BIOPROPERTIES
The Vaccine Innovators

TECH bulletin

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Egg Apical Abnormality

Egg apical abnormality (EAA) is a new syndrome in commercial layers that causes economic loss in affected flocks and has also been called Glass Top Egg syndrome and *Mycoplasma synoviae*-assoziierte Eischalenpoldefekte bei legehennen [*Mycoplasma synoviae*-associated egg-pole shell defects in laying hens]. It was first noticed in 2001 in the Netherlands and since that time has been seen in Belgium, France, Italy, Germany, UK and perhaps Denmark. It has also been seen in Japan and suspected in Indonesia, Mexico, Korea and Turkey.

Tray of reject eggs from affected flock (Japan).

Clinically there is a sudden appearance of glass top eggs. It may not be obvious at oviposition but over 24 hours the eggs become easier to identify. The top 2 cm of the egg (apex) becomes translucent and this area appears to be fragile. There is rarely any acute drop in production but the affected eggs may be up to 4 to 10% of production. The apex of affected eggs is more fragile and can be damaged after inspection during closing of egg carton lids and lead to a further loss of up to 10% of eggs. (In layers eggs are put in cartons with the air-cell down). In some of the latest colony cage facilities with dedicated packing facilities, the problem can lead to the entire production from houses being downgraded to seconds due to the impracticality of selecting out large numbers of affected eggs leading to significant financial losses.

Egg Apical Abnormality was first noticed in the Netherlands and Germany at the beginning of the new millenia

Note shell breaks in the translucent area.

There is usually no egg production drop and the birds often have no clinical signs but overall production may be decreased and FCR has been shown to be compromised. Flocks are affected after the eggs appear for the remaining life of the flock. Subsequent flocks on a site may or may

EAA is caused by *M. synoviae*

not be affected. Response to antimicrobial therapy is variable, shortlived and declines after repeated treatments.

The observation that this condition temporarily responded to anti-mycoplasmal antibiotics and the easy culturing of MS from the oviduct of birds producing affected eggs suggested that this condition was caused by MS. Some other

Translucent apex in affected egg.

conditions can mimic EAA including rough shelled eggs that have their equators smoothed by long egg conveyors. These can be identified as the shell changes are on both ends of the egg. When not specifically candling eggs up to 80% of EAA eggs can be missed.

Researchers at Deventer were able to reproduce the disease with an MS strain that had been isolated from the oviduct of a hen producing glass top eggs. They were able to induce affected eggs in SPF layers at the rate of 40% when the challenge included EAA MS and intratracheal and intramuscular Infectious bronchitis virus (IBV). In a later experiment in SPF broiler breeders using the same challenge they could only achieve a maximum incidence of 2%. There have been field reports of EAA in broiler breeder eggs (in Belgium) but currently the geographic spread of such outbreaks is unclear.

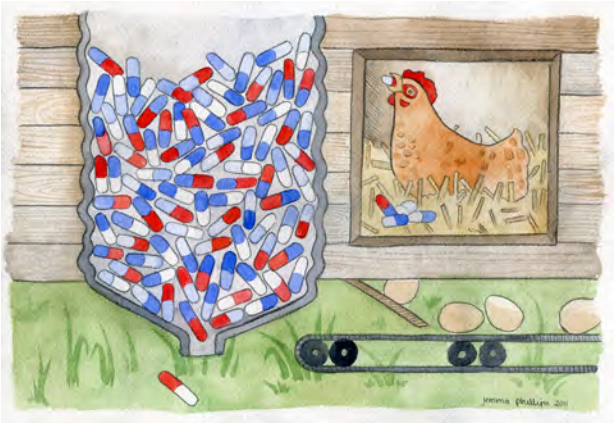
There are a couple of studies that have shown that infections with MS generally increases second quality eggs in flocks without even the appearance of EAA eggs and some authors have estimated that MS infection in layers costs between 5 to 10 eggs per hen housed (MG is estimated to be twice as costly). These estimates are in hens with no clinical signs of infection. These losses are often ameliorated by the routine administration of antibiotics to hens during lay.

Country	Economic Importance	Comments
The Netherlands	2.5 €cents less per EAA egg produced. 2% EAA average on affected (identified) farms.	Layer sector: On affected farms: 330.1€/1000 hens placed (incl. downgrades, lower production and labour). In packing stations receiving eggs from EAA positive flocks: 150€/1000 birds (excl labour). Total 480.1€/1000 birds. National control plan including monitoring. As antibiotic reduction plan is in place, and stamping out is impossible economically due to high prevalence, vaccination of birds considered as best practice.
Italy	Extra labour associated with inspection and removal of affected eggs.	Less than 2% of eggs affected but their identification and antibiotic costs massively decreased profitability.
Belgium	Average 1/3 of second grade eggs are EAA (0.5% of production), and 2% of first grade eggs EAA, total over 2.5% EAA, most of them unidentified at farm level.	Complaints from packing stations and retailers, with difficulties on contract renewals for eggs from affected farms. Layer breeders, commercial layers vaccinated. Antibiotic reduction plan in place.
Japan	Massive economic loss and lost production.	The consumer is often the first person to notice the problem. No antibiotics.
Korea	Cost of antibiotic.	Korean government is trying to decrease the use of antibiotics in food production.

Table showing variable importance of EAA in countries where the condition has been seen.

EAA appears to be associated with one strain of MS

The effect of EAA on the economic egg characteristics of an affected flock, has not been reported. The effect on specific gravity of eggs for example is currently unknown. From experimental studies it was demonstrated the overall strength of apparently normal eggs from affected flocks was reduced by 15%.



Antibiotics are not a long term solution for MS or EAA control.

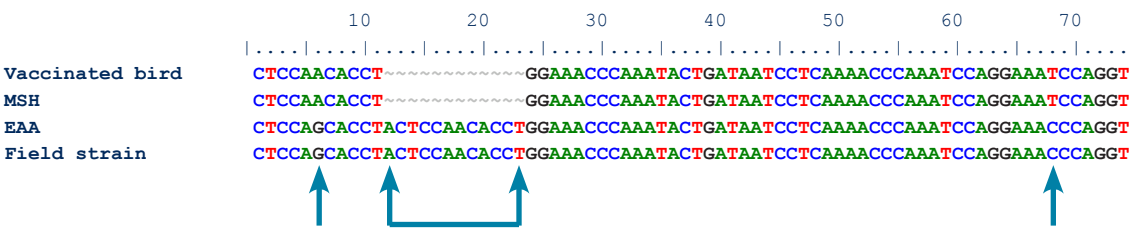
Electron microscope studies have shown a specific shell pathology with the absence of the layer of mammiliform bodies in the shell. Whether this is just in the translucent area is not clear. There are changes in the cilia in the oviduct that have been observed.

Epidemiology

EAA appears to be associated with a single strain of MS that has a tropism for the oviduct. This has been confirmed by field studies characterising the *vlh*A gene of isolates made from the oviduct. Presumably this tropism for the oviduct makes vertical transmission very efficient (approx 5%).

It is not known whether early infection with field strain MS before lay is protective as a natural vaccination but on some farms it seems that only one flock will be affected. On other farms where replacement pullets are delivered free of MS infection may have ongoing problems in each flock. Some farms have no problems and perhaps existing MS strains without the potential to cause EAA are also acting like a natural vaccination. Infected multiage laying sites may explain the observed link between MS infection and *E. coli* peritonitis at the beginning of lay. Again if the hens are reared MS negative then the timing of the initial infection after delivery to the multiage farm is while the reproductive tract is rapidly changing a low grade peritonitis from MS may be set up which predisposes to secondary *E. coli* infection.

This strain has been found in turkeys and broiler breeders but does not seem to cause EAA in these species or is missing some other triggering factor (IBV?). Confirming the involvement of MS in the case of EAA can be difficult. Demonstration of MS in the oviduct is the gold standard.



Part of a vlh*A sequence alignment comparing strains of MS showing that the sample from the vaccinated bird is derived from MSH and clearly different from the field strain from a hen affected with EAA.*

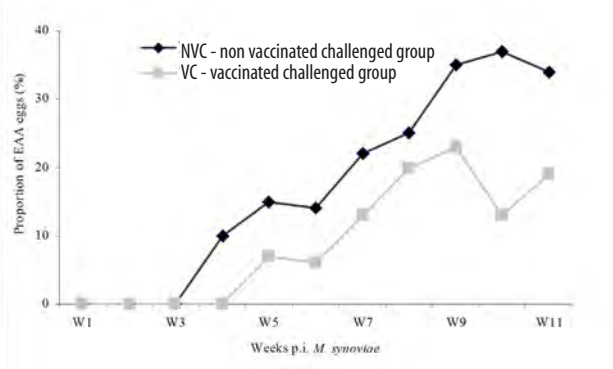
In laboratory trials in Deventer the protective effect of MSH vaccination was demonstrated. Under high level challenge vaccination was able to decrease the incidence of EAA eggs by 50%. Note that the unvaccinated control birds had an incidence of nearly 40%.

EAA has successfully been prevented by vaccination of replacement pullets in Japan. During trials where MSH and ts-11 was being compared to ts-11 only glass top eggs which had been a feature of previous flocks (between 4 to 10% of eggs) completely disappeared in vaccinated flocks.

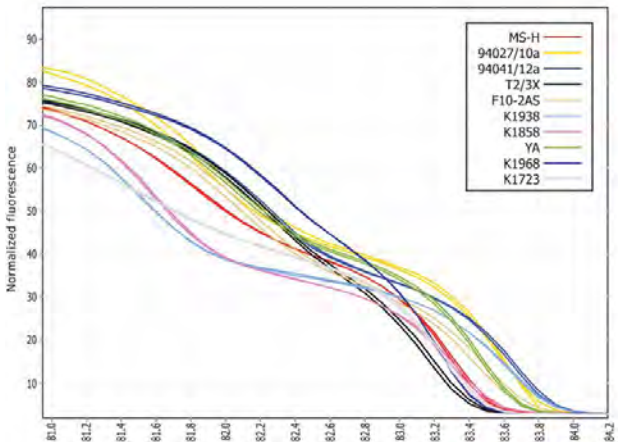
Parameter to 57 weeks	Trial 1 82K sonia grey	Trial 2 Lohmann
Total eggs	+11.4 eggs/HD	+13.4 eggs/HD
Normal eggs	+1.4%	+2.9%
Egg mass	+795g	+787g
FCR	-0.12	-0.07
	More eggs earlier	More eggs earlier

Summary of two MSH trials in Japan. Note EAA eggs had been a feature of previous flocks (controls) and disappeared entirely from vaccinated flocks.

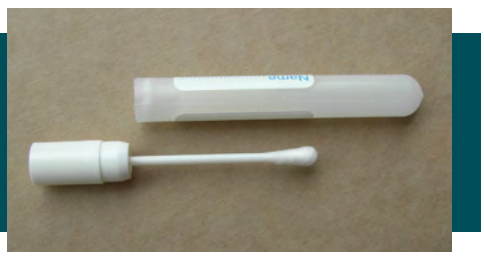
Inactivated vaccines have not been shown to protect against EAA. It is not expected that they would as they do not prevent infection with mycoplasmas.

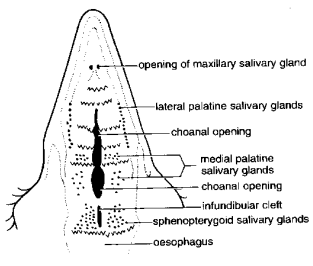


Deventer experiment showing protective effect of MSH vaccination against EAA challenge.



Rapid (1 hour) strain identification by HRM analysis of MS PCR products direct from cloanal swabs showing clear identification of the vaccine strain.



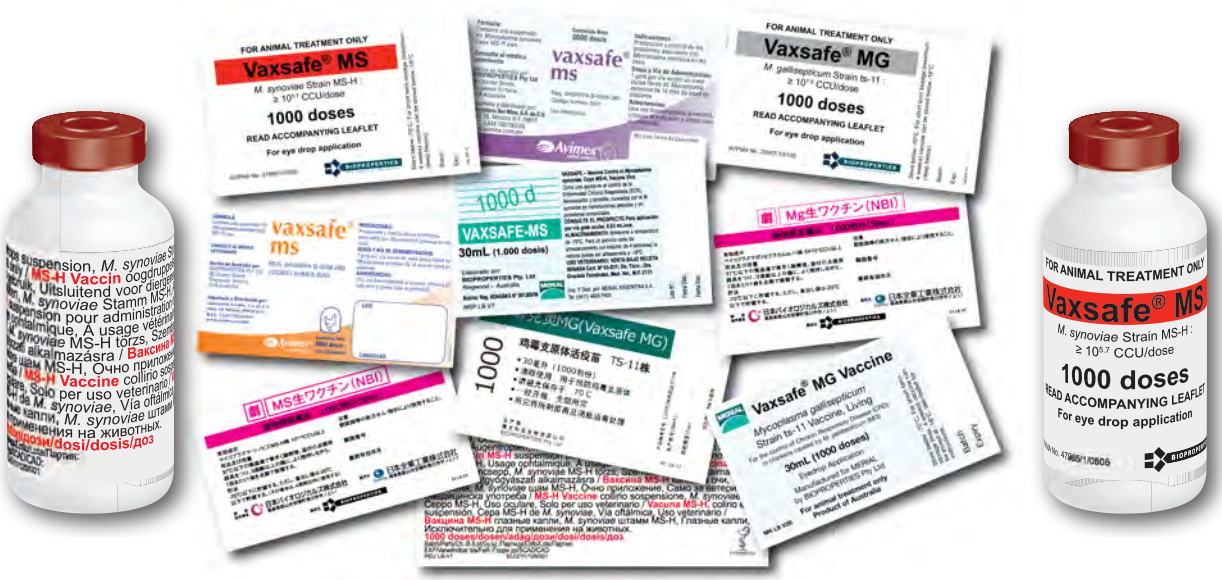


Cloanal swabbing – ordinary swabs can collect samples suitable for PCR-based tests. Avoid contamination with ingesta or blood.

Vaccination to Prevent EAA

MSH is a live mycoplasma vaccine supplied as a frozen culture. It is a sister product of MG ts-11 and has a similar presentation and characteristics. One drop is placed in the eye of the bird after 5 weeks of age and this infects and protects the bird for life. This lifelong infection stimulates a local mucosal immunity in the respiratory tract that is presumed to increase the resistance of the bird to becoming infected with wild strains of MS. It is important that at the vaccine is applied and has time to stimulate immunity (3 weeks) before the bird is challenged with a wild strain. Vaccination of birds already infected will have no deleterious effects but no benefits are expected. After vaccination anti-mycoplasmal antibiotics should be avoided. Chronic administration of these may decrease the mucosal immunity. The vaccine only stimulates moderate amounts of MS humoral antibody but this is not a measure of efficacy. Flock monitoring is by demonstration of the vaccine strain in the upper respiratory system by PCR. This same PCR will detect if field strains are present.

MSH vaccination is one drop of vaccine in the eye after 3 weeks of age (but at least 3 weeks before challenge).



MSH live vaccine is registered in Europe for protecting birds against the development of EAA, making production without antibiotics possible.

Dr Chris Morrow – Global Technical Manager, Bioproperties – inspecting EAA eggs in Japan.

BIOPROPERTIES
The Vaccine Innovators

Bioproperties Pty Ltd
ABN 49 007 303 728

36 Charter Street
Ringwood Vic 3134
Australia

T: +61 (0)3 9876 0567
F: +61 (0)3 9876 0556
W: www.bioproperties.com.au

Europe
andrew.bufton@pharmsure.co.uk