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Molecular epidemiology of unilateral amyloid arthropathy in broiler breeders associated with *Enterococcus faecalis*

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Although symmetrical polyarticular amyloidosis has been described extensively in brown layers, spontaneous unilateral amyloid arthropathy has not been described previously in chickens. Birds from nine flocks of broiler parent stock (PS) had unilateral lameness associated with severe swelling of the left hock joint and the caudal aspect of the metatarsus. Gross pathology was restricted to the left hock joint and the left digital flexor tendons in almost all cases, suggesting an association with administration of Marek's disease vaccine. Amyloid deposits were found in 83% (25/30) of affected joints by histological examination of Congo red stained sections. Systemic amyloidosis, involving mainly the liver and spleen, was found in 59% (10/17) of birds. *Enterococcus faecalis* was isolated from joints in 77% (23/30) of cases and *Staphylococcus aureus* was isolated from the joint in one case (1/30).

Thirty-five *E. faecalis* isolates from joints, tendons and blood samples from birds in five affected PS flocks were compared using pulsed-field gel electrophoresis (PFGE) to separate genomic fragments after digestion with *Sma*I. All but one isolate had identical or closely related restriction endonuclease digestion (RED) patterns that were very similar to a known arthropathic and amyloidogenic *E. faecalis* isolate. A further 30 *E. faecalis* isolates from seven grandparent stock (GPS) flocks and two isolates from two unaffected PS flocks of the same genetic background were analysed by PFGE. Among these isolates, 11 originating from four GPS flocks had RED patterns identical to or closely related to the reference amyloid-inducing strain. Moreover, one *E. faecalis* isolate from amyloidotic joints of brown layers housed in California, USA was included in the analysis and appeared to be identical to the reference strain.

This study showed that the *E. faecalis* isolates involved in these outbreaks of unilateral amyloid arthropathy in broiler breeders belonged to the same clone as that responsible for outbreaks in brown layers.

Introduction

The articular localization of amyloid in chickens was first described in brown layers and was associated with severe growth retardation and lameness (Landman *et al.*, 1994). *Enterococcus faecalis* has been the major pathogen found in cases in field outbreaks of amyloid arthropathy in brown layers (Landman *et al.*, 1994, 1998) and is considered to have a significant role in the induction of this condition (Landman *et al.*, 1997, 1998). In contrast, in broiler breeders with polyarticular amyloid arthropathy, *Staphylococcus aureus* has appeared to be the major agent isolated from affected joints (Landman *et al.*, 1998). Recently, we have also demonstrated experimental induction of amyloid arthropathy with *Mycoplasma synoviae* in brown layers, and the same organism has been isolated from naturally occurring cases (Landman & Feberwee, 2001).

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Table 1. *Flocks affected with unilateral (left leg) AA amyloid arthropathy*

Origin and destination[a]	Date of birth	Breed/genotype	Flock size	Proportion with macroscopic lesions (%)	
				At the rearing farm	At the production farm (30 weeks old)
1–1	2 March 1999	A-1	7800	5	3.5
1–2	2 March 1999	A-1	7300	5	7.5
3[b]	2 March 1999	A-2	720	5	4.0
2–4	2 March 1999	A-3	10 300	Unnoticed	n.r.
2–5	2 March 1999	A-3	8600	Unnoticed	n.r.
3–6	9 March 1999	A-3	12 300	5	7.0
4–7	9 March 1999	A-3	30 700	2.8	none
8[b]	16 March 1999	A-3	10 500	Unnoticed	n.r.[c]
9[b]	14 March 2000	A-3	9700	Unnoticed	9.3

[a] The first figure indicates the farm where affected flocks were reared, the second indicates the broiler breeder farm where affected flocks were housed for production.
[b] Rearing and production farm are the same.
[c] n.r. = not reported

The aim of this study was to investigate a series of outbreaks of unilateral (left leg) amyloid arthropathy in broiler breeder chickens associated with *E. faecalis* joint infection. *E. faecalis* isolates from these cases and two unaffected parent stock (PS) and grandparent stock (GPS) flocks of the same genetic background were characterized by pulsed-field gel electrophoresis (PFGE) and compared with a known arthropathic and amyloidogenic reference strain (Landman *et al.*, 1994, 1997) originating from brown layers.

Materials and Methods

Case history

Unilateral (left side) lameness was observed in birds from nine broiler breeder flocks. The affected PS flocks, which were parental crosses, originated from birds from the same primary breeding company, but were from six rearing farms and three genotypes were involved (Table 1). All affected PS flocks hatched around the same time of year. Eight flocks hatched on 2, 9 and 16 March 1999, and one flock hatched 14 March 2000. Notably, all affected flocks were processed in the hatchery on a Tuesday, the busiest day of the week in the hatchery. Moreover, these flocks were the last processed on that day. The prevalence of lameness varied from 3 to 10% per flock. Lameness was first noticed between 12 and 18 weeks, and the flock size varied from 720 to 30 700 birds. Data on flocks involved are presented in Table 1.

All birds of each flock were vaccinated against Marek's disease (Nobilis® Rismavac + CA 126 Marek vaccine; double dose, batch numbers 98F25-A and 98C13-A in March 1999, and 99I24 in March 2000; Intervet, Boxmeer, The Netherlands). One dose of the Marek's disease vaccine suspension was inoculated intramuscularly in the left gastrocnemius, and the other dose subcutaneously in the neck. Sodium ceftiofur (Excenel®; Pharmacia & Upjohn Animal Health B.V., Woerden, The Netherlands) was added only to the Marek's disease vaccine suspension used for subcutaneous application at a dose of 0.16 mg/chick in eight of nine flocks.

Postmortem examination and further procedures

Birds with left leg lameness from five of the nine flocks were submitted for postmortem examination between 25 and 37 weeks of age. The sample size varied from three to six birds. There were two submissions of birds from flock 9.

Birds were received alive and stunned, using an electric current (10 s at 340 V), and exsanguinated. Jugular vein blood was obtained for serology. Sera from two PS flocks were tested for antibodies against *M. synoviae* (Timms, 1967).

Swabs of joints were taken for bacteriological examination. Enrichment was performed in kanamycin aesculin azide (KAA) broth (Biotrading, Mijdrecht, The Netherlands) for 24 to 48 h at 42°C. After a colour change in the enrichment broth, KAA agar plates (Biotrading) were inoculated using a wire. The plates were then incubated aerobically for 24 h at 42°C. For further identification of micro-organisms, API20 Strep® kits (bioMérieux S.A., Marcy l'Etoile, France) were used. In a few cases, identification of Lancefield group D isolates was carried out using Streptex® (Biotrading).

Blood samples were collected from birds from three flocks by puncture of the ulnar vein after skin disinfection with alcohol (70% ethanol) to examine for bacteraemia. KAA broth was inoculated with 1 to 2 ml whole blood. Further bacteriological examination was performed as described for the joints.

Whole joints and 1 cm^3 samples of liver, spleen, kidney and intestine were fixed in 4% formalin, paraffin embedded and processed for histology. Sections were stained with haematoxylin and eosin, and Congo red.

Pulsed-field gel electrophoresis

PFGE analysis of *Sma*I-digested genomic DNA was performed on 41 *E. faecalis* isolates from amyloidotic joints, from blood and from caeca of birds from five PS flocks. Another 30 isolates originating from yolk sac, liver, tendons, bone marrow or diverticulum of GPS flocks, which were of the same lines but which had not yielded affected progeny, were also examined. Two other isolates from hock joints of two parent flocks, which were also of the same line but which had not had further clinical problems and were unrelated to the flocks involved in the outbreaks, were also analysed by PFGE. All liver and yolk sac isolates originated from 1-day-old chicks. The ages of the birds from which the other isolates were obtained are presented in Table 3. Finally, an *E. faecalis* isolate originating from diseased

Table 2. *Origin of the* E. faecalis *isolates characterized by PFGE after* Sma*I digestion*
Isolates from PS with unilateral amyloid arthropathy

Farm	Isolate	Sample[a]	PFGE type[b]
Reference strain (brown layers)	P	Joint	A
6	1	Hock	A
6	2	Hock	A
6	3	Hock	A
6	4	Hock	A
6	5	Blood	A
6	6	Blood	A
6	7	Caecum	B
6	8	Caecum	B
6	9	Caecum	C
5	10	Hock	A1
5	11	Hock	D
5	12	Hock	A
5	13	Hock	A
5	14	Blood	A1
5	15	Blood	A
5	16	Blood	A
5	17	Caecum	D
5	18	Caecum	D
5	19	Caecum	E
3	20	Hock	A
3	21	Hock	A
3	22	Hock	A
3	23	Hock	A
3	24	Knee	A
1	25	Hock	A
1	26	Hock	A
1	27	Hock	A
California, USA (brown layers)	Q	Joint	A
9 (8 September 2000)	28	Tendon	A
9 (8 September 2000)	29	Hock	A
9 (8 September 2000)	30	Tendon	A
9 (8 September 2000)	31	Hock	A
9 (8 September 2000)	32	Tendon	A
9 (8 September 2000)	33	Hock	A
9 (8 September 2000)	34	Foot	A
9 (8 September 2000)	35	Tendon	A
9 (8 September 2000)	36	Hock	A
9 (8 September 2000)	37	Tendon	A
9 (8 September 2000)	38	Hock	A
9 (13 September 2000)	39	Hock	A
9 (13 September 2000)	40	Blood	A
9 (13 September 2000)	41	Blood	A
ATCC 29212	R		Z

[a] All samples from which *E. faecalis* isolates originated were obtained at 1 day old. The samples collected at another age are mentioned (with age).
[b] Each letter denotes a genetically distinct type.

joints of brown layers with amyloid arthropathy that had been housed in California, USA (kindly provided by H.L. Shivaprasad) was included in the analysis. An arthropathic and amyloidogenic *E. faecalis* strain that had been characterized previously by PFGE (Landman *et al.*, 1998) was used as the reference isolate. The origins of the isolates are presented in Table 2.

Table 3. *Origin of the* E. faecalis *isolates characterized by PFGE after* Sma*I digestion*
Isolates from GPS and two PS farms without clinical problems

Farm	Isolate	Sample[a]	PFGE type[b]
a (PS)	1	Hock(16 days)	B
b (PS)	2	Hock (47 weeks)	C
A	3	Tendon	A
B line a	4	Liver	D
B line a	5	Liver	D
B line y	6	Liver	A
B line y	7	Liver	A
B line y	8	Liver	A
B line t	9	Liver	E
B line t	10	Liver	E
B line t	11	Liver	E
B line c	12	Liver	F
B line c	13	Liver	F
B line c	14	Liver	F
C line t	15	Yolk	G1
C line t	16	Liver	G2
C line c	17	Liver	H
C line c	18	Liver	H
D line c	19	Yolk	I
D line a	20	Yolk	J
D line a	21	Liver	K
D line c	22	Liver	I
D line t	23	Yolk	B
D line c	24	Yolk	I
E	25	Diverticulum (8 weeks)	A2
E line c	26	Liver	A
E line t	27	Liver	A
E line t	28	Liver	A2
E line c	29	Liver	A
E line c	30	Liver	A
F	31	Liver	L
G	32	Bone marrow (4 weeks)	A2

[a] All samples from which *E. faecalis* isolates originated were obtained at 1 day old. The samples collected at another age are mentioned (with age).
[b] Each letter denotes a genetically distinct type.

Prior to PFGE, the identity of all isolates was confirmed by polymerase chain reaction as described by Dutka-Malen *et al.* (1995).

Preparation of agarose plugs (Incert Agarose; FMC Bioproducts Co., Rockland, ME, USA), lysozyme treatment (Lysozyme; Boehringer Mannheim GmbH, Mannheim, Germany) and proteinase K treatment (Proteinase K; Sigma Chemical Co., St. Louis, MO, USA), washing steps and digestion with *Sma*I (Promega Co., Madison, WI, USA) were performed as described by Endtz *et al.* (1997).

Electrophoresis was performed with a CHEF DR III apparatus (Bio-Rad Laboratories, Hercules, CA, USA) (block 1: running time, 10 h; switch time, 5 to 15 sec; block 2: running time, 10 h; switch time, 15 to 45 sec) in a 1% agarose gel (SeaKem GTG agarose; FMC Bioproducts). The gels were stained for 1 h in 1.25 mg/ml ethidium bromide and destained for 2 h in distilled water, then photographed using ultraviolet light transillumination. The PFGE DNA restriction endonuclease digestion (RED) patterns of the isolates analysed were compared with the pattern of the reference *E. faecalis* strain 6085.94, according to the criteria of Tenover *et al.* (1995).

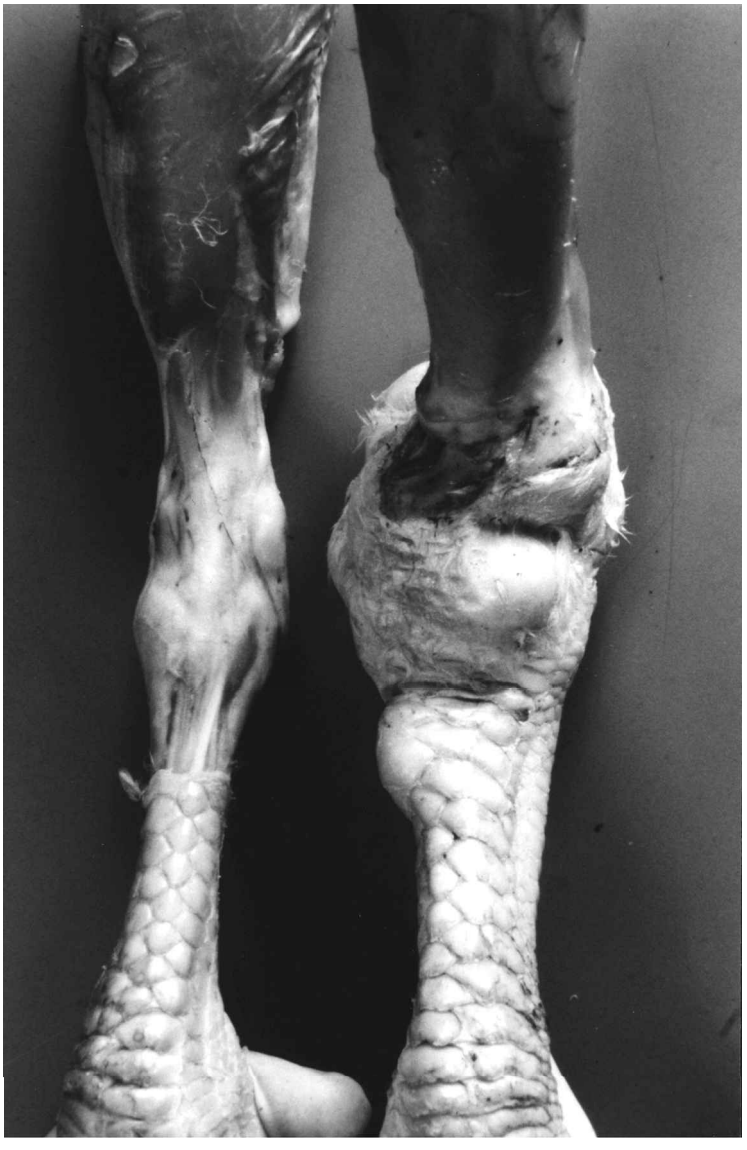

Figure 1. *Anterior view of the hock joint of a bird from flock 9. Note the severe swelling of the left affected hock.*

Results

Clinical examination

In all flocks, the proportion of lame birds during rearing was within normal range until the age of 12 weeks, when the first clinical signs were noticed by farmers, flock managers and/or their veterinarians. The lameness typically involved the left leg, which was consistently held in the air. The birds were reluctant to use the affected limb and moved with the aid of their wings. On closer inspection, severe swelling of the left hock joint was noticed. Often the caudal aspect of the metatarsus was swollen, giving the shanks an enlarged appearance.

Postmortem examination and bacteriology

Extensive postmortem examination of birds from the five affected flocks revealed joint pathology only in the left leg, except in two birds from flock 9 that had swollen right foot joints. In all birds (30/30), the tibio-metatarsal joints, the gastrocnemius and digital flexor tendons were extremely enlarged (Figure 1). In a few birds (3/30), the femoro-tibial joint was also affected. One of the birds had a swollen left foot joint and another two had swelling of the right foot joint. Orange-coloured deposits of amorphous material were found in 28/30 hock joints and most other inflamed joints. Chronic deforming osteoarthritis with concurrent osteomyelitis was present in most birds. The distribution of the amyloidotic joint lesions is shown in Figure 2.

E. faecalis was isolated from hock joint swabs in 77% of cases (23/30), and also from one knee joint and one foot joint. In flock 9 (first submission), the digital flexor tendons were also sampled and yielded *E. faecalis* in 5/6 birds. *S. aureus* was isolated from the hock joint, the digital flexor tendon and foot joint of the remaining bird. *E. faecalis* bacteraemia was detected in 7/17 (41%) birds.

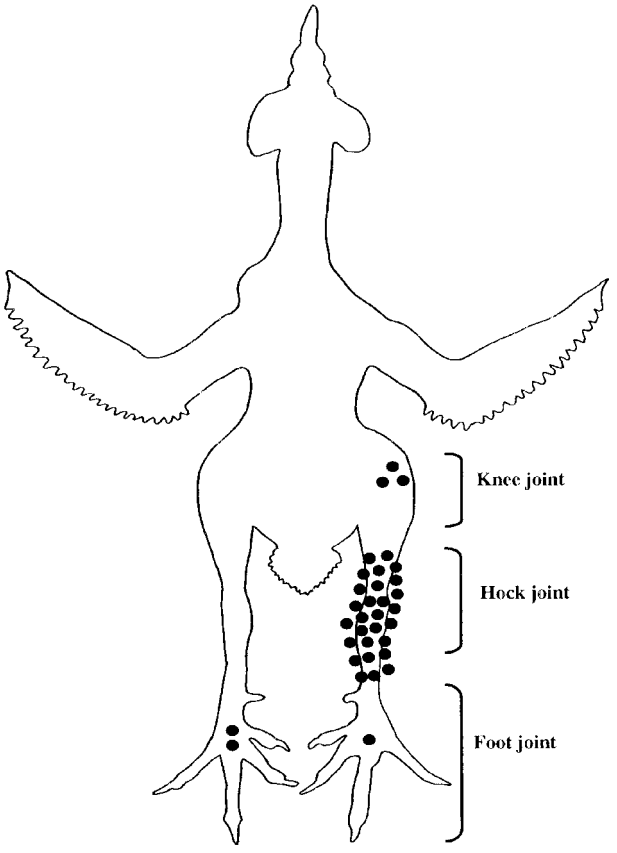

Figure 2. *The distribution of amyloidotic joints in 30 birds sampled from five broiler PS flocks with unilateral lameness. Each black dot represents an affected joint.*

The histopathology of amyloidotic joints and organs was very similar to that described previously in brown layers suffering from naturally occurring and experimentally induced amyloid arthropathy (Landman *et al.*, 1994, 1997; Perperkamp *et al.*, 1997). In joints, the deposits were located in the hypertrophic synovial membrane, the superficial articular cartilage and around nutritional vessels.

The total number of birds with amyloid-positive hock joints was 25/30 (83%), and the number with organ amyloid was 10/17 (59%).

All affected flocks originated from GPS flocks serologically negative for *M. synoviae* by rapid plate agglutination testing. Moreover, 40 lame birds from flock 9 were negative for *M. synoviae* antibodies by rapid plate agglutination 13 weeks after the first sample of birds (submission 1) was received for postmortem examination. Flock 7 was also tested for *M. synoviae* antibodies ($n = 90$) and found to be negative.

PFGE analysis

The distinct DNA RED patterns from GPS and two unaffected PS flocks obtained by PFGE are shown in Figures 3 and 4, and the origin of all isolates and the corresponding pattern types are presented in Tables 2 and 3.

Among the 35 isolates from amyloidotic or arthritic hock and knee joints (22 isolates), digital

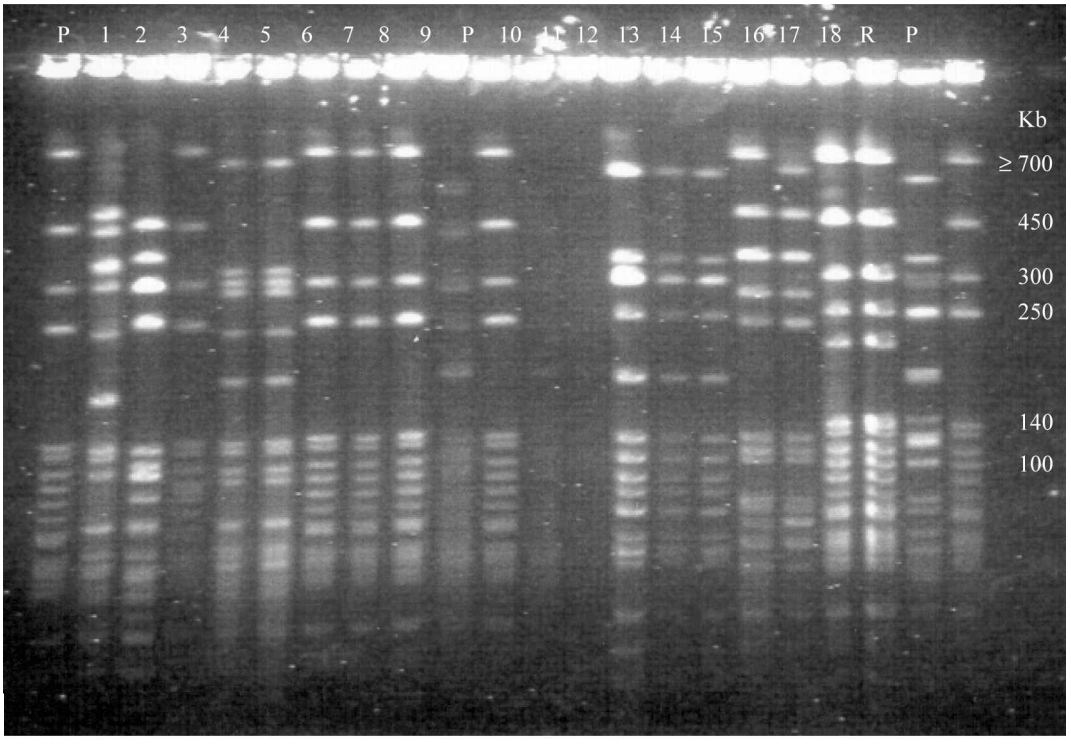

Figure 3. *RED patterns obtained by PFGE of 16* E. faecalis *isolates from birds on three GPS farms and two isolates from birds on two PS farms after digestion with* SmaI*. The origin of the isolates was: 1 to 2, hock joints of birds from PS farm A and farm B, respectively; 3, tendon of a bird from GPS farm A; 4 to 14, liver tissue of birds from GPS farm B; 15, yolk sac of birds from GPS farm C; 16 to 18, liver tissue of birds from GPS farm B. P, Reference arthropathic and amyloidogenic* E. faecalis *strain (6085.94) showing pattern A; R, an ATCC strain. Four GPS isolates, (numbers 3, 6, 7 and 8) have the same RED pattern as the reference strain P. The remaining isolates had distinct RED patterns (B, C, D, E, F, G1, G2, H). Identical patterns were seen for isolates from different samples of birds of the same GPS breeding lines (Table 3). Isolates 10 and 11 had pattern E (as 9) on close inspection of the gel.*

flexor tendons (five isolates), foot joint (one isolate) or blood (seven isolates) from birds from five PS farms, no or minimal differences were found in their PFGE patterns. All these isolates were identical or closely related to the reference arthropathic and amyloidogenic *E. faecalis* strain 6085.94. Only isolate number 11 from a hock of a bird from farm 5 had a PFGE pattern (Table 2) completely distinct from the amyloidogenic reference strain. However, this isolate was similar to two of the caecal isolates from birds on the same farm (numbers 17 and 18). Two isolates (numbers 10 and 14), one from a hock joint and another from blood from a different bird on the same farm, had one band of higher molecular weight. This was confirmed with a second gel (data not shown). These isolates were nevertheless still considered closely related to the amyloidogenic and amyloid-associated strains (RED pattern A). An isolate (Q) originating from the joints of a brown layer with amyloid arthropathy that had been housed on a farm in California, USA also had the same pattern as strain 6085.94.

Isolates from caeca (six isolates) were distinct from those recovered from joints (except one of hock joint, see above) or blood, with two distinct patterns for each farm (in total, four different patterns).

Isolates from GPS and two PS farms without clinical problems

The results of PFGE of 16 *E. faecalis* isolates from birds on three GPS farms and two isolates from birds on two PS farms without clinical problems are shown in Figure 3. Four GPS isolates had RED patterns identical to the reference strain (pattern A). One isolate originated from the tendons of a bird on GPS farm A, the other three from liver tissue of birds on farm B. Although the remaining isolates had distinct RED patterns (B, C, D, E, F, G1, G2, H), isolates from the same GPS lines had similar RED patterns.

The results of PFGE of another 14 *E. faecalis* isolates from birds on four GPS farms are shown in Figure 4. Four isolates from liver tissue of GPS birds on farm E also had pattern A. Two isolates, from the same farm, one from liver and the other from the diverticulum, had a slightly different RED pattern

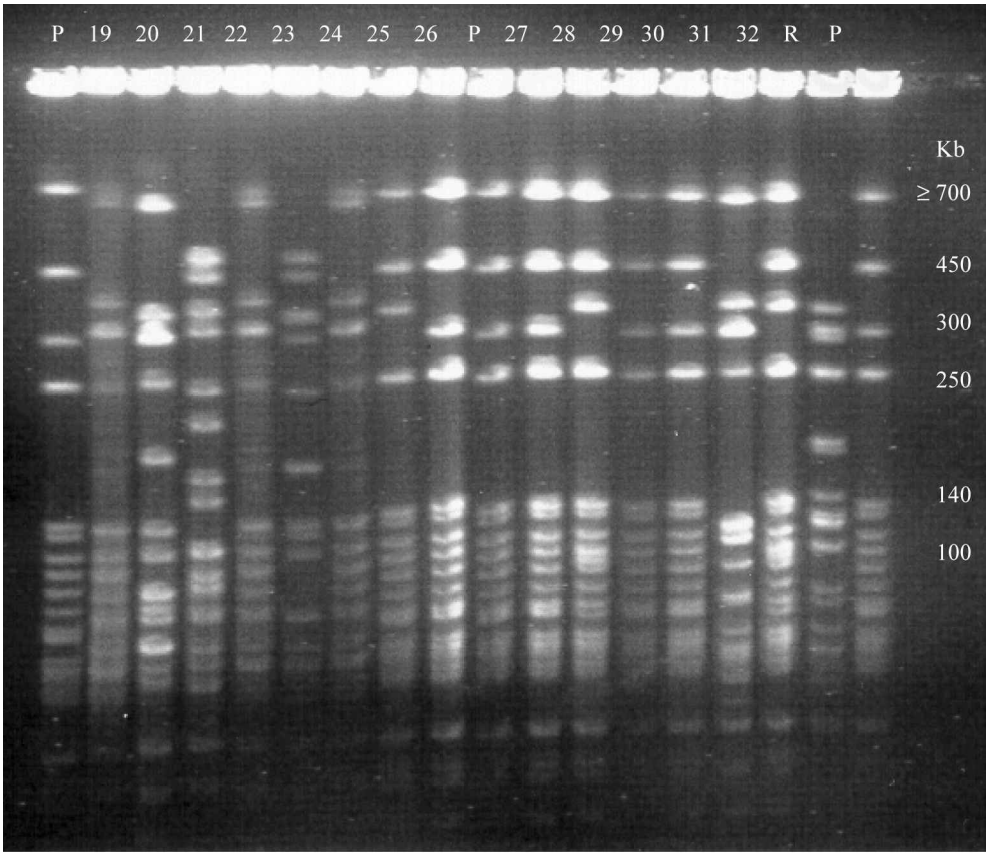

Figure 4. *RED patterns obtained by PFGE of 14* E. faecalis *isolates from birds on four GPS farms with* Sma*I. The origin of isolates was: 19, 20, 23 and 24, yolk sac from birds on farm D; 21 and 22, liver tissue from birds on farm D; 25, diverticulum from birds on farm E; 26 to 30, liver tissue from birds on farm E; 31, liver tissue from a bird on farm F; 32, bone marrow from a bird on farm G. P, Reference arthropathic and amyloidogenic* E. faecalis *strain (6085.94) showing pattern A; R, an ATCC strain. Four GPS isolates from liver tissue (numbers 26, 27, 29 and 30) had RED pattern A, identical to the reference strain. Two isolates (numbers 25 and 28) from the same farm had a slightly different pattern (A2). Pattern A2 was also found in an isolate from bone marrow of farm G (number 32). The remaining isolates had distinct patterns (B, I, J, K, L), one of which (pattern I) occurred in three isolates from birds of the same line (numbers 19, 22 and 24).*

(A2) but this was still closely related to the reference strain. An isolate from the bone marrow of a bird on farm G also had pattern A2. The remaining isolates had distinct patterns (B, I, J, K, L), one of which (pattern I) occurred in three isolates from birds of the same line. Pattern B was also found in an isolate from the hock joint of a bird on a PS farm without clinical problems (Figure 3).

Discussion

Amyloid arthropathy has been extensively described in brown layer chickens (Landman *et al*., 1994, 1997, 1998), where it is considered a complication of a chronic arthritis and features AA amyloid fibril deposition in the joint tissue (Landman *et al.*, 1996). The chronic arthritis can be caused by certain *E. faecalis* pathotypes, although other infectious (bacterial) agents may also play an aetiologic role (Landman *et al.*, 1998). Recently, we have shown naturally occurring and experimental induction of amyloid arthropathy with *M. synoviae* in brown layers (Landman & Feberwee, 2001). The amyloid precursor protein, serum amyloid A, is expressed in both the liver and the altered synovial membrane of amyloidotic birds, suggesting a role for the synovial production of serum amyloid A in the occurrence of joint amyloidosis (Ovelgönne *et al.*, 2001).

The clinical, pathological and histopathological aspects of unilateral amyloid arthropathy in broiler breeders appeared to be very similar to the condition described in brown layers (Landman *et al.*, 1994; Peperkamp *et al.*, 1997). Differences were the lack of growth retardation and ruffled feathers, the late onset of clinical lameness (≥12 weeks of age) and the occurrence of asymmetric arthritis, mainly affecting the left hock joint and digital flexor tendon. The chronic bacteraemia found in birds with active ovaries was also reported previously in brown layer PS inoculated with high doses of *E. faecalis* (Landman *et al.*, 1999) or naturally infected with these bacteria (Landman *et al.*, 2001).

Although polyarticular amyloid arthropathy in broiler breeders has been seen in association with *S. aureus* and *E. faecalis* infections (Landman *et al*., 1998), unilateral disease has not been described. The finding of asymmetric arthritis confined mainly to the hock joint and digital flexor tendon of the left leg in almost all birds necropsied strongly suggests *in situ* deposition of the bacteria by local injection.

The clustering of affected flocks around certain dates and the fact that all flocks had been vaccinated intramuscularly against Marek's disease in their left leg suggests a role for contaminated Marek's disease vaccine suspensions in these outbreaks. Moreover, all flocks were processed on the busiest day of the week and at the end of the day, when infection pressure at the hatchery due to airborne *E. faecalis* might be expected to have reached its maximum. The occurrence of *E. faecalis* in hatchery air samples (up to 10^6 colony forming units (CFU)/m^3 air), in Marek's disease vaccine suspensions (up to 10^6 CFU/ml vaccine suspension) and in injection needles (9500 to 61 000 CFU/needle) during chick processing has been shown in a previous study (Landman *et al.*, 2000).

Unfortunately, in the present cases, no samples of Marek's disease vaccine suspensions were taken to assess whether they were contaminated with *E. faecalis*. In a previous study, PFGE analysis of *E. faecalis* from a hatchery showed that an isolate originating from a contaminated Marek's disease vaccine suspension was closely related to the reference arthropathic and amyloidogenic *E. faecalis* strain 6085.94 (Landman *et al.*, 2000).

Isolates of *E. faecalis* from yolk sac, liver tissue, tendons, diverticulum and bone marrow of 1-day-old GPS chicks belonging to the same line were subjected to PFGE, although they were not the parents of the diseased PS flocks because these had already been slaughtered. The analysis showed that *E. faecalis* strains identical to the arthropathic and amyloidogenic reference strain occurred in 1-day-old GPS chicks of the same line. Contamination of hatching eggs with such strains would enable their spread through the air during hatch, with subsequent contamination of vaccine suspensions and inoculation into 1-day-old chicks.

The present study describes, for the first time, the occurrence of unilateral amyloid arthropathy associated with *E. faecalis* in chickens. The asymmetric distribution of the lesions strongly suggests that they could be iatrogenic in origin, and are likely to have been induced after intramuscular inoculation of contaminated Marek vaccine suspensions. Almost all *E. faecalis* isolates from joint and blood had RED patterns identical or closely related to the arthropathic and amyloidogenic *E. faecalis* found in brown layers, indicating that they belong to the same clone.

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RÉSUMÉ

Epidémiologie moléculaire de l'arthropathie amyloïde unilatérale chez les reproducteurs de type chair associée à *Enteroccus faecalis*

Bien que l'amyloïdose polyarticulaire symétrique ait été largement décrite chez les pondeuses à oeufs roux, l'arthropathie amyloïde unilatérale spontanée n'a pas encore fait l'objet de description chez le poulet. Les oiseaux issus de neuf troupeaux de reproducteurs de type chair (PS) ont présenté un boitement unilatéral associé à un gonflement important de l'articulation du talon gauche et du métatarse caudal. Dans presque tous les cas, les lésions macroscopiques n'ont concerné que l'articulation du genou gauche et des tendons flexeurs digitaux suggérant une association à l'administration du vaccin de la maladie de Marek. Des dépôts amyloïdes ont été trouvés dans 83% (25/30) des articulations qui ont fait l'objet d'un examen histologique utilisant la coloration au rouge Congo. L'amyloïdose systémique concerne principalement le foie et la rate et a été observée chez 59% (10/17) des oiseaux examinés. *Enterococcus faecalis* a été isolé à partir des articulations dans 77% (23/30) des cas et *Staphylococcus aureus* a été isolé à partir d'une seule articulation (1/30).

Trente-cinq souches de *E. faecalis* isolées d'articulations, de tendons et d'échantillons sanguins d'oiseaux appartenant à 5 troupeaux PS affectés ont été comparées en utilisant la technique d'électrophorèse en gel à champ pulsé (PFGE) pour séparer les fragments génomiques après digestion avec *Sma*I. Toutes les souches, a l'exception d'une, ont présenté des profils de restriction enzymatique (RED) identiques ou très proches et étaient très similaires à celui d'une souche d'*E. faecalis* arthropathique et amyloïdogénique. Une autre série de trente souches isolées de sept troupeaux grand parentaux (GPS) et deux souches de deux troupeaux PS non affectés de même variété génétique ont été analysées par PFGE. Parmi ces isolats, onze souches issues de quatre troupeaux GPS ont présenté un profil RED identique ou très proche de la souche de référence induisant une amyloïdose. De plus, une souche *E. faecalis* isolée, d'articulation présentant de l'amyloïde, de pondeuses à oeufs roux élevées en Californie (USA) a été incluse dans l'analyse et est apparue être identique à la souche de référence.

Cette étude a montré que les souches d'*E. faecalis* impliquées dans les cas d'arthropathie amyloïde unilatérale chez les reproducteurs de type chair appartiennent au même clone qui est responsable des cas chez les pondeuses à oeufs roux.

ZUSAMMENFASSUNG

Molekulare Epidemiologie der einseitigen Amyloidarthropathie bei Mastelterntieren in Verbindung mit *Enterococcus faecalis*

Obwohl die symmetrische polyartikuläre Amyloidose bei braunen Legehennen eingehend beschrieben worden ist, ist eine spontane einseitige Amyloidarthropathie bei Hühnern bisher nicht beschrieben worden. Hühner aus neun Mast-Elterntierherden hatten eine einseitige Lahmheit, die mit starker Schwellung des linken Sprunggelenks und der kaudalen Seite des Metatarsus verbunden war. Die makroskopischen pathologischen Veränderungen waren in fast allen Fällen auf das linke Sprunggelenk und die linken Zehenbeugesehnen beschränkt, was auf einen Zusammenhang mit der Verabreichung von Marek-Vakzine schließen lässt. Amyloidablagerungen wurden in 83% (25/30) der erkrankten Gelenke durch die histologische Untersuchung Kongorot-gefärbter Schnitte festgestellt. Systemische Amyloidose, die hauptsächlich die Leber und Milz betraf, wurde bei 59% (10/17) der Tiere festgestellt. *Enterococcus faecalis* wurde in 77% (23/30) der Fälle aus den Gelenken isoliert, und *Staphylococcus aureus* wurde bei einem Fall (1/30) aus dem Gelenk isoliert.

Fünfunddreißig *E. faecalis*-Isolate aus Gelenken, Sehnen und Blutproben von Hühnern in fünf erkrankten Mast-Elterntierherden wurden mit der Pulsfeld- Gelelektrophorese (PFGE) zum Auftrennen der Genomfragmente nach der Spaltung mit *Sma*I verglichen. Alle Isolate außer einem hatten identische oder eng verwandte Restriktions-Endonuklease-Digestions (RED)-Muster, die dem eines bekannt arthropathischen und amyloidogenen *E. faecalis*-Isolat sehr ähnlich waren. Weitere dreißig *E. faecalis*-Isolate aus sieben Großelternherden und zwei Isolate aus zwei nicht erkrankten Mast-Elterntierherden mit dem gleichen genetischen Hintergrund wurden mittels PFGE analysiert. Unter diesen Isolaten hatten elf, aus vier Großelternherden stammende Isolate RED-Muster, die mit dem des amyloidogenen Referenzstammes identisch oder diesem sehr ähnlich waren. Ein *E. faecalis*-Isolat aus amyloidotischen Gelenken brauner Legehennen, die in Kalifornien (USA) gehalten wurden, wurde ebenfalls in die Analyse einbezogen und schien mit dem Referenzstamm identisch zu sein.

Diese Studie zeigte, dass *E. faecalis*-Isolate, die an diesen Ausbrüchen von einseitiger Amyloidarthropathie bei Broiler-Zuchthennen beteiligt waren, zum gleichen Klon gehörten wie das für die Ausbrüche bei braunen Hennen verantwortliche Isolat.

RESUMEN

Epidemiología molecular de la artropatía amiloide unilateral en reproductores de pollo de engorde asociada con *Enterococcus faecalis*

Aunque la amiloidosis poliarticular simétrica ha sido descrita ampliamente en ponedoras rubias, la artropatía unilateral espontánea no ha sido descrita previamente en pollos. Aves provenientes de nueve lotes de un stock de reproductores de pollo de engorde (PS) presentaron cojera unilateral asociada a una tumefacción severa de la articulación tibiotarsal izquierda y de la región caudal del metatarso. La patología macroscópica se restringió a la articulación tibiotarsal izquierda y al tendón flexor digital izquierdo en casi todos los casos, hecho que

sugirió una asociación con la vacuna de la enfermedad de Marek. Los depósitos de amiloide se observaron en un 83% (25/30) de las articulaciones afectadas mediante el examen histológico de preparaciones teñidas con Rojo Congo. Se observó una amiloidosis sistémica, afectando principalmente el bazo y el hígado, en un 59% (10/17) de las aves. *Enterococcus faecalis* se aisló a partir de las articulaciones en un 77% (23/30) de los casos y *Staphylococcus aureus* se aisló de la articulación en un solo caso (1/30).

Treinta y cinco cepas de *E. faecalis* de articulaciones, tendones y muestras de sangre de aves provenientes de cinco lotes PS afectados se compararon mediante un gel de electroforesis de campo pulsado (PFGE) con la finalidad de separar los fragmentos genómicos tras la digestión con *Sma*I. Todos las cepas menos una presentaron patrones de digestión con endonucleasas (RED) muy similares o idénticos y que resultaron también muy similares a una cepa conocida de *E. faecalis* amiloidogénica y artropática. Se analizaron mediante PFGE unas treinta cepas más de *E. faecalis* de siete stocks de abuelas (GPS) y dos cepas aisladas de dos lotes de PS no afectados que tenían el mismo fondo genético. De estas cepas aisladas, once cepas originarias de cuatro lotes de GPS presentaron patrones de RED idénticos o muy similares relacionados con la cepa de referencia inductora de amiloide. Además, una cepa de *E. faecalis* aislada de articulaciones con amiloide de ponedoras rubias provenientes de California (USA) se incluyeron en el análisis y resultaron ser idénticas a la cepa de referencia.

Este estudio demostró que las cepas de *E. faecalis* aisladas e involucradas en estas epidemias de artropatía amiloidea unilateral en reproductores pertenecían al mismo clon que las responsables de las epidemias en ponedoras rubias.