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in the relevant literature, yet in a trial conducted in an isolation room, no serological response was detected (unpublished data).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.

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.

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REFERENCES

1. Barbour, E. K., Hamadeh, S. K. Eid, A., 2000. Infection and immunity in broiler chicken breeders vaccinated with a temperature-sensitive mutant of *Mycoplasma gallisepticum* and impact on performance of offspring. Poultry Science 79:1730-1735.
2. Kleven, S. H., 2000. Mycoplasma update. The Poultry Informed Professional 42:1-4.
3. Kleven, S. H., Garcia, M. L., Tongrui, Beiting, V., Hall B., 2000. Centralized laboratory effort dedicated to the archiving and characterization (fingerprinting) of pathogenic species of avian Mycoplasma. Final Report to U.S. Poultry and Egg Association.
4. Lauerman L. H., Hoer F. J., Sharpton A. R., Shan S. M., Saten V. L., 1993. Development and application of a polymerase chain reaction assay for *Mycoplasma synoviae*. *Avian Diseases* 37:829-834.
5. Paganelli F., Fiorentini L., Leonelli R., 2003. Utilizzo della random amplified polymorphic DNA (RAPD) per differenziare ceppi di *mycoplasma gallisepticum* vaccinali da ceppi di campo. *Large Animals Review*, anno 9, numero 6, bimestrale 2003.
6. Paganelli F., Massi P., Tosi G., 2002. Applicazione del metodo polymerase chain reaction alla diagnosi di *Mycoplasma gallisepticum* e di *Mycoplasma synoviae*. *Large Animals Review*, anno 8, numero 6, bimestrale 2002.
7. Whithear, K. G., Soeripto, Harrigan, K. E., Ghiocas E., 1990 .Immunogenicity of a temperature-sensitive mutant Mycoplasma gallisepticum vaccine. Aust. Vet. J., 67:168-174.

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7) EFFICACY IN THE FIELD OF TWO ANTICOCCIDIAL VACCINES FOR BROILERS

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Summary We compared two attenuated anticoccidial vaccines, administered to broilers by spray into the incubator (88,000 males and 210,100 females). Vaccine A container five species of *Eimeria* and vaccine B three. Zootechnical performance was similar in the two groups, with mean lesion scores no higher than 1; vaccine A caused only duodenal lesions. while vaccine B also caused typhlitis. Maximum oocyst count was 23,000/g feces at age 28 days with vaccine A and 38,000 at 21 days with B. Broilers vaccinated with B had more frequent enteric symptoms, and *C. perfringens.*

Key words: broilers, coccidiosis, vaccine.

Riassunto Sono stati confrontati due vaccini anticoccidici attenuati in broilers (88.000 maschi e 101.000 femmine) somministrati per spray in incubatoio. Il vaccino A conteneva 5 specie di Eimeria e il B 3. Le performance zootecniche sono state simili nei due gruppi, lo score delle lesioni mediamente non superava il valore 1, anche se A dava lesioni solo duodenali, mentre con B era presente anche tiflite. L'emissione massima di oocisti era di 23.000 per grammo di feci (OPG) a 28 gg di vita in A e 38.000 OPG a 21 gg per B. Nei broilers vaccinati con B erano più frequenti sintomi enterici con isolamento di C. perfringens, che ha richiesto terapia specifica per arginare la mortalità.

Parole chiave: pollo da carne, coccidiosi, vaccinazione.

Introduction

Attenuated anticoccidial vaccines containing various species of *Eimeria* pathogenic for poultry are the only alternative to anticoccidial drugs (Chapman *et al*., 2002). Live attenuated vaccines have been under investigation for several years now (Shirley, 1989). Most of these consist of a stabilized suspension of sporulated oocysts of *Eimeria* species from chickens, selected from precocious lines (shorter cycle, reduced reproductive potential, maintenance of the immunogenic capacity). Another method of attenuation is to produce lines of coccidia, especially *Eimeria tenella*, by passages in embryonated hen's eggs (Shirley and Bedrník, 1997). The *Eimeria* lines in commercial vaccines, even though attenuated, can cause some lesions to the intestinal mucosa (Williams and Andrews, 2001) and, in less than optimal breeding conditions, these can interact with other intestinal pathogens, such as *Clostridium perfringens*, facilitating the onset of pathologies such as necrotic enteritis (NE) (Waldenstedt *et al*., 1999).
This study compared the efficacy of two anticoccidial vaccines in eight commercial broiler farms.

Materials and Methods

Vaccines: We used two commercial vaccines, indicated here as A and B. Vaccine A contained five species of a precocious line of *Eimeria* (*E. acervulina*, *E. maxima* (two lines), *E. mitis* and *E. tenella*). Vaccine B container two precocious lines, *E. acervulina* and *E. maxima,* and one egg-adapted line, *E. tenella*. Both were administered using a spray machine (Breuil model), following the manufacturer's instructions, directly into the incubator where all the chicks for controlled breeding were hatched.
Breeding establishments: Eight commercial chicken farms (numbered 1-8) were checked; four only bred males, and four only females, for a total of 298,100 broilers (hybrid ROSS 508). Vaccine A was given to the chicks in farms nos.1 (26,000 males), 2 (24,000 males), 3 (75,600 females), and 4 (46,000 females). Vaccine B was given to the chicks in farms nos. 5 (9,000 males), 6 (29,000 males), 7 (37,500 females) and 8 (51,000 females). Chicks were housed between 31/05/04 and 10/06/04; the breeding density (male:10; female: 16 chicks/sq.m) and feed were comparable in all eight farms.
Laboratory tests: From each shed we took 50 individual samples of feces, and counted the oocysts according to McMaster's method on days 7, 14, 21, 28 and 35 for males and females, and on days 42 and 49 as well for males. At the ages of 21, 28, 35 days for females and 21, 28, 35, 42, 49 days for males, we sampled ten chicks per farm to check for coccidial lesions, using the method of Johnson and Reid (1970). From six chicks for each age group we also removed samples of the intestine (duodenum, jejunum/ileus and cecum), which were fixed in 10% iso-osmotic formalin and examined histologically, according to routine methods. Semiquantitative bacteriological counts were also done on the same animals, as recommended by Elanco Animal Health (2002).

Results and Discussion

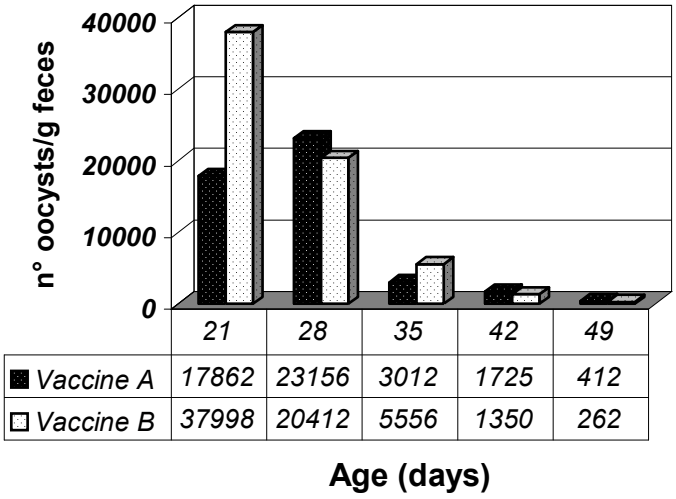
In the farms where chicks had been given vaccine A we found no clinical symptoms of intestinal pathology, and bedding remained in good condition. Table 1 shows the scores for lesions. Chicks vaccinated with A had few lesions due to *E. acervulina* and *E. maxima* and only very few to *E. tenella,* in accordance with the report by Williams and Andrews (2001). Semiquantitative examination of the intestinal flora showed initial mild abnormality with a prevalence of Gram-negative microorganisms up to day 28; from then onwards until slaughter there was an increase in *C. perfringens*, but no gross or microscopic lesions due NE.
There are, however, reports of interactions between field coccidia or attenuated anticoccidial vaccines and NE (Williams et al., 2003). Output of oocysts was near-nil in the first two weeks but peaked at 28 days, as already reported by Williams and Gobbi (2002), declining steeply thereafter (Figure 1).
In three of the breeding farms (nos. 6, 7 and 8) the broilers vaccinated with B had intestinal symptoms (catarrhal enteritis), with a rise in mortality, between the third and fifth weeks. The symptoms stopped only after repeated antibiotic treatments. Bacteriological tests in these B-vaccinated chicks showed a rise in *C. perfringens* already from the third week, when oocyst output was maximum – much higher than in the groups given vaccine A (Figure 1). The lesion score for vaccine B was not very different from A, except for the constant finding of lesions due to *E. tenella,* unlike in the report by Rois *et al*. (2002) in similar settings.

The productive performances of the two groups were in line with the specific literature for this cross. Male and female broilers given vaccine A were slaughtered respectively at 56.7 and 40.85 days, at average weights of 3.719 kg and 1.676 kg, with feed conversion ratio (FCR) 1.87 and 1.74, and mortality 4.05% and 2.46%. Male and female broilers given vaccine B were slaughtered respectively at 52 and 40.3 days, when average weights were 3.244 and 1.676 kg, FCR 2.00 and 1.81, and mortality 6.25 % and 1.78%.

Table 1: Mean lesion scores

	E. acervulina		E. maxima		E. tenella	
Age (days)	**Vacc. A**	**Vacc. B**	**Vacc. A**	**Vacc. B**	**Vacc. A**	**Vacc. B**
21	0.725	0.65	0.35	0.15	0.1	0.125
28	0.75	0.25	0.55	0.45	0.1	0.575
35	0.15	0	0.45	0	0.2	0.05
42	0	0	0	0	0.02	0.05
49	0	0.15	0	0	0	0

Figure 1: Mean oocyst output in eight broiler breeding farms



Conclusions

Attenuated anticoccidial vaccines are the only alternatives to the drug prophylaxis that has for decades permitted the expansion of poultry breeding. These vaccines have been used increasingly in Italy in recent years. Commercial products are safe but must obviously be coupled with good breeding practice for maximum efficiency. The abolition of growth promoter additives, which controlled the anaerobic intestinal flora, particularly *C. perfringens* (Waldenstedt *et al*., 1999), calls for care in selecting the right vaccine.

In this comparison both vaccines effectively controlled coccidiosis in a field setting. Vaccine A, given the same breeding conditions and feed, seemed to interact less on the chicken's intestinal microbial flora, without inducing NE, meaning there was less need for specific antibiotics.

References

1. Anonymous. 2002 "Determinazione di un metodo per la diagnosi in campo della Disbatteriosi intestinale avicola- Eli Lilly Italia, via Gramsci, 733 50019- Sesto Fiorentino, Italia
2. Chapman, H.D., Cherry, T.E., Danforth, H.D., Richards, G., Shirley, M.W., Williams, R.B., 2002. Sustainable coccidiosis control in poultry production: the role of live vaccines. Int. J. Parasitol. 32 :617-629.
3. Rois, O., Rataj, Z., Krapez, A. V., Dovc, U., Cajavec, S. 2002. Evaluation of safety of the anticoccidial vaccine Livacox® T in broiler chickens in experimental conditions. Praxis Veterinaria. 50. 3:231-238.
4. Shirley, M.W. 1989. Development of a live attenuated vaccine against coccidiosis of poultry. Parasite Immunol. 11:117-124.
5. Shirley, M.W., Bedrník, P. 1997. Live attenuated vaccines against avian coccidiosis: success with precocious and egg-adapted lines of Eimeria. Parasitol. Today 13:481-484.

6. Waldenstedt, L., Lunden, A., Elwinger K., Thebo, P., Uggla, A. 1999. Comparison between a live, attenuated anticoccidial vaccine and an anticoccidial ionophore, on performance of broilers raised with or without a growth promoter, in an initially *Eimeria*-free environment. Acta Vet. Scand. 40:11-21.
7. Williams, R.B., Andrews, S. J. 2001. The origins and biological significance of the coccidial lesions that occur in chickens vaccinated with a live attenuated anticoccidial vaccine. Avian Path. 30:215-220.
8. Williams, R.B., Gobbi, L. 2002. Comparison of an attenuated anticoccidial vaccine and an anticoccidial drug programme in commercial broiler chickens in Italy. Avian Path. 31:253-265.
9. Williams, R.B., Marshall, R.N., La Ragione, R.M:. Catchpole, J. 2003. A new method for the experimental production of necrotic enteritis and its use for studies on the relationships between necrotic enteritis. coccidiosis and anticoccidial vaccination of chickens. Parasitol. Res. 90:19-26

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8) ISOLATION OF SALMONELLA IN ANIMAL FOODSTUFF FOR POULTRY

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Abstract Here we have reported data referring to the isolation of *Salmonella spp.* in foodstuffs and non-medicated composite feed destined for poultry. We found that the foodstuffs were frequently contaminated compared to the composite non-medicated feed. The most common species found was S. seftemberg that belongsto E group that was isolated from Soya protein flour and wheat germ. (We are presently putting a monitoring plan into force that deals with the qualitative study of salmonella in animal feed. It should be noted however that quicker and quantitative techniques should also be activated in the future.

Key words: *Salmonella spp.*, raw materials, non-medicated composite feed

ISOLAMENTO DI SALMONELLA SPP. NEGLI ALIMENTI ZOOTECNICI

Riassunto
Questa comunicazione riporta alcuni dati relativi agli isolamenti di *Salmonella spp*. da materie prime e mangimi composti non medicati destinati al settore avicolo. Le materie prime sono risultate maggiormente contaminate dei mangimi composti non medicati con maggior prevalenza della specie *seftenberg* appartenente al gruppo E, isolata da farina di soja proteica e da crusca. Attualmente viene attuato un piano monitoraggio che prevede la ricerca qualitativa della Salmonella negli alimenti destinati all'alimentazione zootecnica, anche se è auspicabile che in futuro saranno previste tecniche di tipo quantitativo e più tempestive.

Introduction
There is presently a monitoring programme in force throughout the European Union that has been designed to evaluate the contamination levels of *Salmonella spp.* in raw materials used to produce vegetarian animal feed used either as they are or in composite food stuffs for poultry, pigs, cattle, birds, goats, fish and rabbits. It is clear that even vegetable based feed can contain pathogenic agents for animals and man that therefore create food and public health problems. At the moment there are no Community laws referring to zoonotic agents in animal feed specifically from vegetarian based origins. It is for this reason that this monitoring programme could be useful to integrate into the hygiene guidelines and quality control programs of production, transport, storing and handling of zoo-technical food products, that will enter into force on 1st of January 2006. Therefore, the microbiological controls have recently been intensified in order to measure the frequency and level of Salmonella *spp* contamination in vegetarian based animal feed. From 1st January 2003 to 10th July 2004, the IZSLER microbiology laboratories at Forlì analysed 304 samples; 125 of which were foodstuffs and 179 complete non-medicated animal feed all of which were destined for poultry. These were all samples supplied directly from the producers and by the breeders according to strict internal quality control.

Materials and Methods.
The feed samples were analysed according to a five consecutive step microbiological programme: