Field trials with the use of a live attenuated temperature-sensitive vaccine for the control of *Mycoplasma gallisepticum* infection in meat-type turkeys

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ABSTRACT

Mycoplasma gallisepticum (MG) continues to be an important pathogen of poultry, causing significant production losses in many parts of the world. Eradication is the preferred method of control but it could result impractical after the organism has been introduced in an area with high density of poultry farms. TS-11®, a temperature-sensitive live attenuated MG vaccine, is currently utilized in several countries for the control of MG infections in commercial layers and broiler breeders. In the present field trial, conducted in an industrial meat-turkey farm (belonging to an integrated company), previously affected by severe MG infections, the ability of TS-11® in effectively colonizing the upper respiratory tract in a turkey flock was evaluated ("TS-11®" flock). A second flock grown in an adjacent pen of the same farm was vaccinated with an inactivated MG vaccine ("Inactivated" flock). Polymerase Chain Reaction (PCR) and Random Amplified Polymorphic DNA (RAPD) analysis were applied for the detection and differentiation of TS-11® from other MG strains possibly present in the same flocks, such as the field strains and the 6/85 live vaccine strain currently utilized in commercial layers in Italy. PCR-RAPD results achieved in the "TS-11®" flock were compared with those of a flock of turkey grown in the same farm but vaccinated with an inactivated MG vaccine. Encouraging results were achieved by means of PCR-RAPD detection of TS-11® from all of the samples up to eight weeks post vaccination, whereas it was never detected in the "Inactivated" flock. Moreover, the field strain was never detected in the "TS-11®" flock but in the "Inactivated" one it was detected either 5 and 8 weeks after the vaccination. The aggregate production data of the two flocks resulted significantly improved when compared to the performance of the previous flocks grown in the same farm and similar to the production standard of the integrated company.

Key Words: Turkeys, *Mycoplasma gallisepticum*, Live vaccine, Polymerase Chain Reaction (PCR), Random Amplified Polymorphic DNA (RAPD).

RIASSUNTO

PROVE DI CAMPO NELL'IMPIEGO DI UN VACCINO VIVO ATTENUATO TERMO-SENSIBILE PER IL CONTROLLO DELL'INFEZIONE DA *MYCOPLASMA GALLISEPTICUM* IN TACCHINI DA CARNE

Mycoplasma gallisepticum *(MG) continua ad essere un importante agente patogeno del pollame, dove provoca gravi perdite produttive in diverse aree del mondo. L'eradicazione rappresenta il miglior metodo per il controllo di MG, tuttavia può risultare difficilmente praticabile una volta che l'infezione è stata introdotta in un'area caratterizzata da elevata concentrazione di allevamenti avicoli. TS-11®, un vaccino MG vivo attenuato termo-sensibile, viene attualmente impiegato in diversi Paesi del mondo per il controllo delle infezioni da MG nelle galline ovaiole commerciali e nei riproduttori pesanti. Nella prova di campo, condotta in un allevamento commerciale di tacchini da carne (appartenente ad un gruppo avicolo integrato), colpito nei cicli produttivi precedenti da gravi infezioni MG, è stata valutata la capacità di TS-11® di colonizzare efficacemente le vie respiratorie superiori (gruppo "TS-11®"). Un secondo gruppo allevato in un*

capannone adiacente nella stessa azienda è stato vaccinato con un vaccino inattivato (gruppo "Inattivato"). Le tecniche PCR e RAPD sono state applicate per l'individuazione e la differenziazione di TS-11® da altri ceppi di MG eventualmente presenti negli stessi gruppi, quali i ceppi di campo ed il vaccino vivo 6/85 attualmente utilizzato nelle ovaiole commerciali in Italia. I risultati dei test PCR-RAPD ottenuti nel gruppo "TS-11®" sono stati comparati con quelli del gruppo "Inattivato", allevato in un capannone adiacente, nella stesso allevamento. Sono stati così ottenuti risultati incoraggianti, infatti TS-11® è stato individuato sistematicamente su tutti i campioni prelevati tra le 2 e le 8 settimane dopo la vaccinazione mentre non è mai stato identificato nei tamponi prelevati dal gruppo "Inattivato". Inoltre, il ceppo di campo non è mai stato evidenziato nel gruppo "TS-11®" mentre nel gruppo "Inattivato" i tamponi tracheali sono risultati positivi rispettivamente a 5 e 8 settimane post-vaccinazione. I dati produttivi complessivi sono risultati significativamente migliori rispetto alle performance dei cicli precedenti allevati nella stessa azienda ed in linea con lo standard produttivo del gruppo avicolo integrato.

Parole chiave: *Tacchino,* Mycoplasma gallisepticum*, Vaccino vivo,* PCR, RAPD.

Introduction

Mycoplasma gallisepticum (MG) plays an important role as pathogen of poultry, causing significant economic losses, particularly in the areas where higher is the concentration of industrial farms. In meat-type turkeys infected with MG, severe respiratory syndromes occur with losses due to increased mortality rate and condemnation of carcasses, poor performance and increased medication costs.

Strategies to minimize if not eliminate the impact of MG infections can include eradication programmes, specific antimicrobial treatments and immunization with live or inactivated vaccines.

TS-11® is a temperature-sensitive live attenuated MG vaccine, currently utilized in Italy and many other Countries for the control of MG infections in commercial layers. The vaccination of broiler chicken breeders prevented the infection by field MG in their tracheas and infraorbital sinuses and in the vitelline membrane of their embryos (Barbour *et al.*, 2000).

In spite of its use on a large scale in commercial layers in the United States, it was never isolated from field turkeys (Kleven *et al.*, 2000).

In a vaccination trial conducted by Kleven (2000), administration of TS-11® to meat turkeys resulted safe and there was some protection detected against lesions in the upper respiratory tract, thus indicating a potential use in certain conditions. During the last years, in some densely poultry populated areas of Northern Italy, an increasing number of severe MG outbreaks, occurred consecutively in the same industrial turkey farms; either individual and mass antimicrobial treatments were not able to control the clinical disease. The promiscuity of the staff and the equipment utilized in the farms of the same area was probably critical in perpetuating the infection, in spite of an all in-all out policy applied in every single farm.

The main purpose of the present trial, conducted in one of the farms, (belonging to an integrated company), affected by MG infections in the previous cycles, was to examine the ability of TS-11® in colonizing in the mucosa of the upper respiratory tract of turkey and to evaluate the persistency and its possible effect in inducing protection against field MG strains.

PCR and RAPD analysis (Paganelli *et al.*, 2003) applied on tracheal swabs drawn at regular intervals from turkey flocks, enabled the detection and the genetic differentiation of TS-11® from the field strains.

Material and methods

MG vaccines

TS-11® (Merial Italia S.p.A.), a frozen temperature-sensitive live attenuated MG vaccine (Whithear *et al.*, 1990), was stored at –20°C then thawed in a water bath at 30 to 35°C just prior to the administration; each bird of the first flock ("TS-11®") received one dose, containing at least $10^{7.4}$ Colour-Changing Units (CCU) in 50 microlitres, applied by ocular instillation.

One dose (0.5 ml) of a commercial inactivated oil emulsified MG vaccine was administered subcutaneously to the second flock ("Inactivated") in the same farm.

MG serological tests

Rapid plate agglutination test (RPAT) for MG antibodies was carried out with a commercial antigen.

A commercial Enzyme-Linked Immuno-Sorbent Assay (ELISA) test kit was used to detect MG antibodies.

DNA extraction and MG identification

The Polymerase Chain Reaction (PCR) protocol was adapted from the procedure described by Lauermann *et al.* (2003) and performed on DNA after extraction from tracheal swabs (Paganelli *et al.*, 2003). Each sample (100-2000 ng/µl) was treated with PCR reagents included in Accuprime TaqPCRx DNA Polymerase (Invitrogen®,USA). We used primers "forward" MG-F+ and primers "reverse" MG-R (Table 1). DNA amplification is performed in a thermocycler (TpersonalTM, Biometra, Germany). The optimized PCR program was as follows: 5 minutes at 94°C (hot start), 35 cycles with 3 steps: 94°C for 30 sec. (denaturation), 55°C for 30 sec. (annealing), 72°C for 1 minute (extension); finally 1 minute at 72°C for final extension. PCR products were separated using electrophoresis on a 1,7% agarose gel containing ethidium bromide for 30 minutes at 80V. MG amplicons were visualized with an ultraviolet transilluminator along with control samples (positive and negative) and PCR marker GeneRuler 100bp DNA ladder (Fermentans®, Lithuania). When MG positive, amplified product presented a 185bp fragment (Paganelli *et al.*, 2002).

RAPD analysis

A further PCR on extracted DNA was performed using a commercial kit (Ready-to go RAPD Analysis – Pharmacia Biotech®, Piscataway, USA) containing a set of primers. Primer 6 was used for the first reaction. A combination of primers 3 and 4 was used for the second reaction (Table 1) (Paganelli *et al.*, 2003). RAPD was performed in a thermocycler (TpersonalTM, Biometra®, Germany). RAPD program was as follows: 5 minutes at 95°C (hot start), 45 cycles with 3 steps: 95°C for 1 minute, 36°C for 1 minute, 72°C for 2 minutes; finally 1 cycle at 4°C. Each reaction included the vaccine strains TS-11 and 6-85.

RAPD products were separated using electrophoresis on a 2% agarose gel containing ethidium bromide for 30 minutes at 80V. MG amplicons (185 bp) were visualized with an ultraviolet transilluminator, comparing *patterns* each other, along with marker GeneRuler 100bp DNA ladder (Fermentans®, Lithuania) (Paganelli *et al.*, 2003).

Field trial design

8500 day-old turkey toms were divided in two flocks of the same size and housed in adjacent pens in the same farm (belonging to an integrated company), previously undertaken to cleaning, disinfection and downtime. Before vaccination 15 swabs were randomly taken from trachea and choanal cleft respectively and 20 blood samples were randomly drawn from the turkeys of each flock to exclude any MG positivity. At three weeks of age "TS-11®" flock was eye-drop vaccinated while "Inactivated" flock was vaccinated subcutaneously. At the age of 5, 8, 11 and 15 weeks respectively, from each of the two flocks were randomly drawn 15 blood samples and 15 tracheal swabs; from every set of 15 swabs 3 pools were obtained and tested by PCR-RAPD.

Due to practical reasons, the production parameters of the two flocks could not be registered separately but the aggregate data were compared with the correspondent data of the previous production cycle.

Table 1. Sequence of RAPD primers.

MG-F	5′ GAGCTAATCTGTAAAGTTGGTC 3′
MG-R	5′ GCTTCCTTGCGGTTAGCAAC 3′
RAPD 3	5′-d [GTAGACCCGT]- 3′
RAPD 4	5′-d [AAGAGCCCGT]- 3′
RAPD 6	5′-d [CCCGTCAGCA]- 3′

Results and discussion

Clinical monitoring detected no reaction after TS-11® vaccination and no considerable treatments were needed during the whole production cycle.

The results of PCR and RAPD monitoring on pooled tracheal swabs are summarized in Table 2. Table 3 shows the serological results. In Table 4 the aggregate performance of the two flocks are compared to the results of the previous cycle and to the production standard of the Integrated Company.

PCR and RAPD analysis enabled the identification of TS-11® in the trachea of turkeys up to 8 weeks after the vaccination. The live vaccine strain was detected only in the TS-11® flock whereas the field infection was demonstrated only in the "Inactivated" flock. Serological results showed a late response in the TS-11® flock, more evident with the RPA Test; however, a possible effect of the field infection could not be ruled out even if not detected by PCR-RAPD techniques. To the knowledge of the authors, data on serological response of turkeys vaccinated with TS-11® are not reported in the relevant literature, yet in a trial conducted in an isolation room, no serological response was detected (unpublished data). In any case the protection against MG seems non correlated with serological titres. Production results of the two flocks, although were not registered separately, showed a significant improvement of all the parameters when compared to those of the previous cycle grown in the same farm and were similar to the production standard of the integrated company.

Table 2. Results of PCR and RAPD tests applied on pools of tracheal swabs drawn at different ages from TS-11® and Inactivated flocks.

Age	3 weeks		5 weeks		8 weeks		11 weeks		15 weeks	
Test	PCR	RAPD	PCR	RAPD	PCR	RAPD	PCR	RAPD	PCR	RAPD
TS-11®	Neg.	-	Pos. 3/3	TS-11®	Pos. 3/3	TS-11®	Pos. 3/3	TS-11®	Neg.	-
Inactivated	Neg.	-	Neg.	-	Pos. 1/3	'Field' MG	Pos. 2/3	'Field' MG	Neg.	-

Table 3. Percentage of turkeys sero-positive to MG at different ages in TS-11® and Inactivated flocks.

Age	3 weeks		5 weeks		8 weeks		11 weeks		15 weeks	
Test	RPAT	ELISA	RPAT	ELISA	RPAT	ELISA	RPAT	ELISA	RPAT	ELISA
TS-11®	0	-	0	0	0	0	100	60	100	0
Inactivated	0	-	0	0	0	80	27	87	100	53

RPAT: Rapid plate agglutination test.

Table 4. Performance compared the previous cycle and to the Company production standard.

Parameters	Age days	Mortality %	Avg. body weight Kg	Feed Conversion	Medication costs Euro/kg
Field trial	137	8.82	18.7	2.57	0.017
Previous cycle	134	13.88	16.0	2.62	0.076
Company standard	135	9.50	18.5	2.56	0.025

Conclusions

The data of PCR and RAPD analysis, even if achieved only by a field trial, enable to suppose the ability of infection of TS-11® in the upper respiratory tract of turkey and the possibility of a protection against the field infection. It is fair to consider that the traditional microbiological techniques make it possible to isolate MG strains with the capability of replication whereas the PCR test can only detect the bacterial genome; however, the recovery of TS-11® repeated during a long time interval would give evidence of its ability in the tracheal colonization and persistence.

Good production performance in spite of the field infection in the farm, support the hypothesis of the protective effect of this live Mg vaccine.

Further trials are needed to confirm the results achieved, before considering the possibility of utilization of TS-11® on a large scale as an additional tool for the control of MG infections in turkeys.

The authors would like to thank the technical staff of the Sezione Diagnostica di Forlì (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna) *for the valuable support.*

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