Field evaluation of a Live Mycoplasma synoviae vaccine in turkey breeders, by MS Specific PCR and Serology

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Abstract

Mycoplasma synoviae (Ms) is a major poultry pathogen. In turkeys, Ms can cause significant mortality associated with airsacculitis, infra-orbital sinusitis, arthritis and/or synovitis. The Ms infection is one of the main causes of early slaughter of turkey breeder flocks in France. Thus, it was decided to evaluate an attenuated live vaccine, authorised in chickens, in turkey breeders from a hatchery historically confronted with episodes of Ms infections. Between June 2017 and August 2021, 90 flocks free of Ms were vaccinated at 7 weeks of age with MS-H Vaccine by eye drop (+ booster at 65 weeks in case of extended laying periods). Monitoring was organised to assess vaccine uptake and identify field strain Ms challenge: PCR was performed from tracheal swabs every 4 weeks on all flocks and on 6 flocks of broiler turkeys at 1 and 21 days of age from vaccinated parents. The DNA obtained from these PCRs was then tested with a DIVA PCR. Ms ELISAs were performed from 20 flocks of turkey breeders and from 6 flocks of broiler turkeys sampled at 1 and 21 days of age from vaccinated parents. The first observation was that there was no clinical reaction after administration of the vaccine. The vaccine strain was not detected in progeny by PCR. MS-H PCR positivity rate is variable but on average very good 9 weeks after vaccination (96%). This positivity rate decreases with increasing age and increases again after the booster vaccination carried out in flocks with extended laying periods. The Ms ELISA titres were very low and progressively increased with age. A positive titre was detected in one single day-old turkey from parents at 64 weeks of age. These maternally derived antibodies disappeared after 21 days of age. Since 2017, no contamination with field strains of Ms was identified in the company. In conclusion, PCR monitoring is an appropriate method to assess the vaccination uptake of turkeys vaccinated with MS-H Vaccine.

Introduction

Mycoplasma synoviae (Ms) is a major and widely distributed pathogen in poultry. In broiler turkeys, Ms can cause significant mortality following vertical or horizontal contamination. Clinical signs associated with infection with this bacterium can be airsacculitis (increased condemnations at the slaughterhouse), infraorbital sinusitis, arthritis or even synovitis, requiring costly antibiotic treatments (Van Meirhaeghe *et al.* 2015).

The infectious pressure of Ms in France is high, as highlighted by previous prevalence studies: in particular, 60% seroprevalence in laying hens (Bouchardon *et al.* 2012) and 9% (Dheilly, 2003) to 10% (Kermogant, 1998) in broiler turkeys. Although the prevalence varies from one year to the next, the infectious pressure on breeding turkey farms remains high because the number of birds potentially Ms positive and living outdoors is increasing.

In turkey breeders, the prevalence is very low. Infected animals are often older than broiler turkey when infected by Ms and show no symptoms. However, Ms infections are one of the main causes of early slaughter of turkey breeders in France because the risk of vertical contamination has a significant impact for broiler turkey farms.

In France and other countries around the world, vaccines are used to protect poultry against the clinical and economic consequences of Ms infections.

MS-H vaccine (MS-H) is indicated for use in future laying hens and broiler/layer breeders. This vaccine has been authorised in the European Union since 2011 with an indication to reduce air sac lesions and the number of eggs with eggshell apex abnormalities due to Ms.

In 2007, this vaccine was tested in broiler turkeys to assess its effectiveness against the clinical consequences of Ms infections (Noormohammadi *et al.* 2007). According to this trial, MS-H Vaccine by eye drop or spray colonised the upper respiratory tract and induced a serological response, without causing damage to the air sacs, joints, or tracheas. Histopathological examination of turkeys vaccinated after exposure to a virulent Ms strain revealed that administration of MS-H by eye drop or spray, at the recommended dose for chickens, protected turkeys from macroscopic/microscopic lesions and colonisation of the trachea by field Ms strains. Spray administration of MS-H to broiler turkeys placed in an isolator had provided better results than the eye-drop application. However, it is likely that in the field this would have been different because the spray administration technique in commercial livestock farming results in a significant loss of vaccine dose (de Wit *et al.,* 2013).

Following this finding, it was decided to evaluate the safety, efficacy and vaccine response to MS-H in turkey breeders from a hatchery historically confronted with episodes of Ms infection.

1. Materials and Methods

1.1. Choice of farms

A French hatchery for turkey breeders was chosen because this company has historically been confronted with episodes of Ms on its farms, resulting in the slaughter of flocks (5 contaminated by Ms between 2014 and 2017). Among these cases, a farm had been contaminated twice in a row without the farmer being at fault. For the company, it is very complicated to no longer work with certain farms even if there are recurrences. The farms are all located in the North-West of France, an area with a very large density of poultry and therefore high pressure of Ms.

The biosecurity of farms is therefore important (showering, change of clothing, etc.) but not infallible in the face of a pathogen that can spread by air up to 8 km on dust or dander particles (Hy-Line International 2020).

In addition to the economic impact of early slaughter, there is a significant human impact on the company's staff, due to the significant disruptions to the production schedule, and for farmers who lose animals early without apparent symptoms.

1.2. Animals

The genetics (male and female) of the turkey breeders were *Premium* (Aviagen Turkeys) and *Grade Maker* (Hybrid). Between June 2017 and August 2021, 430,000 animals (90 flocks of males and females in production) were followed after their vaccination with MS-H. Their feed was classic. The animals were transferred to the production site at circa 29 weeks of age and the start of production was at circa 32 weeks of age. The end of normal hatching egg production for these turkey breeders was circa 55-57 weeks of age. Some flocks were in lay for an additional egg-laying period of 67-70 to 87-90 weeks of age.

1.3. Choice of vaccine

MS-H is a commercial vaccine against Ms authorised in chickens and consists of a live attenuated thermosensitive vaccine strain called MS-H. Each 30µL dose of vaccine contains a minimum of $10^{5.7}$ colour changing units. The name of this vaccine is MS-H Vaccine eye drops suspension (Pharmsure Veterinary Products Europe Ltd).

All animals were vaccinated at 7 weeks of age by eye-drop (one dose per animal as in chickens and as had been successful in the trial of Noormohammadi *et al.* 2007) and for some flocks that were expected to do an additional egg-laying period, a second eye-drop application was made at around 65 weeks of age.

A PCR Ms analysis was performed 2-3 days before vaccination to ensure that all animals to be vaccinated were Ms negative.

1.4. Choice of PCR kits

The PCR kit chosen to detect field or vaccine Ms strains was a classic real-time Mg/Ms PCR kit targeting 16s RNA: *Adiavet® Myco Av Fast Time* (Adiagene). The DNA obtained from this PCR was then tested with a DIVA

(Differentiating Infected from Vaccinated Animals) real-time PCR: *Adiavet™ MS-H DIVA Fast Time* (Adiagene). This kit specifically detects Ms strains (100% in the validation dossier, reference ADI561-100), while differentiating infections by field Ms strains from the MS-H vaccine strain.

1.5. Choice of ELISA kits

The Ms ELISA kit used in this study was from Biochek because it clearly specifies the possibility of antibody analysis from turkey serums. According to the supplier's specifications, a titre was considered positive from 594.

1.6. Monitoring of vaccinated flocks

The purpose of this monitoring was to assess vaccine take and to identify field strains of Ms.
For the PCR analysis, 40 tracheal swabs were taken in females and 20 in males (analysed in pools of 3) every 4 weeks for all flocks.

Concerning the broiler turkeys from vaccinated parents, 10 tracheal swabs were taken on 6 flocks at the age of 1 and 21 days.

Blood samples for Ms ELISA serology were taken from 10 turkey breeders per flock (19 flocks of females and 1 flock of males) at 28, 32, 44 and 52 weeks of age.

For broiler turkeys from vaccinated parents, 20 blood samples per flock (6 flocks) were taken at 1 and 21 days of age.

2. Results and Discussion

MS-H PCR results in female turkey breeders (Figure 1) at 16 weeks of age were very positive (96% on average). Only 2 negative flocks at 24 weeks of age had to be revaccinated to detect the strain by PCR. The lack of detection of the vaccine strain by PCR in these two flocks could be due to an error in the use of the vaccine, or the levels present being below the level of detection of the PCR technique, which must be applied within 2 to 3 hours after thawing in water below 35°C to avoid a decrease in the viability of the vaccine strain.

The rate of positive MS-H PCR decreased with age (approximately 61% MS-H positive PCR 49 weeks after vaccination (= 56 weeks of age): the end of a normal production period) and increased again after a booster vaccination performed at 65 weeks in flocks with extended laying periods (Figure 2).

Broiler turkeys 1 or 21 days old from vaccinated parents were all PCR negative (data not shown).
The MS-H positive PCR rate in males (Figure 3) is higher than in females (p-value=8.33×10^{-21} Aspin-Welch).

Ms ELISA titres increased gradually with age (Figure 4) even though the correlation between the Ms ELISA positivity rate and the age of the animals was weak (23% of sera weakly positive 20 weeks after vaccination and 55% 45 weeks after vaccination). Seropositive females appeared to be able to transmit detectable antibodies to day-old turkeys (Figure 5), although this was rare and more likely at the end of egg-laying when parental titres were at their maximum. Maternally-derived antibodies were detected only in a 1-day-old turkey from 64-week-old parents; unless it was a possible non-specific reaction.

Maternally-derived antibodies were undetectable when the offspring were 21 days old (data not shown).

The first observation of this study was that MS-H Vaccine by eye-drop did not cause any adverse vaccine reaction in turkey breeders. The vaccine strain was also not detected in the offspring by PCR (no vertical transmission of the vaccine strain).

Secondly, regarding vaccine take, the percentage of positive MS-H swabs (PCR) was very high at the beginning of the production period and then decreased with age. In males, these results were somewhat higher than in females, perhaps

because these animals were vaccinated more slowly and are housed in smaller bird groups that females that facilitate easier handling of birds; unless there is a gender related effect.

The variability of the PCR results (R2 < 0.006) could be explained by the sampling technique (tracheal sampling potentially being more complicated/less sensitive than in the cleft palate).

For comparison, the percentage of positive MS-H PCR in turkey breeders was 97% 10 weeks post-vaccination and 61% 49 weeks post-vaccination. In the study by Moronato (2018), 100% of PCRs were positive 10 weeks after MS-H vaccination of broiler breeders and up to 49 weeks post-vaccination (vaccine done at 4 weeks of age).

Regarding the Ms ELISA titres of the turkey breeders in our study, they were low and increased slowly with age. Compared to results obtained in broiler breeders (Moronato *et al.,* 2018 and Todte, 2014), humoral antibody titres therefore appear to be lower in turkey breeders; an observation that should perhaps be put into perspective because the ELISA kit contains antibodies against chicken antibodies. In broiler breeders, 72% of ELISA titres were positive 5 weeks after MS-H vaccination (mean Biochek titres 1540 versus 400 in turkey breeders and 0% positive ELISA) and 100% positive at 37 weeks after vaccination (mean Biochek titres 2851 versus 670 in turkey breeders and 55% positive ELISA). Thus, the ELISA titres of the offspring appeared to be higher in chickens (37% positive ELISA for the offspring of broiler breeders vaccinated 37 weeks earlier and 100% positive ELISA for the offspring of broiler breeders vaccinated 47 weeks earlier; compared to 0% positive ELISA for 1-day-old broiler turkeys from turkey breeders less than 57 weeks of age). It should be noted that the field challenges of Ms may not be detected by PCR and could potentially influence Elisa titres.

At 57 weeks of age, the veterinarian working for the hatchery concerned decided to revaccinate flocks with extended egg-laying periods with MS-H by eye-drop. This increased PCR positivity rates and perhaps thanks to this, better protected the animals. As studied in chickens (Feberwee *et al.* 2017), we can hypothesize that the presence of the MS-H strain in the upper respiratory tract may be correlated with the reduced ability of field Ms strains to colonize the respiratory tract of birds and thus provide better protection against Ms.

Since 2017, no contamination with a field Ms strain has been identified in the company concerned, which seems to demonstrate the effectiveness of MS-H in protecting turkey breeders against infections by field Ms strains. The company's staff is therefore less stressed by possible contamination by a field Ms strain since the implementation of vaccination with MS-H.

Conclusion

PCR monitoring is an appropriate tool to assess the vaccination take of turkey breeders vaccinated with MS-H by eye-drop.

Despite the registered efficacy claim for MS-H in chickens, the vaccine strain appeared to colonize the upper respiratory tract of turkey breeders less intensely and durably than in broiler breeders, resulting in fewer humoral antibodies, unless the sampling technique or the nature of the ELISA kit were responsible for these differences.

References

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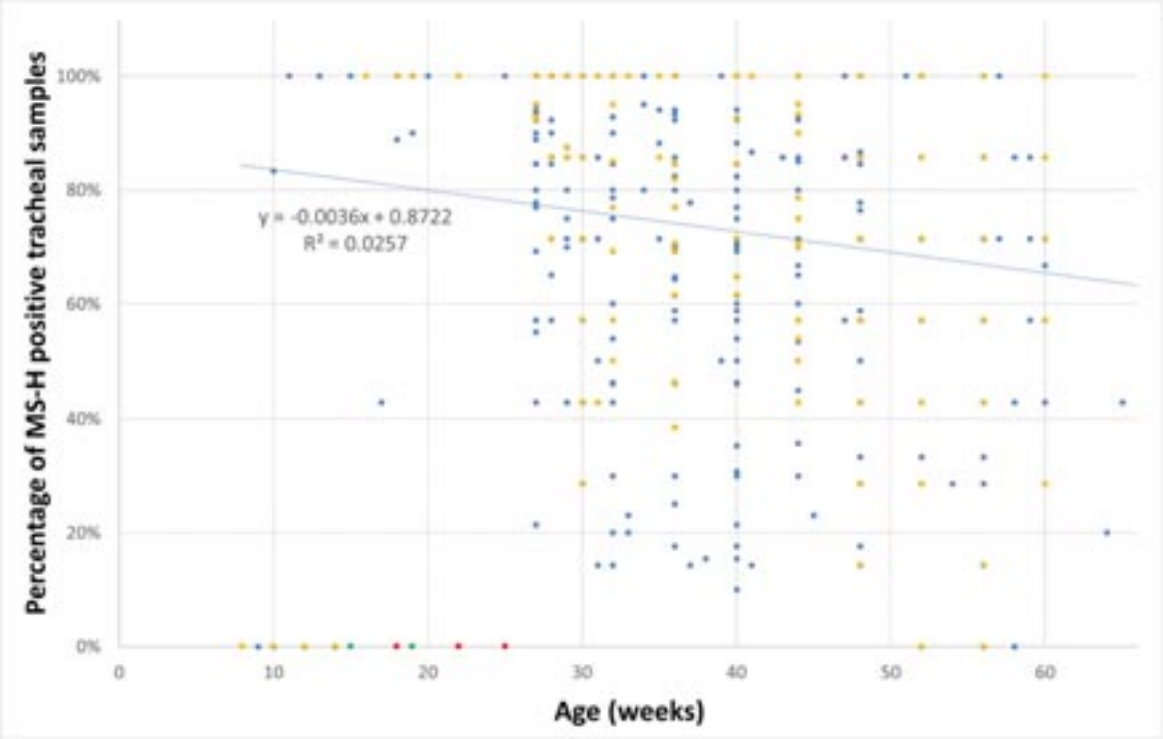
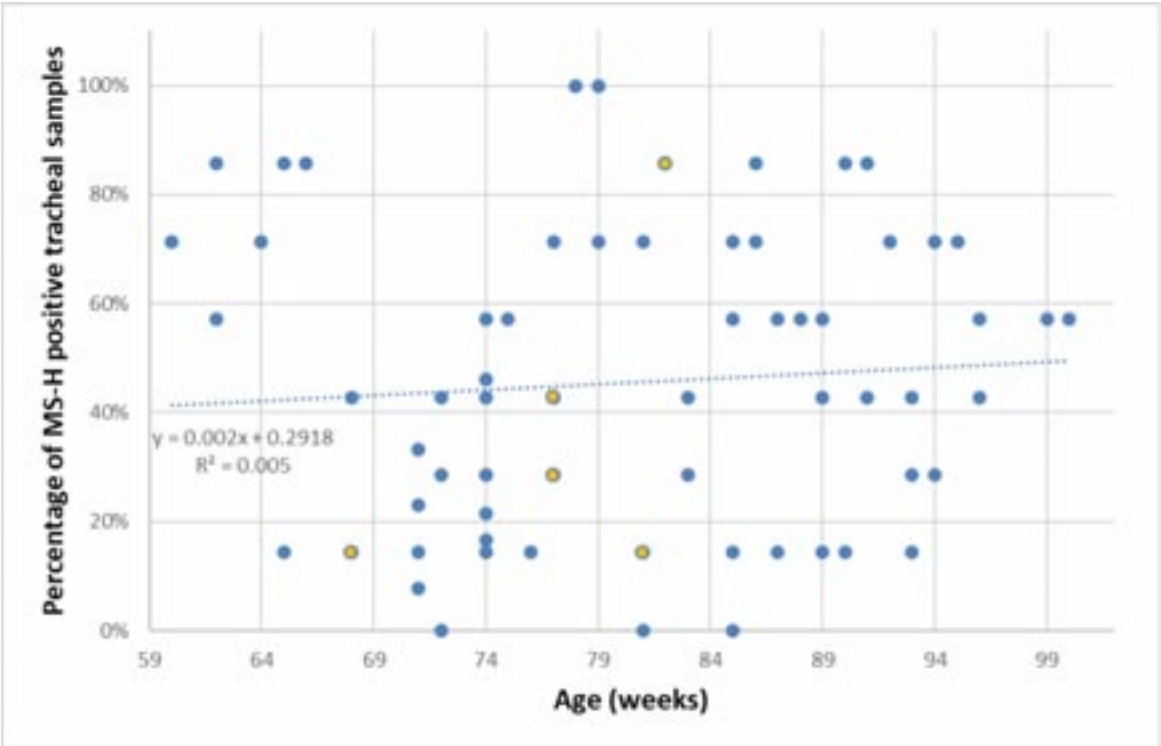
Figure 1. *Percentage of positive PCR MS-H tracheal swabs for females (normal production period)*



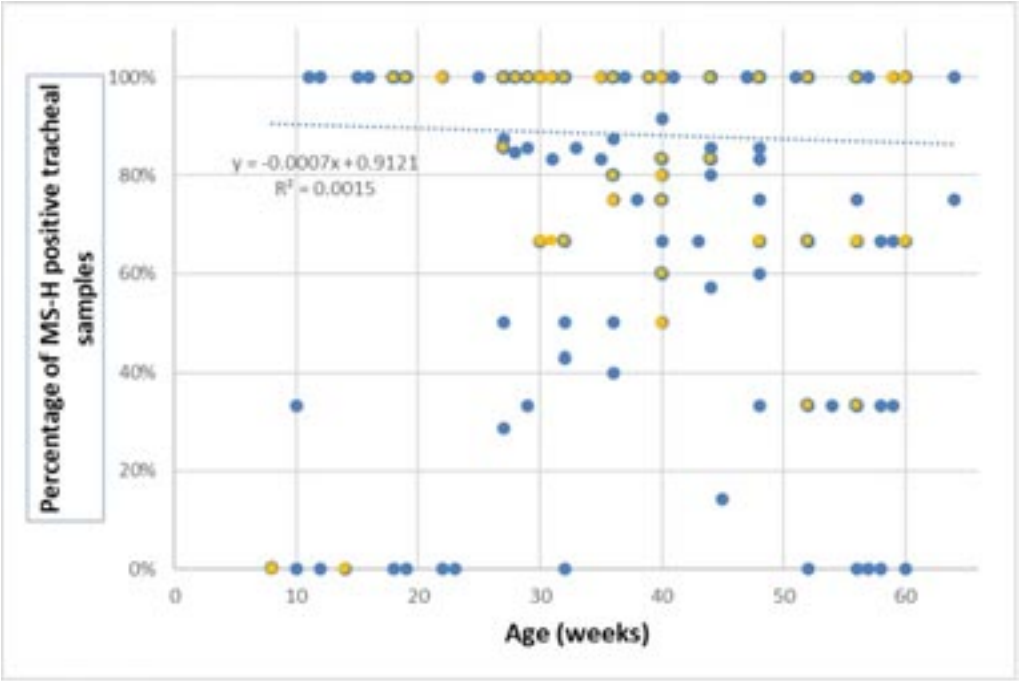
● First flock vaccinated twice ● Second flock vaccinated twice
● Means one flock ● Means several flocks with the same results

Figure 2. *Percentage of positive PCR MS-H tracheal swabs for females (2nd egg-laying period)*



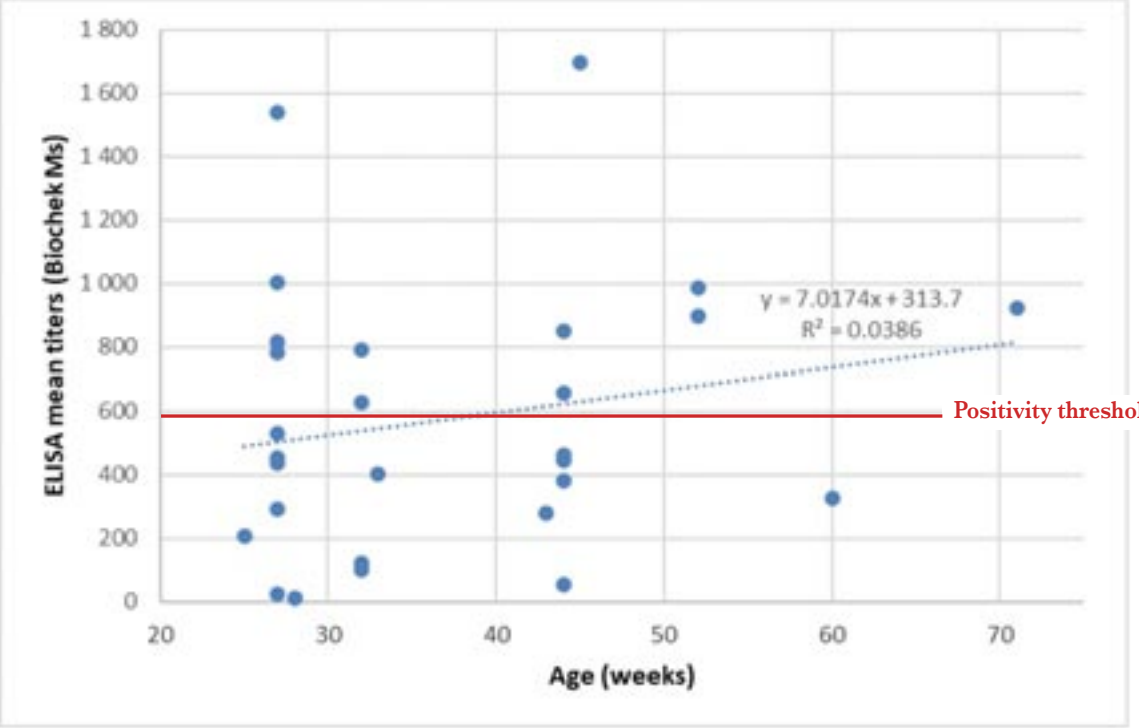
● Means one flock ● Means several flocks with the same results

Figure 3. *Percentage of positive PCR MS-H tracheal swabs for males*



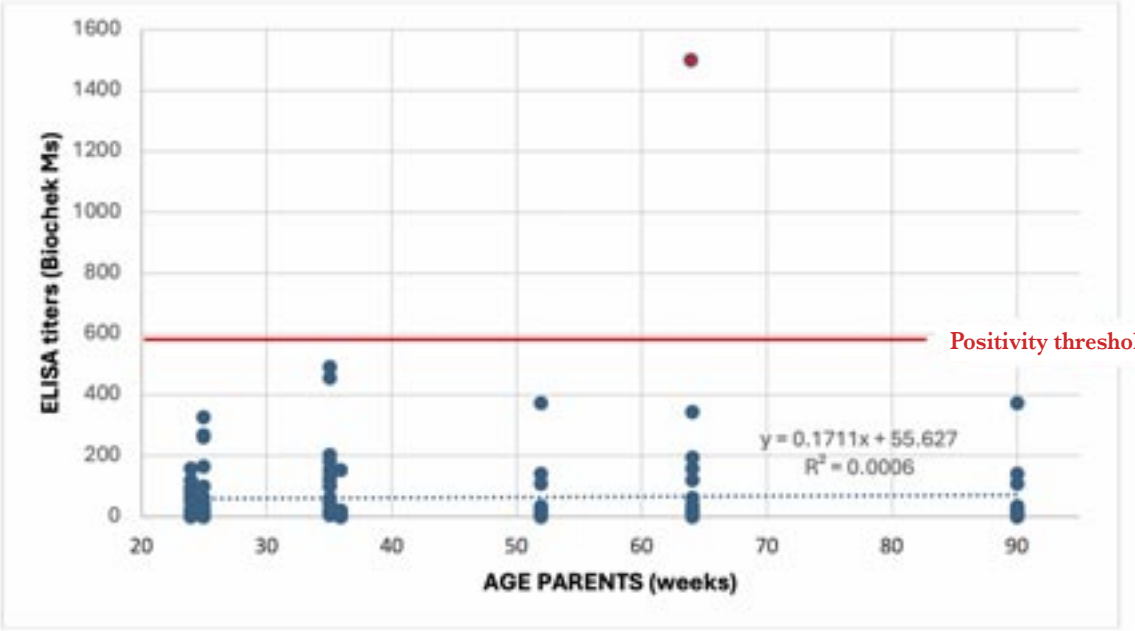
● Means one flock ● Means several flocks with the same results

Figure 4. *Mean ELISA Ms titres (normal egg-laying period, females and males)*



● One flock

Figure 5. *ELISA Ms titres on day-old turkeys (depending on the age of vaccinated parents)*



● Seropositive turkey poult ● Individual titre of turkey poults