Detection of *Erysipelothrix rhusiopathiae* in environmental samples from organic laying hen farms affected by erysipelas

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The objective of this study was to investigate where *Erysipelothrix rhusiopathiae* can be found in the environment during outbreaks of erysipelas in organic laying hen flocks. Since *E. rhusiopathiae* was found in manure and dust from affected flocks these materials may constitute a risk for transmission of the infection.

Introduction

Erysipelas, caused by infection with *Erysipelothrix rhusiopathiae*, has been reported from several countries with non-caged laying hens (Eriksson *et al.*, 2013, Vet Rec, 173, 18). Outbreaks are associated with high flock mortality and a sudden egg drop.

Figure 1. *High flock mortality during an outbreak of erysipelas in a laying hen flock in an aviary. Photo: S. Mattsson.*

Materials and methods

Four organic laying hen farms (A-D) with ongoing outbreaks and two farms with clinically healthy flocks (E-F) were visited. In total, 233 samples were collected (Table 1). Bacteriological investigations were performed using pre-enrichment in a selective crystal-violet sodium-azide broth followed by culture on horse blood agar and selective blood agar containing kanamycin (400 µg/ml) and neomycin (50 µg/ml). Bacterial growth was harvested, boiled lysates were prepared and PCR analysis targeting a fragment of the ERH_0856-gene of *E. rhusiopathiae* (Shimoji *et al.*, 1998, J Clin Microbiol, 36, 86-89) was performed.

Results

Results of bacteriological culture are presented in Table 1. All samples from which *E. rhusiopathiae* could be isolated were also positive by PCR. In addition, seven samples (three intestinal, two manure and two dust samples) were positive by PCR but the bacterium could not be isolated from these samples.

	A	B	C	D	E	F
Laying hen – spleen	**5/5**	**5/5**	**5/5**	**5/5**	0/5	0/5
Laying hen – intestine	0/5	**1/3**	**3/5**	**4/5**	0/2	0/2
Mouse – nasopharynx	-	-	-	0/5	0/5	-
Mouse – intestine	-	-	-	0/5	0/5	-
Sock swabs[1]	0/5	0/6	0/6	0/5	0/6	0/6
Nipple drinker swabs	0/5	0/5	0/5	**4/5**	0/5	0/5
Dust (air inlet)	-	0/6	-	-	-	-
Dust (exhaust fans)	0/2	0/3	0/2	**1/3**	0/3	0/2
Soil (outside pen)	0/5	0/5	0/5	0/6	0/5	0/5
Manure (heap/storage)	0/3	**1/3**	**2/3**	**2/4**	0/3	0/3
Insects (manure heap)	0/1	0/1	0/1	0/1	-	-
Fly trap in hen house	0/2	0/1	0/2	-	0/1	-
Other arthropods	0/1	0/2	0/1	0/2	-	-
Other	-	0/3	-	0/3	-	0/4

Table 1. *Bacteriological culture of organ and environmental samples (no. of samples positive for E. rhusiopathiae/ no. of investigated samples). A-D represents farms with ongoing outbreaks of erysipelas and E-F farms with clinically healthy flocks. [1]Swabs from the ground outside the entrance door, the floor of the anteroom, litter bed, winter garden and the outside pen.*

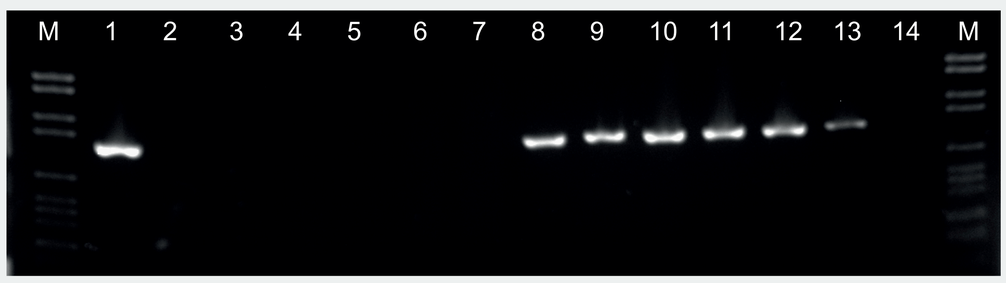

Figure 2. *PCR results after analysis of some of the samples from Farm D. The figure shows an agarose gel. M, size marker, lane 1 positive control (E. rhusiopathiae ATCC 19414[T]), lanes 2-7 sock swabs and lanes 8-14 nipple drinker swabs. For the positive control and the samples in the lanes 8-13, bands of a size corresponding to the expected 937 bp are seen.*

Discussion

Erysipelothrix rhusiopathiae was isolated from manure and dust from affected flocks. Therefore, these materials may constitute a risk for transmission and special precautions might be needed when handling manure from an infected flock. In this study, the *E. rhusiopathiae*-specific PCR was more sensitive than culture for environmental samples.

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