Mortality in organic free-range chickens and molecular characterization of the involved pathogens

N. M. Eigaard, A. Permin, J. P. Christensen & M. Bisgaard

Department of Veterinary Microbiology - The Royal Veterinary & Agricultural University, Stigbøjlen 4, DK-1870 Frederiksberg C, Denmark

Introduction

Free-range poultry farming has expanded in Denmark within the last 10 years mainly due to public pressure for improved animal welfare. Especially organic table egg production has increased from almost nothing in 1996 to 14% of the current marketed eggs. However, high mortality contributes to economic losses in organic laying flocks compared to other production systems. According to the annual report from The Danish Poultry Council,17.1% of the hens died in the production period in organic systems, while 5.4% and 12.1% died in cage and deep litter systems, respectively. To date, no systematic study has explained the higher mortality in organic layer flocks. An investigation on causes of diseases is highly relevant to develop strategies to improve disease prevention in free-range layer farms.

Materials and methods

A longitudinal study

A longitudinal investigation on the causes of mortality was carried out at a Danish farm with organic layers. At the time of purchase, the two study flocks consisted of 18 week-old pullets with 2,428 and 3,564 chickens, respectively. At the time of purchase the pullets were vaccinated against *E. rhusiopathiae* using an inactivated vaccine containing serotype 1 and 2. In addition, the pullets were vaccinated against *Pasteurella multocida subsp. multocida* using a bacterin. The pullets were vaccinated as fowl cholera and erysipelas had been observed in the previous flocks at the farm.

Recorded mortality, post-mortem and sampling

The two flocks were followed for one year and mortality was recorded daily. Dead chickens were submitted weekly for *post-mortem* examination and samples for microbiological examination were taken where indicated.

Characterization of isolates

Detailed bacteriological examinations were carried out as phenotypic and genotypic characterization by Pulse Field Gel Electrophoresis (PFGE), Restriction Endonuclease Analysis (REA) and Restriction Fragment Length Polymorphism (RFLP). In addition, isolates from a previous outbreak of fowl cholera from wild birds were included for comparison with isolates originating from the organic farm. The outbreak clone was involved in two outbreaks of fowl cholera in wild birds in Denmark in 1996, from an outbreak among domestic ducks in 1998 and, subsequently, from an outbreak in the Swedish avifauna in 2001.

Results

Mortality

The observed mortality rates for the two flocks were 63% and 91%, respectively.

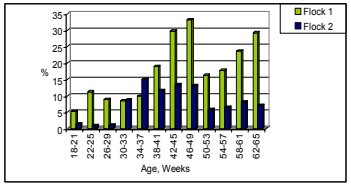

Figure 1. *Mortality based on daily-recorded dead chickens for the 2 layer flocks.*

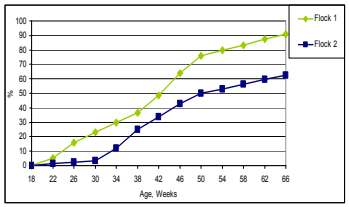

Figure 2. *Total mortality cumulated over time for the 2 layer flocks.*

Post-mortem and sampling

Post mortem was performed on 16% of the dead chickens from each flock. The results of the bacteriological isolates obtained from the dead chickens during the study period are presented in table 1.

Isolates obtained out of total number of submitted chickens	Flock 1[a]	Flock 2[b]
Erysipelothrix rhusiopathiae[c] (single infection)	166/359 (46.2%)	
Pasteurella multocida[d] (single infection)	69/359 (19.2%)	160/349 (45.8%)
E. coli (single infection)	61/359 (17.0%)	53/349 (15.2%)
P. multocida[d] & *E. coli* (dual infection)	6/359 (1.7%)	4/349 (1.1%)
P. multocida[d] & *E. rhusiopathiae*[c] (dual infection)	5/359 (1.4%)	0/349 (0%)
P. multocida[d] & *Gallibacterium* (dual infection)	1/359 (0.3%)	0/349 (0%)
Streptococcus (single infection)	1/359 (0.3%)	1/349 (0.3%)
Gallibacterium (single infection)	1/359 (0.3%)	1/349 (0.3%)
P. multocida[d] & *Streptococcus* (dual infection)	0/359 (0%)	1/349 (0.3%)
Staphylococcus aureus (single infection)	0/359 (0%)	2/349 (0.6%)

[a] 359 layers were submitted for post-mortem and 348 layers were subjected for bacteriology (36 samples (10%) were sterile).
[b] 349 layers were submitted for post-mortem and 316 layers were subjected for bacteriology (34 samples (26.9%) were sterile).
[c] Isolates of *E.rhusiopathiae* were serotyped to 1ab.
[d] Isolates of Pasteurella were identified as P. multocida subsp. multocida by biotyping.

Table 1. *Results of bacteriological examinations for chickens submitted for post-mortem examination.*

P. multocida subsp. multocida and *E. rhusiopathia* were the two most frequent isolated pathogens. The first isolation of *P. multocida* was at 24 and 31 weeks of age from flock 1 and 2, respectively. *E. rhusiopathia* was first isolated when flock 1 was 35 weeks old. Both pathogens persisted on the farm till slaughter of the remaining chickens. The gross pathological findings associated with these two pathogens were typical and varied from acute septicaemia to more chronic and localised lesions. In addition, 20% of the chickens infected with erysipelas showed valvular endocarditis.

Characterization of isolates

1. Restriction Endonuclease Analysis (REA) and Restriction Fragment Length Polymorphism (RFLP)

In total, 14 out of 81 and 23 out of 165 isolates of *P. multocida subsp. multocida* originating from flock 1 and 2, respectively were analysed by REA and RFLP using the restriction enzyme *HpaII*. The analysed isolates were obtained with regular intervals from the flocks. The DNA fingerprints obtained by *HpaII* REA were identical for the 37 isolates investigated, but the profiles differed from the clone obtained from the previous outbreak in Denmark and Sweden by one band.

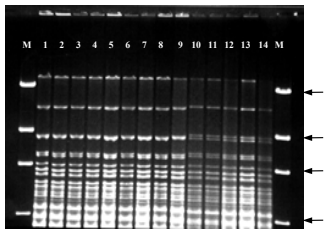

Figure 3. *HpaII REA profiles of P. multocida. Lanes M: λ-size marker. Lanes 1-9: successful clone from an outbreak of fowl cholera in Sweden in 1998 and in Denmark in 1996 and 2001, respectively. Lanes 10-14: outbreak clone in the two investigated layer flocks. The two clones varied with only one fragment as the clone originating from the layers contained a double fragment of approximately 9,2 kb.*

2. Pulse Field Gel Electrophoresis (PFGE)

PFGE using restriction enzymes *Sma1* and *Apa1* demonstrated that 76 selected isolates obtained with regular intervals from the outbreak of erysipelas in flock 1 had identical patterns. The analysed isolates were selected from the 171 isolates obtained over time.

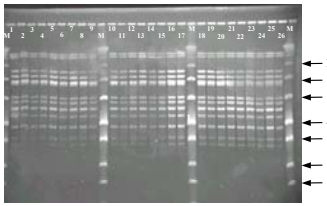

Figure 4. *SmaI1 PFGE profiles of E. rhusiopathia. Lanes M: λ-size marker. Lanes 2-10 & 12-19, 21-29: outbreak clone in the affected layer flock.*

Discussion

In free-range, including organic egg production systems where the hens are offered access to outdoor facilities appear to result in re-emerging classic disease conditions including fowl cholera and erysipelas which seldom are seen in conventional indoor systems. A recent Danish field observation has shown that about 80% of the diagnosed cases of fowl cholera in domestic poultry appeared in flocks that had access to outdoor facilities, which indicates that there is a significant risk of transmission of *P. multocida* between the avifauna and free-range poultry.

In the present study *P. multocida* and *E. rhusiopathia* were established in layers in spite of vaccination. This might indicate the possibility that these two disease agents have been built up on the farm as the previous flocks were infected too. Therefore the challenge dose of the infectious agents might be so high that the normal vaccination response will be overwhelmed. Furthermore, the pullets were infected with a relatively high number of the nematode *Ascaridia galli* at the time of vaccination which might have caused immune suppression of the pullets and resulted in ineffective protection.

All investigated *P. multocida* isolates originating from the farm were shown to be identical and closely related to the unique clone obtained from the previous outbreak in Denmark and Sweden. Furthermore, previous studies have compared the Danish/Swedish clone genotypically with isolates submitted to the Danish Veterinary Institute from 1995 to 1997 and it can be confirmed that none of these are identical to the clone from the organic layers. However, as the clone originating from the organic layer farm is very similar to the Danish/Swedish clone it may be possible that the Danish/Swedish clone has undergone successful modification.

The clonality aspect of the isolates obtained in the present study is interesting and indicates that some clones have a potential to infect and successfully colonise a flock. Several introductions of the same pathogen, resulting in more genotypes in the flock have not been observed.

Further investigations are highly relevant in order to examine the possibility of disease transmission between wild birds and domesticated poultry as high mortality contributes to significant problems in especially organic laying flocks compared to conventional indoor production systems.

Conclusions

This longitudinal study on the causes of mortality at a Danish farm with organic egg-laying chickens demonstrated establishment and spread of a successful clone of *P. multocida spp. multocida* and *E. rhusiopathia,* respectively.

In addition, it was demonstrated that these two pathogens might cause severe losses in free-range chickens with a mortality rate as high as 91%.