Veterinary Microbiology 212 (2017) 1–6

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Staphylococcus agnetis, a potential pathogen in broiler breeders

Louise Ladefoged Poulsen[a,*], Ida Thøfner[a], Magne Bisgaard[b], Rikke Heidemann Olsen[a], Jens Peter Christensen[a], Henrik Christensen[a]

[a] University of Copenhagen, Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, Stigbøjlen 4, DK- 1870, Frederiksberg, Denmark
[b] Bisgaard Consulting, Viby Sjælland, Denmark

ARTICLE INFO

Keywords:
Poultry
Pathogen
Disease
Endocarditis
Septicaemia

ABSTRACT

In this study, four broiler parent flocks have been followed from the onset of the production period (week 20) until slaughter (week 60). Every week, approximately ten dead broiler breeders, randomly selected among birds dead on their own, were collected and subjected to a full *post mortem* analysis including bacteriological examination. In total 997 breeders were investigated and for the first time *Staphylococcus agnetis* was isolated in pure culture from cases of endocarditis and septicemia from 16 broiler breeders. In addition, the cloacal flora from newly hatched chickens originating from the same four flocks were characterized and *S. agnetis* was found in pure culture of several newly hatched chickens (n = 12) and only in one case in combination with another species. Clonality of the isolates was examined by pulsed-field-gel-electrophoresis which showed indistinguishable patterns in isolates from both broiler breeders and broilers. Three isolates were whole genome sequenced to obtain knowledge on virulence genes. The isolates harbored a number of genes encoding different fibrinogen binding proteins and toxins which might be important for virulence. The present findings demonstrate that *S. agnetis* may be associated with mortality in broiler breeders. No disease was associated with the broilers which were found positive for *S. agnetis* in the cloaca.

1. Introduction

Staphylococcus agnetis, a Gram positive coagulase-variable staphylococcus, was described as a separate species in 2012 (Taponen et al., 2012). It is closely related to *Staphylococcus. hyicus,* the aetiology of exudative dermatitis in pigs, and *Staphylococcus chromogenes* (Devriese et al., 1978; Lamers et al., 2012) a coagulase-negative staphylococcus commonly isolated from cows mastitis (dos Santos et al., 2016). *Staphylococcus agnetis* was isolated from bovine mastitis and fell into the group of coagulase-variable staphylococci which is the most frequently isolated group of bacteria from bovine milk (Pyörälä and Taponen 2009; Gillespie et al., 2009). In 2015 *S. agnetis* was described as an aetiology of lameness in broiler chickens causing bacterial chondronecrosis with osteomyelitis (Al-Rubaye et al., 2015) and it was later on confirmed experimentally that *S. agnetis* has the potential of causing bacterial chondronecrosis (Al-rubaye et al., 2017). Species of *Staphylococcus* and *Enterococcus* involved in poultry disease are mainly associated with chronic infections and septicemia (Christensen and Bisgaard, 2016) which corresponds with the studies demonstrating *S. agnetis* as a cause of bacterial chondronecrosis. In this study, *S. agnetis* has for the first time been associated with bacterial endocarditis and septicaemia in broiler breeders. In staphylococcus isolates from humans it has been demonstrated that fibronectin binding proteins are important for adhesion to endothelial cells (Peacock et al., 1999). Fibronectin binding proteins play an important role in the adhesion to mammalian cells (Joh et al., 1999). Therefore three isolates were whole genome sequenced and analyzed for the presence of genes associated with fibronectin binding proteins. Toxins causing damage to membranes, interference with receptors and affecting defense mechanisms of the host are important in the pathogenesis of diseases caused by Stapylococcus aureus (Otto, 2014). Therefore the genomes were analyzed for all genes which were annotated as related to toxin production.

2. Materials and methods

2.1. Sampling/study design

Four broiler breeder flocks (Ross 308) were followed from the onset of the production period (week 20) until slaughter (week 60). It was planned to collect 10 dead birds per week from each broiler breeder farm for *post mortem* and bacteriological examination. Farmers collected dead birds 2–3 times per day and immediately stored dead birds at − 20 °C until *post mortem* and bacteriological examination.

Newly hatched chicks originating from each of the four flocks were

* Corresponding author.
E-mail address: Ladefog@sund.ku.dk (L.L. Poulsen).

http://dx.doi.org/10.1016/j.vetmic.2017.10.018
Received 3 July 2017; Received in revised form 20 October 2017; Accepted 24 October 2017
0378-1135/ © 2017 Elsevier B.V. All rights reserved.

swabbed in the cloaca just before leaving the hatchery (n = 120 per flock per sampling). Samplings were carried out at parent ages of 30, 40, 50 and 60 weeks. Additionally, chickens were swabbed 24 h before leaving the hatchery at two occasions, namely at parent age 30 weeks and 60 weeks. When swabbing chickens 24 h before leaving the hatchery, 240 samples were collected per flock due to an expected low prevalence of bacteria. A grand total of 3840 newly hatched chickens were sampled. The bacterial flora was subsequently analyzed. At broiler breeder ages of 30 and 60 weeks, chickens originating from each of the four parent flocks were followed to the broiler farm where all chickens dying during the first week of life were collected and stored at −20 °C until *post mortem* examination. The causes of first week mortality (FWM) were determined by *post mortem* and bacteriological examination.

2.2. Post mortem examination

Collected broiler breeders (age 20–60 weeks) and chickens dead during the first week of life underwent a full *post mortem* examination including bacteriological sampling when macroscopic signs of infection were present (vascular disturbances, discoloration, exudations and enlargement of liver and spleen). Samples for bacteriological examination were collected when macroscopic lesions typical of infection were present (e.g. necrosis in liver, general vascular stasis, pulmonary stasis, hyperemia and generalized organ swelling).

2.3. Bacteriology

Bacteriological samples were collected during *post mortem* with a sterile cotton swab after sterilizing the surface of the relevant organ with a hot iron. Broiler breeders were sampled from liver and/or spleen and/or endocardium. Broiler chicks were sampled from liver or yolk sac. Samples collected during cloacal swabbing, *post mortem* examinations of dead broiler breeders and broilers were immediately plated on blood agar plates (BA) prepared with 5% calf blood in a blood agar base (Oxoid, CM0055). Plates were incubated aerobically overnight at 37 °C. From each plate showing abundant and pure growth of presumptive *S. agnetis* colonies (irregular to circular colonies of 2–3 mm in diameter, slightly convex with a smooth surface and greyish color) a single colony was sub-cultured in Heart Infusion Broth (HIB) (Oxoid, CM1032) and stored in 15% glycerol at −80 °C.

2.4. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS)

The species identity of all presumptive *S. agnetis* isolates was confirmed by MALDI-TOF MS (Vitek MS, Biomérieux) after the type strain CCUG 59809 of *S. agnetis* was included in the library for the MALDI-TOF MS for identification. Presumptive *S. agnetis* isolates were grown on BA at 37 °C for 18 h and the procedure described by Kudirkiene et al. (2015) was followed. In brief, a small amount of bacterial culture from each isolate was transferred to the MALDI-MS target plate by touching a bacterial colony on the agar plate by a 1 μl loop and then touching the MALDI-MS target plate with the same 1 μl loop. To each spot on the MALDI-MS target plate 1.0 μl of 0.25% aqueous formic acid was added (Vitek® MS-FA: bioMérieux SA). Samples were dried at room temperature and overlaid with 1.0 μl CHCA matrix solution (Vitek® MS-CHCA: bioMérieux SA) and dried at room temperature. Subsequently the MALD-MS target plate was inserted in the MALDI-TOF MS and data exported and analyzed in the SARAMIS software where fingerprints of each bacterial isolate were matched against the library in the database (Vitek MS, Biomérieux).

2.5. Pulsed-field-gel-electrophoresis (PFGE)

The clonality of the isolates from the broiler breeders and the newly hatched chicks (n = 29) were investigated by PFGE according to Murchan et al. (2003). The gels were run on a CHEF DR III System (BioRad). However, the running conditions were modified as follows: the initial switch time was 3 s; final switch time 33 s and total run time was 18 h. The voltage for the run was 6 V/cm with a 120° angle. Gels were analyzed by GelCompar II (Applied Math) by DICE similarity analysis (1% optimization, 1% tolerance).The criteria defined by Tenover et al. (1995) were used to describe the relatedness of the isolates. The Lambda PFG marker (New England Biolabs) was used as a reference on the gels.

2.6. Genome analysis

Three isolates representing the most prevalent PFGE type (B) (722-260714-1-8 heart, 722-230714-2-5 spleen and 723-310714-2-2 spleen) were whole genome sequenced using Illumina Miseq sequencing by Macrogen (Seoul, Korea). DNA was extracted using DNeasy blood and tissue kit (Qiagen). Sequences were trimmed and assembled using CLC Gemonic WorkBench 7.0 (Qiagen). For species identification the genomes were uploaded to Genome-to-Genome Distance Calculator (GGDC) (http://ggdc.dsmz.de/) described by Auch et al. (2010). Genomes were annotated in RAST (http://rast.nmpdr.org/rast.cgi) (Aziz et al., 2008) and all genes annotated as toxins and fibronectin binding proteins where listed. DNA and protein sequences from all genes which were annotated as toxins or fibronectin binding proteins in RAST were aligned in MEGA7 (Kumar et al., 2016). Proteins were further identified by BLAST search in UniProt (www.uniprot.org).

3. Results

3.1. MALDI-TOF MS

All presumptive *S. agnetis* isolates which were confirmed to be *S. agnetis* by MALDI-TOF MS were included in the study for further analysis (n = 29) (Table 1).

3.2. Post mortem examination and bacteriology

The cumulated mortality in the four broiler breeder farms ranged between 5.4 and 6.1% (Poulsen et al., 2017) which is within the range of expected mortality according to Aviagen (www.aviagen.com). From the four broiler breeders flocks, 997 hens were collected for *post mortem.* Of these, 596 had died due to an infection and in 16 cases; *S. agnetis* was the etiological agent (2.7%). The most common bacterial pathogen isolated was *E. coli* which was responsible for 62% of the mortality (Poulsen et al., 2017). The most common lesion associated with *E. coli* was salpingitis-peritonitis (Poulsen et al., 2017). These lesions were characterized by the presence of exudation, whereas, infections caused by *S. agnetis* were characterized by focal liver necrosis and pronounced enlargement of the liver and spleen and absence of exudation. Isolates of *S. agnetis* were obtained from liver and/or spleen and/or endocardium (Table 1). In six cases, valvular endocarditis was caused by *S. agnetis* and in four out of these six cases disseminated focal necroses were found in the livers, which is a thromboembolic sequel to haematogenous dissemination of bacteria. Three hens were diagnosed with septicemia with enlargement of liver and/or spleen and circulatory shock. From one of these, *S. agnetis* was also isolated from the endocardium even though no macroscopic valvular endocardial lesions were present.

From the remaining seven hens, isolates of *S. agnetis* were obtained in pure culture from liver and/or spleen and in one case also from the endocardium. In these seven cases, *S. agnetis* infection could be secondary to the primary pathology which was considered to be; congestive heart failure together with hepatic lipidosis diagnosed in two birds. In addition, one of these demonstrated hepatic subcapsular hemorrhage. Cannibalism caused the death of one bird which was also

Download English Version:

https://daneshyari.com/en/article/8505690

Download Persian Version:

https://daneshyari.com/article/8505690

Daneshyari.com