OUTBREAK OF CLINICAL SALMONELLOSIS IN LAYING HENS

By Lis Olesen1, Gitte Sørensen2, Klara Tølbøll Lauritsen3, Jeppe Boel2, Elisabeth Holm3 and Karl Pedersen3

1LVK, Fynsvej 8, DK-9500 Hobro, 2National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg, 3National Veterinary Institute, Technical University of Denmark, Bulowsvej 27, DK-1870 Frederiksberg C.

Each year, layer flocks in Denmark are infected with Salmonella. These infections, which are caused by several different serological types, are usually detected by means of compulsory Salmonella monitoring samples, and usually do not cause any clinical symptoms in the hens. Eggs from such Salmonella-infected flocks constitute a well-known risk to humans who eat raw or insufficiently heat-treated eggs (EFSA/EDC 2013). In 2013, however, the infection-source report for Salmonella showed that very few (0.6–2.7%) of human Salmonella cases in Denmark were attributable to the consumption of Danish eggs (anonymous, 2014), which emphasises the great success of the Salmonella action plans. Clinical salmonellosis in poultry does occur, however, although quite rarely, and in Denmark it is only seen at multi-year intervals.

Thus, it is all the more remarkable that in the summer of 2014, a case of Salmonella was found in a layer flock, resulting in an outbreak of clinical salmonellosis.

Symptoms
The flock involved was large, comprising more than 60,000 hens in enriched cage systems. The hens were 36-weeks old in mid-August 2014, when the mortality rate started to increase to around 30 hens a day. Therefore, the flock manager summoned a veterinarian who performed a clinical diagnosis and autopsy of 10 dead hens on site. The veterinarian discovered widespread peritonitis with yellowish green pus and fibrin coatings on internal organs. The liver and spleen were swollen and enteritis was seen in the initial intestinal sections, whereas appendices appeared normal. Also, salpingitis and inflammation around the ovarian follicles were seen. The cause of death of all the autopsied hens was assessed to be an acute bacterial sepsis, such as that seen in coli-septicaemia or fowl cholera. Bacterial analysis with seeding on blood agar and an enteric medium (SSI) quite surprisingly showed both greyish-white colonies and black colonies with a sulphurous odour. As such colonies are suspected of Salmonella, both the flock manager and the local veterinary inspection office were notified. Concurrent to this, colonies suspected of Salmonella were also detected by cultivating the most recently taken routine boot swab samples, pursuant to the executive order on the Salmonella action plan, and were sent to the Eurofins laboratory. This find, when compared to the
clinical picture, could not rule out that this could be fowl typhoid, a notifiable illness caused by *Salmonella Gallinarum*, or an infection caused by *Salmonella Enteritidis*. As a result, the flock was put under public supervision and at the same time strain isolates from both the monitoring samples and the dead hens were sent for serological typing at DTU Food/National Food Institute. At the Institute, it was possible to ascertain that *S. Enteritidis* was involved, and subsequent phage typing showed that it was phage type 21.

**Additional analyses**

Afterwards, the task of putting down the flock was promptly initiated, but in conjunction with this, 10 random, killed hens and 20 eggs were sent to DTU Vet/National Veterinary Institute for autopsy and serological analysis respectively. The autopsy of the 10 hens confirmed the autopsy finds in the flock. The outer inspection showed that the comb was not red, as is normal, but rather remarkably pale.

Otherwise, no additional pathological finds were made. At the autopsy, fibrinous peritonitis was detected in 8 of the 10 hens, with large quantities of pus and fibrin in the abdominal cavity. There were also fibrin secretions on the hepatic serous coat. Seven hens also showed pronounced fibrinous pericarditis, and all showed varying degrees of vascular injection and hyperaemia of the ovary and fallopian tube. The texture of the liver was reduced whereas the spleen was either slightly swollen or normal. One hen had a ruptured liver and pronounced fatty liver. The intestines and intestinal contents of all hens were normal.

Material was taken from the small intestine of four birds and from the liver of five birds for general bacteriological analysis and analysis for salmonella. Two pools of liver were taken from each of 5 birds and 2 pools of intestines from each of 5 birds for selective bacteriological salmonella analysis. *Salmonella* was found in only 3 of the 5 non-pooled livers by means of direct seeding and in 2 out of the 4 intestinal samples. The other non-pooled samples were sterile. *Salmonella* was detected in the pool samples of all 4 pool samples, both intestinal and hepatic. Serological typing of representative isolates showed that this was *S. Enteritidis*.

**Serological testing**

By means of serological analysis of the 20 eggs in Salmonella MIX ELISA (Feld et al., 2000), a reaction was found in 3 eggs with OD values of 33, 85 and 86 respectively. Values of less than 20 are considered negative, values between 20 and 40 as uncertain, whereas values over 40 are positive. In other words, two of the eggs were positive, and one was uncertain, i.e. suspicious.

Previously Denmark’s serological monitoring involved the taking of random samples of 60 per house to be tested for salmonella. If two of the random samples
were positive, the flock was considered positive until the opposite had been proven by means of bacteriological suspicion-based sampling. The fact that in this instance 2 out of the 20 eggs were serologically positive was therefore serological verification that the flock was infected.

Unknown source of infection
The source of the infection is still unknown. The fact that Salmonella was not detected in the preceding monitoring samples submitted 2 weeks previously, compared to the acutely emerging illness in the flock, could indicate that this was a relatively recent introduction. By contrast, the clear serological response in 3 out of 20 eggs indicates that the infection must have been in the flock for a certain period of time, roughly 14 days, in order for the hens’ immune response to form antibodies (Jouy et al., 2005; Mizumoto et al., 2004).

Salmonella Enteritidis PT21 detected only a few times in Denmark
Salmonella Enteritidis PT21 has only been detected six times in poultry over the past decade in Denmark, and in only one layer flock since 2010 and again in the same flock in February 2013. This type is also familiar abroad in poultry, including layer flocks (Anon., 2007; Fisher, 2004). As it is regularly detected in human cases in Denmark, it should therefore be assumed that these instances are travel-related.

Previous history of the flock
It is part of the story that, at the age of 23 weeks, the same flock was afflicted by an acute outbreak of necrotising enteritis caused by Clostridium perfringens, an outbreak that was stopped by penicillin treatment. Although it is possible for necrotising enteritis to occur in young hens early in the laying period, it is otherwise a typical pullet illness most frequently seen in broiler chicks around the age of 2–3 weeks, after which the birds usually build up natural immunity to the toxins of Cl. perfringens. One week after the end of treatment, an outbreak of coccidiosis caused by Eimeria necatrix occurred, which was treated with amprolium for one week. In spite of the treatment, sporadic deaths of hens caused by coccidiosis occurred over the subsequent month or so. The coccidiosis outbreak occurred in spite of vaccinations against coccidiosis. The cause of the lack of the vaccine’s effect is a matter of conjecture, but it could have been caused by a failure to comply with the prescribed vaccination procedures or by the vaccine oocysts did not recirculate ideally in the cage system. In all probability, however, the immune response of the flock concerned had been generally poor.

Conclusion
Clinical salmonellosis emerged in a large layer flock in Denmark in the summer of 2014. The last reported outbreak of salmonellosis in a layer flock in Denmark is uncertain. The outbreak was caused by S. Enteritidis, phage type 21. The flock had
experienced other problems with illness, and presumably its immune response was not ideal.

References:


