. The vaccination protocol of broilers turkeys consist of intramuscular or subcutaneous administration of a dose 0.3 ml from 3 weeks of age, with booster 3 to 4 weeks later.

ANIMALS

A total of 150 turkeys were used in this study and divided as follow:
- 30 turkeys aged 10 weeks to determine the challenge dose.
- 70 turkeys aged 4 weeks to determine the vaccine formulation by challenge test.
- 50 turkeys of 4 weeks of age to determine efficacy and immunity duration.

A field trial was conducted on 30.000 turkeys aged 3 to 4 weeks, on Ouled Moussa Farm, Settat region supervised by Dr Ahmed Achhal.

METHOD

Animal testing

1) Determination of challenge dose

30 turkeys at 10 weeks of age seronegative Pasteurelle before experimental infection, were divided to six groups of five animals each. Group 1 to Group 5 were inoculated with serial dilutions of the Pasteurella multocida strain from 10^1 CFU/ml to 10^5 CFU/ml, respectively.

Group 6 was not inoculated and remained as control.
Animals were monitored during 14 days post infection: mortality, clinical signs, necropsy and sampling of tissue: liver lung and bone marrow.

2) Determination of vaccine dose by challenge test

Seventy turkeys, aged 4 weeks were randomized into 4 groups as described in table 1.

Group	Number	Vaccine	Age of vaccination	Dose/ route	Blood samples	Challenge	Post challenge monitoring
I	20	Avipast 10^7CFU	4 weeks 8 weeks	0.3 ml via intramuscular	D0 (4 weeks) D28 (8 weeks) D49 (11 weeks)	*11 weeks of age:* 1 ml via IM 10^1CFU/ml	14 days: mortality, clinicalsigns and necropsy with tissue sampling(lung, live and bone marrow) for bacteriology and PCR
II	20	Avipast 10^8CFU					
III	20	Avipast 10^9CFU					
IV	10	Controls	-	-			

Table 1: Dose escalation study design

3) Vaccine efficacy and immunity duration

Fifty turkeys, aged 4 weeks were randomized into 3 groups, as follow.

Group	Number	Vaccine	Age of vaccination	Dose/ route	Blood samples	Challenge	Post challenge monitoring
A	20	Avipast 10^9 CFU	4 weeks 8 weeks	0.3 ml via intramuscular	D0 (4 weeks) D28 (8 weeks) D56 (12 weeks) D77 (15 weeks)	*15 weeks of age:* 1 ml via IM 10^1CFU	14 days: mortality, clinical signs and necropsy with tissue sampling (lung, live and bone marrow) for bacteriology and PCR
B	20		4 weeks 8 weeks 12 weeks				
C	10	Controls	-	-			

Table 2: Efficacy test study design at 15 weeks of age

Field study

The aim of the field study was to verify under field conditions the safety and immunogenicity of Avipast vaccine in turkeys. The Avipast inactivated vaccine was administered to a total of 30.000 turkeys according to the recommendation of the manufacturer, as follow:
Primary-vaccination at 3 to 4 weeks of age: 0.3 ml of Avipast vaccine by intramuscular route in the wishbone.

Second vaccination at 7 to 8 weeks of age: 0.3 ml of Avipast vaccine by subcutaneous route in the lower part of the neck. Blood samples were taken, from a sample of 20 vaccinated animals before vaccination at D0, at one week (D7), at two weeks (D14), and at seven weeks (D49) post vaccination. Blood samples were transferred to serology Laboratory, at MCI Santé Animale for ELISA test, ID Screen® Pasteurella multocida Chicken and Turkey Indirect (IDvet).

Results

1) Determination of challenge dose

Group	Number	Strain dose	% of mortality	% of strain reisolation
1	5	10^1CFU/ml	80%	100%
2	5	10^2CFU/ml	100%	100%
3	5	10^3CFU/ml	100%	100%
4	5	10^4CFU/ml	100%	100%
5	5	10^5CFU/ml	100%	100%
6	5	Controls	0%	0%

Table 3: Determination of challenge dose results

Considering these results, the dose of 10^1 CFU/ml was chosen and selected as a dose of challenge to test the efficacy of the Avipast vaccine.

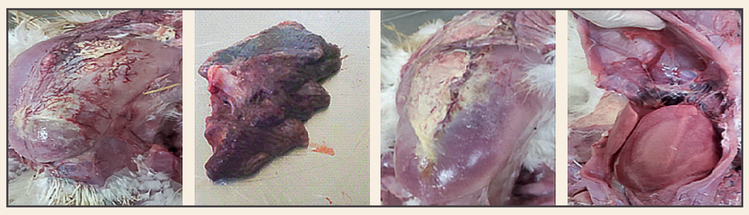

Figure 1: Lesions recorded in infected turkeys with Pasteurella multocida strain

2) Determination of vaccine formulation by challenge test

Figure 2: Percentage of seropositive animals after vaccination with AVIPAST vaccines at different doses

Good serological response was noted in vaccinated turkeys from GIII (109 CFU) with 65% of seroconversion after the first vaccination.

Gr	Number	Vaccine dose	% of mortality	% of strain reisolation and PCR	% of protection
I	20	10^7CFU/ml	45%	100%	55%
II	20	10^8CFU/ml	5%	90%	95%
III	20	10^9CFU/ml	5%	25%	95%
IV	10	Controls	70%	80%	-

Table 4: Mortality, bacterial isolation and PCR results recorded in dose escalation study

Under the conditions of the study, the Avipast inactivated vaccine induced a good level of protection (95%), as shown by the rate of protected animals vaccinated with a dose of 10^9 cfu. Considering these results, it can therefore be concluded that 10^9 cfu was chosen dose for vaccine formulation.

Vaccine safety

Following vaccination of turkeys with AVIPAST vaccine there were no evidence of an alteration of the general condition in all turkeys.

Vaccine efficacy and immunity duration

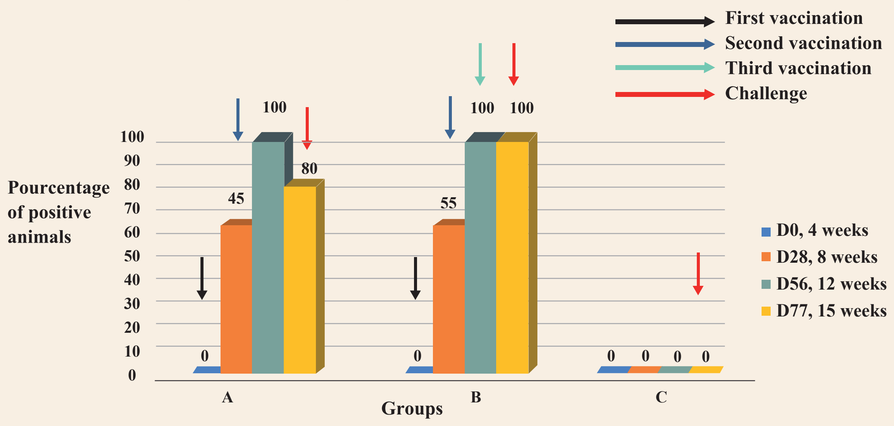

Figure 3: Percentage of seropositive turkeys from groups A, B and C after vaccination with AVIPAST vaccines, using ELISA test

The serological results revealed a significant development of a good level of specific anti- *Pasteurella multocida* antibodies in the vaccinated animals especially after the second vaccination of groups A and B with an advantage of Group B with the third vaccination. All control animals remained seronegative.

Gr	Number	Vaccine	Age of vaccination	% of mortality	% of strain reisolation and PCR	% of protection
A	20	Avipast	4 and 8 weeks	20%	30%	80%
B	20		4, 8 and 12 weeks	0%	5%	100%
C	10	Controls	-	80%	80%	-

Table 5: Mortalities and percentage of strain reisolation recorded in inoculated turkeys of different groups after experimental infection

Based on these results, it was concluded that the Avipast vaccine protects vaccinated turkeys at 15 weeks of age with two-vaccine administration, with better protection with three vaccinations at 4, 8 and 12 weeks of age.

Field study

Following the monitoring of the vaccinated turkeys, a weakness during the first 12 hours was recorded. All the turkeys remained in good health with no mortality or any local reactions after the administration of the Avipast vaccine were observed.

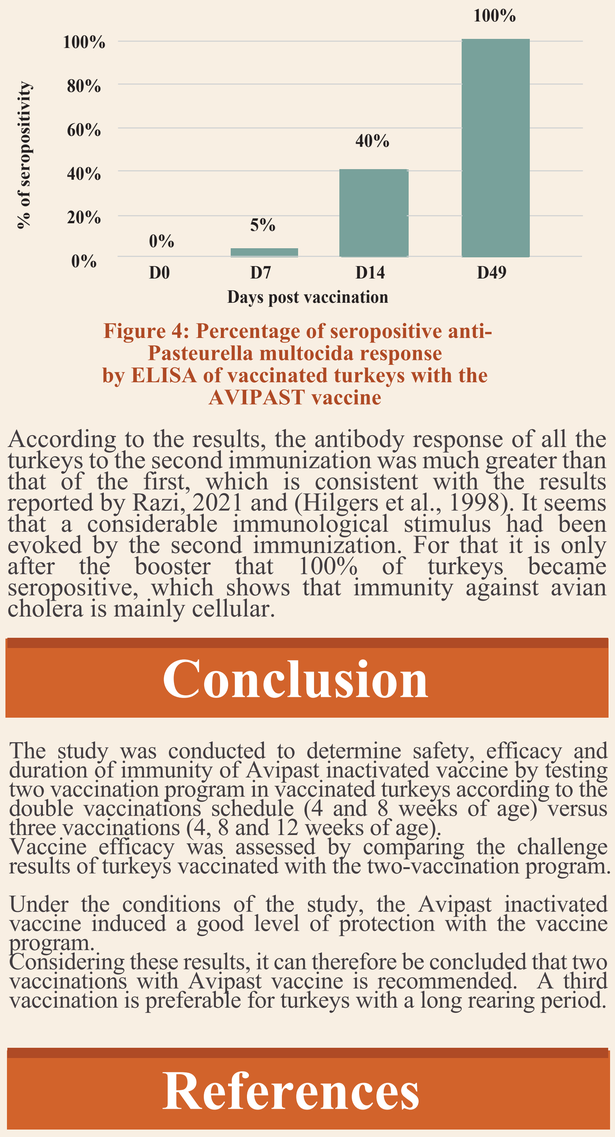

Figure 4: Percentage of seropositive anti-Pasteurella multocida response by ELISA of vaccinated turkeys with the AVIPAST vaccine

According to the results, the antibody response of all the turkeys to the second immunization was much greater than that of the first, which is consistent with the results reported by Razi, 2021 and (Hilgers et al., 1998). It seems that a considerable immunological stimulus had been evoked by the second immunization. For that it is only after the booster that 100% of turkeys became seropositive, which shows that immunity against avian cholera is mainly cellular.

Conclusion

The study was conducted to determine safety, efficacy and duration of immunity of Avipast inactivated vaccine by testing two vaccination program in vaccinated turkeys according to the double vaccinations schedule (4 and 8 weeks of age) versus three vaccinations (4, 8 and 12 weeks of age).
Vaccine efficacy was assessed by comparing the challenge results of turkeys vaccinated with the two-vaccination program.

Under the conditions of the study, the Avipast inactivated vaccine induced a good level of protection with the vaccine program.
Considering these results, it can therefore be concluded that two vaccinations with Avipast vaccine is recommended. A third vaccination is preferable for turkeys with a long rearing period.

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