

## THE POTENTIAL ZONOTIC RISK DUE TO CLOACAL FLORA IN INTENSIVELY RAISED BROILERS

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### Abstract

*Escherichia coli* is one of the main inhabitants of the intestinal tract of most mammalian species and birds. In veterinary medicine, the presence of microbial carrier estate led to numerous studies on the presence, persistence and importance of *E. coli* in broiler chickens and has motivated epidemiological studies on bacterial contamination levels on the farms.

Swabs from cloaca of intensively raised broiler chickens were randomly (2%) sampled along with sanitation samples after disinfection on the same farm. All samples were processed by use of conventional, OIE approved, bacteriological techniques to identify bacteria indicating faecal pollution of zoonotic importance. Strains passed to selective media were biochemically tested and identified by use of API20E kits (France, Lyon).

The most important bacterial strain in the cloaca isolates was *E. coli* (68.75%), followed by *Proteus vulgaris* (18.75%) and *Escherichia hermannii* (12.5%). After disinfection, the swabs from surfaces tested constantly positive for *Proteus* spp, which proved to resist to broad spectrum disinfectants applied repeatedly, according to the technology. Both bacteria with pathogenic potential from the carried microbial flora and those isolated from surfaces represented a major risk, and could constitute a major cause of epidemic outbreaks under inappropriate technological circumstances.

**Key words:** broilers, *E. coli*, intensive farming, zoonotic risk

### INTRODUCTION

*Escherichia coli* represents one of the main commensal inhabitants of the intestinal tract of most mammalian species and birds. Nevertheless, some strains are important intestinal and extraintestinal pathogens (Karmali, 1989). Human and animal-origin pathogenic *E. coli* strains are able to cause a broad spectrum of illnesses ranging from self-limiting gastrointestinal infections to bacteremia leading to death. Usually, commensal *E. coli* isolates harbor no or only

very few virulence factors (VFs), while ExPEC isolates have specialized VFs enabling them to colonize host surfaces, injure host tissues, and avoid or subvert host defense systems (Corstes et al., 2010, Johnson and Stell, 2000). In veterinary medicine, the presence of microbial carrier estate resulted in numerous studies on the presence, persistence and importance of *E. coli* in broiler chickens and motivated epidemiological studies on bacterial contamination levels on the farms (Baykov and Stoyanov, 1999). The sources of *E. coli* in air of chicken houses, and subsequently on the walls and ceiling in these shelters, were the chicken, either directly or indirectly, by their feces (Chinivasagam et al., 2009). Similarly, in automated chicken egg layer management systems, the main sources were the live birds with both the feces and the birds linked to the contribution of *E. coli* to aerosols (Venter et al., 2004). In conclusion, as a direct consequence of the association of *E. coli* with chickens, these organisms can be inhabitants of the immediate poultry environment. It has been demonstrated that multidrug resistant producing *E. coli* isolates could be found at every level of the broiler production pyramid. On broiler farms these strains spread very fast, leading to high prevalence, which causes serious concern by entering the food chain.

The aim of the study was to isolate and identify bacterial strains of potential zoonotic importance from apparently healthy broilers raised under intensive conditions.

### MATERIALS AND METHODS

Birds. The research was carried out on a farm on a farm accommodating 80,000 intensively

raised broiler chicken distributed in 4 chicken houses, per each series.

**Sampling.** Cloacal swabs were sampled randomly, including in the trial 2% of the birds in each chicken house, from day-old, 17 and 31 days old birds. At the end of the production cycle, sanitation samples (n=10 from each chicken house) during and after the disinfection were collected on the same farm. All samples were processed by use of conventional, OIE approved, bacteriological techniques to identify bacteria indicating fecal pollution of zoonotic importance.

**Methods.** Laboratory standard protocols were used for identification of both Gram- and Gram+ bacterial strains. Firstly, the samples were inoculated on meat broth, a culture medium that allows the growth for various bacterial species, and incubated for 24 h at 37°C under aerobic conditions. Subsequently, the bacterial culture was examined microscopically following the Gram stain to establish further appropriate selective growth media. The next stage of identification included inoculation on various selective media: nutritive agar medium with glucose for a better observation of the colonies, MacConkey agar for lactose fermentation of Gram - bacteria (Messaudi et al., 2009). The inoculated plates were incubated for 18-36 h at 37°C in aerobic atmosphere.

Strains passed to selective media were biochemically tested and identified by use of API 20E kits and specific software (France, Lyon). Only those species of Gram- bacteria were considered that posed potential zoonotic risk to birds and consumers.

## RESULTS AND DISCUSSIONS

Vertical transmission, horizontal transmission as well as recirculation of *E. coli* isolates on chicken farms and hatcheries may play a role in spreading of strains with potentially pathogenic role in animal and human infections (Dierikx et al., 2013). For chickens at flock level, a very high prevalence (63.4%) of more pathogenic *Enterobacteriaceae*, extended spectrum  $\beta$ -lactamase producing, therefore multi-resistant to antibiotics, was determined (Geser et al., 2012). Furthermore, some researches indicated that levels of

highly antibiotic resistant CTX-M extended spectrum  $\beta$ -lactamase producing *E. coli* were higher in chicken cecal contents and pig feces than in samples from cattle feces (Horton et al., 2011). Extended-spectrum  $\beta$ -lactamase- and AmpC-producing *Proteus mirabilis* was found on chicken carcasses (reich et al., 2013). *Escherichia hermanii* was rarely quated in literature on chicken farms but was isolated from egg shells in Correa (Chang, 2000).

Opposingly, investigations carried out on fecal samples from different species of domestic animals revealed no pathogenicity genes in chicken feces isolates. Similarly, surveys of farms with multiple animal types indicate that the prevalence of *E. coli* O157 in chickens is low. Nevertheless, *E. coli* strains of the O157:H7 serotype differ widely in their ability to cause human disease, colonize animal carriers, and survive in the environment (Ferens and Hovde, 2011). Therefore, researches should not only evaluate the spread of bacteria in broilers, but should also take into account the potential persistence in the shelters after disinfection. Samples taken from day-old chicken allowed the identification of *Escherichia coli* in all cloacal swabs. In 17 days old birds the bacterial flora changed somewhat, the isolates including, according to cultivation on selective media and API 20E software, *Escherichia coli* (22 strains) and *Proteus mirabilis* (4 strains).

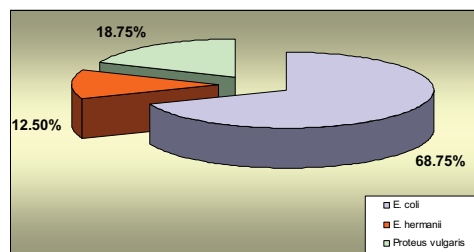


Figure1. Distribution of zoonotic strains isolated from chicken and shelters on a broiler farm

At 31 days of age, *Escherichia coli* (36 strains) as well as *Proteus mirabilis* (7 strains) were identified.

The results indicated the persistence of *E. coli*, as dominant bacteria in the cloacal

microflora from one to 17 days of age and the appearance of *Proteus mirabilis*, along with the increase in the numbers of *E. coli* strains towards 31 days of age. Several researchers indicated, based on quantitative and qualitative microbiological analyses carried out on broiler chickens during their first days of life, that the cloacal microflora was composed mainly of *Bacteroidaceae*, *Lactobacillus* and *E. coli*, considering the latest, to a certain extent, as a participant to normal local microflora (Miyamoto et al., 1998). Although a conditioned pathogen, *E. coli* present exclusively in the isolated flora from the cloaca in the experimental birds suggested that these could represent a source of infection for susceptible individuals. An underdeveloped immune system at this age, connected with high infectious pressure and stressful changes during the first days of the exploitation cycle of the birds could lead to severe infections in chickens later on.

The intervention of *Proteus mirabilis* was noticed with day 17 zile, and its persistence was observed after day 31 of age. The number of isolated strains increased from eight to twelve, during that period of time. The vaccinations included in the technology (anti-Newcastle, anti-Gumboro, anti-bronchitis) probably exerted an immune suppressive effect, depending on the administration route, and led to the increase of the numbers of *E.coli* isolates.

The sanitation sample taken seven days after the first disinfection of the premises contained *Escherichia hermannii* (10 strains), while the samples taken subsequent to the final disinfection contained *Proteus mirabilis* (5 strains)(Figure 1).

Subsequent to the first step of disinfection, *Escherichia hermannii*, an atypical biogroup of *E. coli*, with different biochemical reactions was identified. This bacteria was mentioned as an agent of secondary infections following surgery, during respiratory and digestive infections in humans. The atypical character of this bacteria could lead to diagnostic errors and inadequate prevention and control measures, furthermore atypical disease outbreaks.

Disinfectants used on the farm possessed a broad antibacterial spectrum, including

*Enterobacteriaceae* family. Nevertheless, after disinfection, the swabs from surfaces tested constantly positive for *Proteus spp*, which proved to resist to broad spectrum disinfectants applied repeatedly, according to the technology. Both bacteria with pathogenic potential from the carried microbial flora and those isolated from surfaces represented a major risk, and could constitute a major cause of epidemic outbreaks under inappropriate technological circumstances.

These results indicated a non-proficient disinfection technique applied on the farm, possibly combined with the presence of multi-chemo-resistant bacteria. The results stressed the importance of very thorough combined examinations, both for the detection of *E.coli* and *Proteus*, in chicken and on the surfaces, after performing the disinfection procedure, by cultural and identification (API 20E) tests. The samples obtained after the final disinfection indicated the persistence of *Proteus mirabilis* in the chicken houses, although the disinfectants used were of broad antibacterial spectrum (Virucidal and Virucidal Extra). This could lead to the persistence of pathogenic agents on the farm, posing a risk of disease not only to the birds, with subsequent economic losses but also to the workers and their contacts. Correctly implemented technological measures, such as the acquisition of chickens from a secure source, use of appropriate food, a safe microclimate with appropriate ventilation and productive gaps of at least two weeks between chicken series, allowing the appropriate disinfection and sanitation control could represent some of the steps to be taken to avoid an increase in pathogenicity of commensal bacteria.

## CONCLUSIONS

*E. coli*, *E. hermannii* and *P. mirabilis* with pathogenic potential from the carried microbial flora and those isolated from surfaces represent a major risk for chickens and their human contacts, and could constitute a major cause of epidemic outbreaks under inappropriate technological circumstances. Increased incidence of two *Escherichia* type bacteriae and of *Proteus*, suggested an

increased resistance of these microbes in the environment and draws the attention to the necessity and usefulness of periodical microbial examinations for each exploitation series of chicken and also complete and thorough sanitation checks on broiler chicken farms.

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