Veterinary Microbiology 153 (2011) 257–263

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Use of ovotransferrin as an antimicrobial in turkeys naturally infected with *Chlamydia psittaci*, avian metapneumovirus and *Ornithobacterium rhinotracheale*

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ARTICLE INFO

Article history:
Received 8 July 2010
Received in revised form 6 May 2011
Accepted 10 May 2011

Keywords:
Ovotransferrin
Chlamydia psittaci
Turkeys
Ornithobacterium rhinotracheale
Avian metapneumovirus

ABSTRACT

Respiratory pathogens are difficult to control in large-scale turkey production. This report describes a clinical trial of antimicrobial ovoTF aerosol on a large Belgian turkey farm. ovoTF was administered to reduce *Chlamydia psittaci* (*C. psittaci*) infections and to study the impact of this action on the occurrence of *Ornithobacterium rhinotracheale* (*O. rhinotracheale*) and avian metapneumovirus (aMPV) infections. Two subsequent broods were included; (i) a control brood receiving no ovoTF and (ii) an ovoTF brood receiving ovoTF aerosol (5 mg/animal) at the age of 2 weeks, continuing daily for 12 days. Twenty-four one-day-old toms of the control and ovoTF brood were tagged and monitored for 15 weeks. The control brood experienced two periods of respiratory disease, the first (2–3 weeks of age) due to *C. psittaci* and the second (8–17 weeks of age) in the presence of *C. psittaci*, *O. rhinotracheale* and maybe aMPV. Extensive antibiotic treatment was needed in 2, 8 and 9 week-old toms. In the ovoTF brood, toms stayed healthy until the age of 9 weeks, whereafter respiratory disease occurred in the presence of *C. psittaci*, *O rhinotracheale* and aMPV. OvoTF administration: (i) reduced the amount of *C. psittaci* in the air as demonstrated by bioaerosol monitoring, (ii) prevented respiratory disease during the first half of the brood period, (iii) was associated with 46% reduction of mortality, and (iv) reduced the antibiotic cost. Our results justify additional clinical trials to explore the use of this innovative antimicrobial strategy for poultry.

1. Introduction

Respiratory disease is of major economical importance in turkey production. *Chlamydia psittaci*, until recently named *Chlamydophila psittaci* (Kuo and Stephens, 2011), plays a key role (Van Loock et al., 2005a). *C. psittaci* is an intracellular zoonotic pathogen that presents an occupational risk to veterinarians and poultry workers (reviewed in Beeckman and Vanrompay, 2009; Dickx et al., 2010). No *C. psittaci* vaccine is available.

Belgian turkey farms are like the rest of Western European and U.S. turkey production units, modern industrial farms. We do implement Sanitel, a traceability system that tracks products through the entire supply chain. It is a prerequisite to an effective supply chain and quality management. In Belgium, we do not raise turkey breeders (parent turkeys). We buy our eggs from other European countries, mostly France. Turkey eggs are brought to Belgian industrial hatching facilities and one-day-old meat turkeys are transported to the Belgian farms.

Belgian turkey farms experience two *C. psittaci* infection waves, i.e. at 3–6 and 8–12 weeks of age (Van Loock et al., 2005a). Doxycycline administration is mostly needed to lower mortality. Outer membrane protein A (*ompA*)

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doi:10.1016/j.vetmic.2011.05.016

genotype D and A strains are often involved. These highly virulent *C. psittaci* genotypes induce IL-10 production by infected macrophages (Beeckman and Vanrompay, 2010), which can lead to inhibition of antigen presentation, lowered MHC class II antigen expression, inhibition of the CD4Th1 response and deactivation of macrophages, rendering the animals more susceptible to concurrent infections. Indeed, the first chlamydial infection wave is usually closely followed by an *O. rhinotracheale* infection at the age of 6–8 weeks. The second *C. psittaci* infection wave, at the age of 8–12 weeks is usually accompanied or followed by a subtype B aMPV infection, regardless of an earlier subtype A aMPV vaccination. The first and second chlamydial infection waves were often due to the same *ompA* genotype, suggesting that it might be the same strain (Vanrompay et al., 1997; Van Loock et al., 2005a; Verminnen et al., 2008).

Formerly, we described the anti-*C. psittaci* activity of ovotransferrin (ovoTF) *in vitro* in chicken macrophages (HD11 cells) (Beeckman et al., 2007). ovoTF inactivated extracellular *C. psittaci* and blocked bacterial internalization into host cells through interference with the bacterial type III secretion system, leading to the inhibition of actin polymerization underneath the bacterial attachment site. The latter is essential for bacterial cell entry. Subsequently, we performed a pre-clinical trial in specific pathogen free (SPF) turkeys. An ovoTF aerosol, administered to SPF turkeys prior to a *C. psittaci* infection, significantly reduced clinical signs, lesions, bacterial replication and excretion (Van Droogenbroeck et al., 2008).

This report describes a clinical trial of antimicrobial ovoTF aerosol on a large Belgian turkey farm. ovoTF was administered to reduce *C. psittaci* respiratory infections in turkeys. The impact of this action on the occurrence of *O. rhinotracheale* and aMPV infections was examined.

2. Materials and methods

2.1. Farm management

The study was conducted on a Belgian industrial turkey farm located in West-Flanders. Farm selection was based on: (i) the occurrence of respiratory disease due to *C. psittaci*, *O. rhinotracheale* and/or aMPV during all former broods and (ii) the farmer's cooperativeness to nebulize ovoTF. The farm applied an all-in all-out management schedule with a sanitary service period of 2 weeks between slaughter and restocking (3 broods/year) during which the 2 barns were cleaned and disinfected with formalin 40% + MS Megades (Schippers, Bladel, The Netherlands) using the MS Powermister (Schippers).

British United Turkey (BUT) Big-6 turkey eggs originating from French layers were transported to Belgium and incubated for 28 days in a hatchery in West-Flanders. One-day-old turkeys were transported to the farm. Stocking densities were approximately 3 and 6 turkeys/m^2 for toms and hens, respectively. Upon arrival, all turkeys were vaccinated against aMPV (Nobilis® RTV 8544, subtype A strain, Intervet, Boxmeer, The Netherlands) and against Newcastle Disease (NCD) (Nobilis® ND LaSota, Intervet). Turkeys received enrofloxacin (Enroxil®, Quinolone antibiotic, Eurovet, Bladel, The Netherlands) orally (0.5 l/1000 l water) during the first four days, as a preventive measure against bacterial infections. During the first week, turkeys were raised at an ambient temperature of 34–35 °C. Natural and mechanical ventilation was regulated as required. Hens and toms were raised in two separate groups housed in the same climate-controlled barn having a soft floor covered with wood chips. From the age of 2 and 4 weeks, turkeys were raised at an ambient barn temperature of 20 °C and 18 °C, respectively. At the age of 4 weeks turkeys received an aMPV and NCD booster vaccination. Hens then stayed in barn 1 and toms were moved to the neighbouring barn 2. Hens and toms were slaughtered at the age of 15 and 17 weeks, respectively. The farmer provided daily information on clinical symptoms and mortality, as well as on medication. The farm tested negative for *Salmonella*.

2.2. Ovotransferrin administration

From the age of 2 weeks, all turkeys of the ovoTF brood (6650 hens and 6650 toms; $n = 13{,}300$) received a daily ovoTF (Fordras, Lugano, Switzerland) aerosol for 12 subsequent days. Therefore, 5 mg ovoTF/animal was solubilized daily in 4 l tap water and completely nebulized in the complete barn by the use of the Atomist 1026 (Desvac, Pellouailles-les-Vignes, France), generating 50 μm droplets. The administration dose and regime were based on a pre-clinical study in SPF turkeys (Van Droogenbroeck et al., 2008). The previous brood (5140 hens and 5000 toms; $n = 10{,}140$), which received no ovoTF, served as negative control (control brood).

2.3. Study concept

We decided to monitor antibiotic treatment and the occurrence of clinical signs, mortality and the presence of *C. psittaci*, *O. rhinotracheale* and aMPV in toms, as according to the farmer, toms were more susceptible to these pathogens than hens. We also determined the amount of live *C. psittaci* organisms in the air of the barn by using a bioaerosol monitoring technique, recently developed by Van Droogenbroeck et al. (2009). The experimental set-up is presented in Table 1.

At the age of 2 days, 24 randomly selected one-day old toms were individually tagged with a leg number. They were also marked with blue ink on their feathers to allow on sight, rapid identification amongst others, as they were allowed to move freely throughout the tom compartment. Animals were sampled at the age of 2 days and at the age of 2, 3.5, 6, 10 and 15 weeks. Bioaerosol monitoring was performed at the same moments, plus just before stocking, sampling air in empty barn 1 and 2.

The following samples were collected: (1) blood for *C. psittaci*, *O. rhinotracheale* and aMPV antibody titration, (2) pharyngeal swabs for *C. psittaci* isolation and subsequent *ompA*-based molecular characterization of isolates, (3) pharyngeal swabs for *O. rhinotracheale* 16S rDNA PCR and (4) pharyngeal swabs for the detection of aMPV subtypes A, B, C and D by using real-time RT-PCR.