Safety and efficacy of 9R Live attenuated vaccine against fowl typhoid in partridges species

Zohra Bamouh[1], Noha Semmate[1], Mohamed Mouahid[2], Ismail Kerbal[1], Khalid Omari Tadlaoui[1], Mehdi El Harrak[1]

[1] Department of Research and Development, MCI Santé Animale, Lot. 157, ZI Sud-Ouest P.O. Box 278, Mohammedia 28810, Morocco

[2] Cabinet Vétérinaire Dr MOUAHID, Route Sidi Yahia Zaires 20 Apt N° 1 Lot Abbadi, Temara, Morocco

Background

Salmonella enterica serovar *gallinarum* (*S. gallinarum*) is the causative agent of fowl typhoid, a severe systemic disease which affects mainly chickens and turkeys, however other birds species can be affected, especially partridges, which are a prestigious species in Morocco. The mortality rate on farms is high, and the use of antibiotics is not effective against this disease. Vaccinating partridges against fowl typhoid is therefore an absolute priority if this breed is to be preserved in Morocco. However, the effectiveness of vaccines against fowl typhoid in this species has not been extensively studied.
Due to its negative impact on partridge farms in Morocco, a study was carried out to assess the benefits of managing the fowl typhoid in partridges via vaccination with a 9R vaccine produced locally: AVIVAX SG-9R.

Objective

The aim of this study is to evaluate efficacy of vaccination with 9R strain « AVIVAX SG-9R » against fowl typhoid in partridges species at recommended age.

Materials and Methods

CHALLENGE STRAIN:
Salmonella gallinarum MSG1 strain, isolated from case of fowl typhoid, provided by Pr. K.BOUZOUBAA. The strain was refreshed on pullets to increase its virulence.

VACCINE:
The AVIVAX SG 9R live attenuated bacterial vaccine against avian typhoid (MCI Santé Animale, Mohammedia, Morocco), was used in this study.
The vaccination protocol consist of a subcutaneous administration of a 0.2 ml dose (2.10^7CFU) in partridges at the minimum recommended age for vaccination (6 weeks), followed by revaccination at 12 weeks of age.

ANIMALS:
A total of 235 partridges were used in this study:
- 60 partridges of 14 weeks old, were divided in 4 groups of 15 each to determine the challenge dose.
- 175 partridges of 6 weeks of age, were divided in 3 groups to evaluated safety and efficacy of the AVIVAX SG 9R vaccine.

METHOD:

Determination of challenge dose

60 partridges, divided into 4 groups of 15 each inoculated with serial dilutions of MSG1 strain:

Table 1: groups of animals with serial dilutions of MSG1 challenge strain

Groups	Number	Strain MSG1 dose
1	15	10^4CFU/ml
2	15	10^5CFU/ml
3	15	10^6CFU/ml
4	15	10^7CFU/ml

Inoculated animals were monitored during two weeks post infection for clinical signs, mortality and lesions. Died animals were autopsied and the following organs were collected for bacterial isolation: spleen, liver, caecas and bone marrow.

VACCINATION:
At D0 of the study, 175 partridges, 6 weeks old, were divided into three groups as follow:

Table 2: efficacy study design

Groups	Number	Vaccination at 6 and 12 weeks of age	Challenge at 14 weeks of age
I	105	+	+
II	35	-	+
II	10	-	-

Results

Determination of challenge dose

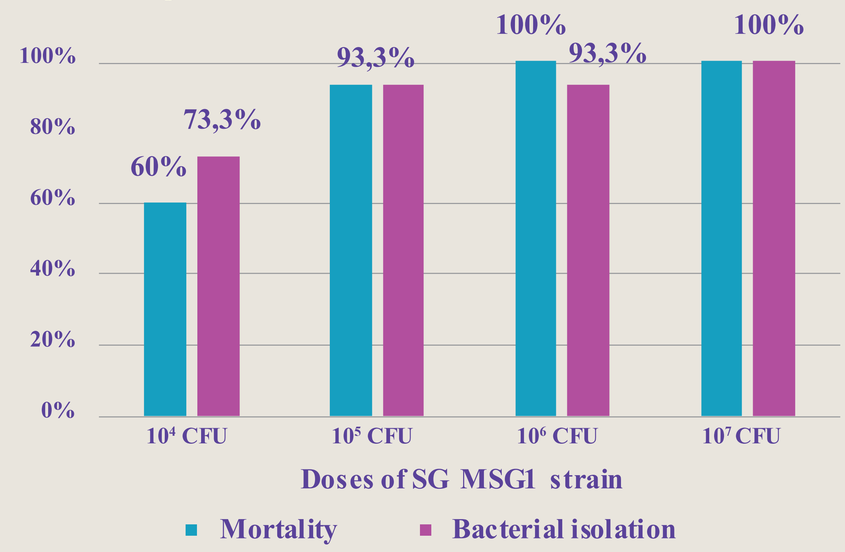

Figure 1: Percentage of mortality and bacterial isolation obtained after inoculation of partridges with serial dilutions of MSG1 strain

The challenged dose chosen for efficacy test was 10^4CFU/ml.

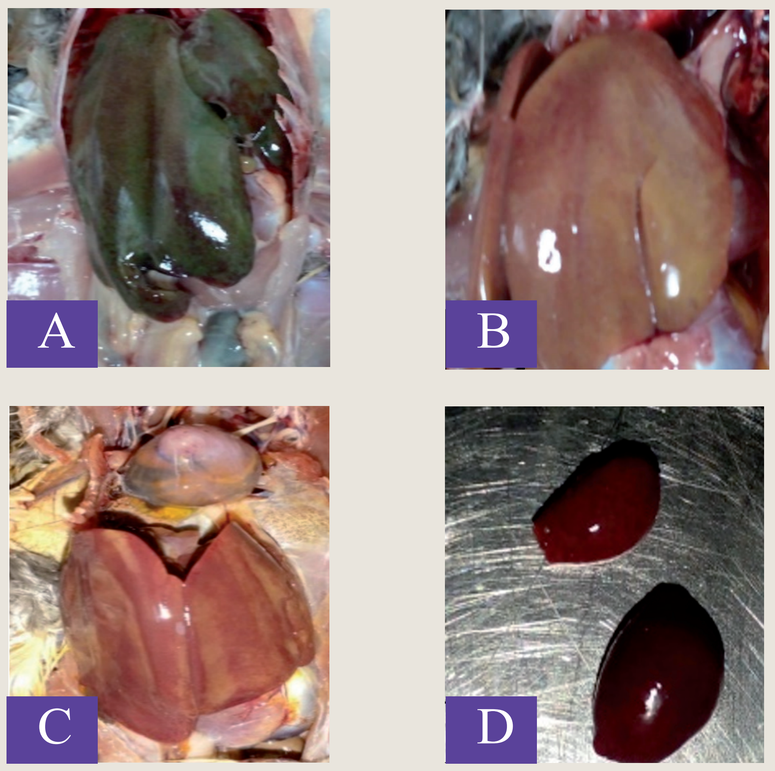

Figure 2: Lesions observed in infected partridges by Salmonella Gallinarum MSG1 strain. (A): greenish, enlarged liver. (B): discolored liver. (C): discolored liver and hydropericarditis (D): splenomegaly

Efficacy

Clinical signs
All challenged control partridges (GII) showed clinical signs after infection: anorexia, depression and ruffled feathers.
Some vaccinated and challenged partridges showed slight clinical signs similar to GII.
No clinical signs were observed unvaccinated and unchallenged control group (GIII).

Mortality
The mortality rates of the control and vaccinated groups were 70.6% and 35%, respectively. No mortality was noted in unvaccinated and unchallenged group (GIII).

Reisolation and PCR
The *Salmonella gallinarum* was reisolated from 100% of challenged partridges from control group (GII), however, 75% of strain reisolation was recorded in vaccinated partridges (GI). No *Salmonella gallinarum* was detected in control partridges from GIII. These results were confirmed by PCR test.

Table 4: Mortalities and bacterial isolation recorded in challenged partridges of different groups

Groups	Number	Mortality	Bacterial isolation	Protection (%)
Vaccinate GI	20	7	15	65%
Inoculated control GII	17	12	17	-
Uninoculated control GIII	10	0	0	-

Materials and Methods

At D2, D3, D5, D7, D14, D21, D28, D42, D44, D45, D47, D49 and D56 post vaccination, 5 vaccinated partridges from GI were euthanized. A complete autopsy was carried out to examine macroscopic lesions. Samples were collected from spleen, liver, caecal junction and bone marrow for bacterial isolation.

CHALLENGE:

Table 3: groups used to control efficacy of 9R vaccine

Groups	Number	Age	Challenge dose
I	20	14 weeks	10^4CFU/ml, via oral route
II	17		
II	10		-

Partridges were observed during 14 days post challenge (clinical signs, mortality and lesions).
Dead and sacrificed animals were autopsied and samples were collected for bacteriological isolation and PCR from spleen, liver, caecal junction and bone marrow.

Discussion

In the present study the protection rate achieved seems lower than that obtained in the only similar study conducted in Morocco (Lahouideg et al. 2009), which showed full protection in vaccinated partridges.
These results can be attributed to both the virulence and the dose of the challenge strain.
In our study the MSG1 strain was refreshed twice on the target species to increase its pathogenicity. Indeed, control partridges were challenged with a DL60% (10^4CFU), while in the study of Lahouideg et al. 2009, the challenged dose was DL50% (8.71 x 10^5CFU).
In the other side, the high mortality recorded in this experiment indicated that partridges species are more susceptible to *Salmonella gallinarum* than chickens. Using the same MSG1 challenge strain with a high dose of 10^9 CFU we obtained only 10 to 20% of mortality in pullets.

Conclusion

The vaccine has been shown to be effective in preventing mortality and reducing clinical signs and lesions of fowl typhoid in partridges.
An adjustment of the minimum age of vaccination of partridges with AVIVAX SG-9R vaccine to 6 weeks plus a booster at 12 weeks of age is acceptable based on the results of the safety and efficacy studies. Two doses provide better protection than a single dose. The vaccine is given subcutaneously.
AVIVAX SG-9R vaccine, based on the *Salmonella gallinarum* 9R strain, can reduce flock losses due to fowl typhoid and contribute to the reduction of infection. Vaccination should be considered as part of a fowl typhoid eradication programme.

References

Lahouideg, T., Darkaoui, S., Kadiri, A., Amar, O., Id sidi yahia, K., Mouahid, M., 2009. Effectiveness of vaccination against fowl typhoid in the partridge (Alectoris barbara).
Bouzoubaa, K., Lemainguer, K., Bell, J.., 1992. Village chickens as a reservoir of Salmonella pullorum and Salmonella gallinarum in Morocco. Prev. Vet. Med. 12, 95–100.
Díaz-Sánchez, S., Moriones, A.M., Casas, F., Höfle, U., 2012. Prevalence of Escherichia coli, Salmonella sp. and Campylobacter sp. in the intestinal flora of farm-reared, restocked and wild red-legged partridges (Alectoris rufa): is restocking using farm-reared birds a risk? Eur J Wildl Re 58, 99–105.
Feberwee, A., de Vries, T., Hartman, E., de Wit, J., Elbers, A., de Jong, W., 2001. Vaccination against Salmonella enteritidis in Dutch commercial layer flocks with a vaccine based on a live Salmonella gallinarum 9R strain: evaluation of efficacy, safety, and performance of serologic Salmonella tests. Avian Dis 45, 83–91.
Javed, T., Hameed, A., Siddique, M., 1990. Status of Salmonella in indigenous (domestic) chickens in Pakistan. Veterinarski Arhiv 60, 251–255.
Lee, Y., Mo, I., Kang, M., 2005. Safety and efficacy of Salmonella gallinarum 9R vaccine in young laying chickens. Avian Pathol 34, 362–366.
Lucientes, J., 1998. Las principales patologías de la perdiz roja silvestre., in: La Perdiz Roja, I Curso, FEDENCA/Grupo Editorial V, Madrid, Spain.
Mastroeni, P., Chabalgoity, J.A., Dunstan, S.J., Maskell, D.J., Dougan, G., 2001. Salmonella: Immune responses and vaccines. Vet. J. 161, 132-164.
Revolledo, L., 2018. Vaccines and vaccination against fowl typhoid and pullorum disease: An overview and approaches in developing countries. Journal of Applied Poultry Research 27, 279–291.