

Application of MS DIVA-PCR in the Monitoring of *Mycoplasma Synoviae* Vaccination (Strain MS-H) in a Broiler Breeder Farm in Malaysia

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Incidence of *Mycoplasma synoviae* (MS) infection is often underestimated because of the widespread use of antibiotic treatments in breeder and layer flocks. The harsh reality remains that MS is an economically important disease of poultry industry, thus warranting effective preventative and control measures. In the Malaysian context, there has been an increasing awareness and subsequently a growing concern regarding MS particularly from the commercial sector as current available options and/or approaches to effective control and prevention are limited. Several published reports have shown that the application of a live MS vaccine (MS-H) has shown to be an effective tool in the control of MS challenge in the field.

In the present field study, the use of a live vaccine (MS-H) (Vaxsafe® MS) in a commercial broiler breeder farm in Malaysia was evaluated by means of a quantitative real-time PCR method to differentiate between field MS and the MS-H live vaccine strain (MS DIVA-PCR method). Under a special permit, the Department of Veterinary Services Malaysia has approved the use of the MS vaccine in this farm as a control measure due to the observable fact that using anti-mycoplasma drugs were deemed to be resistant and recurrence was regularly seen shortly after the treatment regime.

Two out of six vaccinated breeder flocks were randomly chosen for monitoring purposes. Prior to administering the MS-H vaccination, birds were monitored by real-time PCR to exclude the presence of MS infection. Twenty choanal swabs were randomly collected from day old chicks and one to two weeks prior to vaccination. At six weeks of age, birds were administered with the Vaxsafe MS vaccine following the manufacturer's recommendation. At around 6 weeks after the MS-H vaccination (twelve weeks of age), twenty choanal swabs were collected again to determine the colonization of the vaccine strains in the trachea. All MS positive samples were further tested with MS DIVA-PCR to confirm the presence of a vaccine/field strain or both. The MS DIVA-PCR test was developed in accordance to the method described by Dr. Chris Morrow in 69th Western Poultry Disease Conference (2020) — 'Diva Testing of Flocks Vaccinated with Live Mycoplasma Vaccines'. Apart from real-time PCR screening, flock health monitoring was conducted by the farm veterinarian on a weekly basis from day-old to fifty-two weeks of age. The presence of any suspected clinical symptoms or post-mortem lesions which resembled mycoplasma infections were recorded and samples such as organ(s) or serum were also collected for further diagnosis to rule in/out MS (serology, RT-PCR, etc.).

MS PCR and MS DIVA-PCR

The overall test results of MS PCR and MS DIVA-PCR coincided with our expectations. Field MS was not detected in the day-old chicks' choanal samples and therefore, the likelihood of vertically transmitted MS contamination was omitted. Similarly, choanal swabs collected prior to Vaxsafe MSH vaccination (four weeks of age) showed PCR-negative results. The absence of wild-type MS would provide a more favourable condition for the colonization of MS-H when it is administered. On the contrary, high percentages of MS positives were detected by means of RT-PCR screening in the samples collected around six weeks post-vaccination (80% to 100%). The subsequent MS DIVA-PCR tests showed all the positive samples were of vaccine origin (MS-H) while field MS was not detected in any of the samples. The results presented compelling evidence to indicate the successful vaccination whereby both flocks showed high vaccine takes. Throughout the weekly monitoring period from day old to fifty-two weeks of age, no clinical cases of MS was diagnosed in the assessed flocks. Upon further investigation, the farm veterinarian indicated that similar findings in other four vaccinated flocks.

The study findings clearly indicate that the use of MS-H vaccine is safe as the vaccinated flocks did not show any adverse post-vaccination reactions or pathological signs of MS infection throughout the field study. Furthermore, the MS-H vaccine showed a wide dissemination and a great persistence in the vaccinated flocks, evidenced by high recovery of vaccine origin MS. Consequently, as evidenced here, the use of the MS PCR and MS DIVA-PCR could represent an effective laboratory technique to improve monitoring vaccinated populations.

Keywords: *Mycoplasma synoviae* (MS), Vaxsafe® MS (strain MS-H), MS DIVA-PCR, broiler breeder.