

What do we know about coli infections in commercial layers?

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Introduction

Escherichia coli is a gram negative bacterium of the family Enterobacteriaceae and is a pathogen of high importance in both human and veterinary medicine. These bacteria are part of the normal gut flora and they can be found frequently in the environment. There are many different strains which vary in their host specificity and pathogenicity, e.g. their potential to induce diseases. Thus, a complete bacteriologic diagnosis requires the identification of pathogenicity factors. A range of methods is established for this purpose (GYLES, 1994).

Extensive work has been done on the interrelation between germ and host in mammals. Therefore, several relevant pathogenicity factors could be routinely evaluated, e.g. toxins which can induce food poisoning in humans (BOCK-EMÜHL et al., 1997). Other important pathogenicity factors are structures on the surface of the bacteria, which allow the adhesion at the host cell (adhesins, fimbriae) or which induce an increased resistance to antimicrobial factors in the blood (serum resistance). Strains with pathogenic potential to humans (enterohemorrhagic *E. coli*) were not found in poultry (WASTLHUBER et al., 1998).

To date, the knowledge about *E. coli* related diseases in commercial layers is relatively scarce. In mammals, coli infections are mostly affecting neonates and young individuals. However, high losses in adult chickens are of major importance. In young chickens yolk and navel inflammations can be found. These could be successfully reduced by good hygiene during hatching, egg collection, and incubation (BARNES and GROSS, 1997). The use of antibiotics in young chicks is tolerable regarding withdrawal periods and economics, treatments are a possibility as well. During rearing of pullets, clinical diseases caused by *E. coli* are rare.

Frequent post mortem findings in layers are generalised, fibrinous-exsudative inflammations of the air sacs, peritoneum and laying organs. These findings are usually referred to as colibacillosis or egg peritonitis. Fibrinous inflammations of the peritoneum and the air sacs are not very specific but rather a sign of generalised bacterial inflammations which could be caused by infections with *Pasteurella*, *Salmonella* or *Erysipela* as well as *E. coli*. Therefore, laboratory examinations are essential for the correct diagnosis.

In case of reduced resistance of the birds due to primary infections (e.g. IB, TRT, Mycoplasma), vaccine reactions or stress situations, coli infections can be found more frequently. Poor environmental conditions (e.g. ammonia, dust, changing temperatures) and social stress (feather pecking, cannibalism) represent important stress factors. Injuries of the skin caused by pecking allow direct infections, which might ascend or generalise.

Especially under the Central European conditions of egg production losses caused by *E. coli* are particularly difficult to control:

- In cages increased losses by *E. coli* peritonitis are less frequently found as compared to floor, and free range systems (BÖHLAND, 1999). Therefore, in these alternative systems the problems are strongly increasing.

- Beak trimming reduces pecking injuries, which are supporting infections. Thus, keeping more and more flocks without beak trimming leads to a higher risk of coli infections.
- Consumer protection is aiming for food free of drug residues. Due to the necessary withdrawal periods the use of systemic acting antibiotics cannot be accepted in egg production.

Which possibilities exist for egg producers and their veterinarians for prevention and treatment?

- Primary infections have to be controlled as good as possible by acceptable hygienic conditions, regular health control and optimal vaccination programs. Of special importance is the control of climatic conditions in the poultry houses.
- Acute disease outbreaks could be treated by antibiotics. Of practical importance (in Germany) are only Colistine and Neomycine, as with these antibiotics there is no withdrawal period for eggs.
- Autogenous vaccines against colibacillosis are available. Since several years especially flocks in floor and free range systems are vaccinated.

Putting the above mentioned factors into practice is often difficult. Preventing the introduction of pathogens is impossible in free range systems, same as the reduction of dust levels in floor systems compared to cages (STEIN, 1997). Therefore, expectations focus on the success of vaccination programs.

The veterinary laboratory of Lohmann Tierzucht GmbH produces inactivated, autogenous vaccines since several years. *E. coli* strains isolated from affected birds are grown, inactivated and emulsified for injection. It is recommended to vaccinate pullets twice before onset of production with an interval of 4-6 weeks. The increasing demand for these vaccines indicates their efficacy, although precise data about the level of protection are scarce. Experimental data shows good protection which is, however, limited to homologous strains (DHO-MOULIN and FAIRBROTHER, 1999).

Own studies

Since 1999, in our lab isolated *E. coli* strains are evaluated for phenotypic parameters which may indicate possible interactions in pathogenicity of these strains. For the production of a vaccine batch, we aim to select the most "serious" strains present in a flock in order to use these as vaccine antigens.

Material and methods

Isolates

In the years 1999 and 2000 in total 442 *E. coli* isolates from commercial layers, parent and grandparent flocks have been obtained or submitted to our laboratory. The material originated from 53 poultry companies from 11 different countries. Only isolates from the heart or bone marrow were included in the study. All isolates were identified by the API 20E System (Bio Merieux, Nürtingen,

Germany). Hemolysis was evaluated on columbia agar plates with 5 % sheep blood (Oxoid, Wesel, Germany) and motility microscopically in fresh bouillon cultures.

Serotyping

From 339 isolates the serotype of the somatic antigens (O-antigen) was identified by coagglutinating reagents against the O-antigens O1, O2 and O78 (BioVac, Beaucouzé, France). In case of all negative reactions, isolates were sent to a specialized laboratory for further typing (CVL, Weybridge, UK).

Sensitivity test against antimicrobials

From 178 isolates sensitivity tests were performed by the micro dilution method, using commercially available kits (Merlin, Bornheim-Hersel, Germany).

Embryo pathogenicity tests

The method described by WOOLEY et al. (2000) was modified and used on 31 isolates to obtain an embryo pathogenicity index. Approximately 300 colony forming units (CFU) of each isolate were injected into the allantoic cavity from 11 nine-day old SPF embryos (Valo, Lohmann Tierzucht, Cuxhaven). The infected eggs were further incubated and over a period of 6 days embryo mortality was recorded. Results were expressed as an embryo pathogenicity index, the number of dead embryos was counted daily and added over the observation period of six days. Thus, in case of mortality of all embryos on the first day p.i., the maximum value of 66 is achieved.

Results

All 442 strains were tested for hemolysis. Positive reactions were only seen with two strains of one submission, which were serologically not typable. The motility test of 315 isolates revealed 213 positive and 84 negative results (Table 1).

Table 1: Hemolysis and motility in *E. coli* isolations from laying hens

	Positive	Negative
Hemolysis	0.45 %	99.55 %
Motility	67.6 %	32.4 %

Of the 339 isolates used for serotyping in 303 cases the O-antigen type could be detected, the other 33 isolates were not typable. In total 40 different O-antigens were identified, of which the serotypes O2, O78, O1 and O8 were identified most frequently (Figure 1).

The antibiotic resistance of 278 isolates tested against a range of antimicrobials used in poultry is shown in Figure 2. Three isolates were resistant to all antibiotics tested with the exception of Colistine. One isolate was sensitive to Colistine and Ampicilline and another one to Colistine and Gentamicine. Eight further isolates were sensitive only to Colistine and two other antibiotics.

The embryo pathogenicity index was tested from 31 isolates of which 9 belonged to serotype O2 and eight to serotype O78. From five isolates, the serotype was not known. The average pathogenicity index for all isolates was 51, for O2 isolates 42 and for O78 isolates 53. Results are shown as a histogram in Figure 3.

Figure 1: Detection frequencies of various O-serotypes

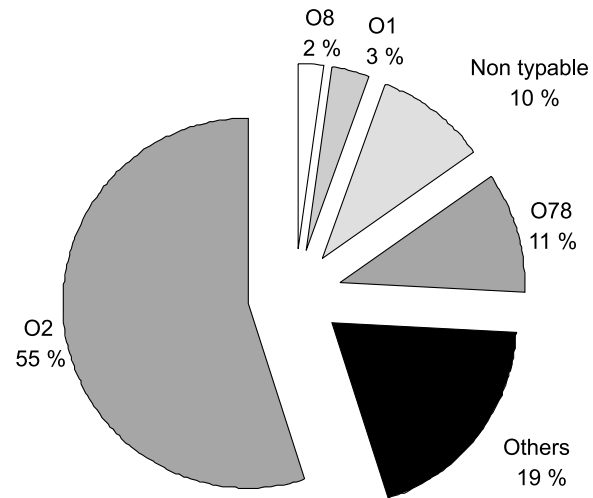


Figure 2: Resistance against antimicrobials in %

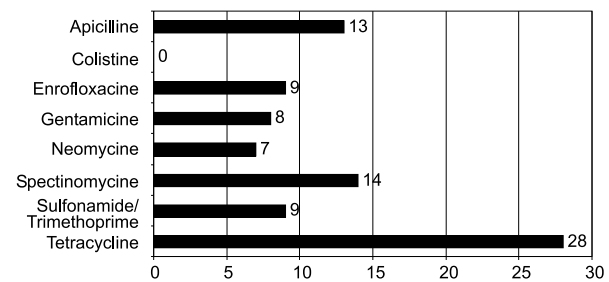
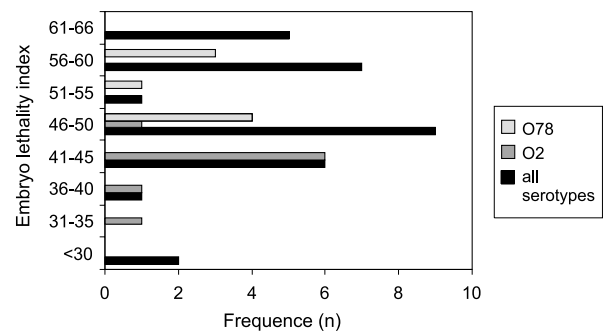


Figure 3: Embryo lethality indices of different O-serotypes



Discussion

In the routine work of our poultry diagnostic laboratory, *E. coli* is of outstanding importance. This is true for the post mortem room, where typical lesions are frequently seen, as well as the bacteriology, where many strains are isolated, characterised if possible, and stored in our archive. The number of submissions from many countries demonstrates the world wide importance of coli infections. By serological and virological examinations we try to identify possible primary infections in the affected flocks. In

some cases increased antibody levels against Infectious Bronchitis Virus or Avian Pneumovirus indicate earlier infections. But very often coli infections with high losses occur also in flocks which have no indication for primary infections, including *Mycoplasma gallisepticum* and *Mycoplasma synoviae*.

This leads to the question, to which degree particular *E. coli* strains can induce diseases without primary alteration of the host. Those strains would need to have pathogenicity factors which affect especially layers in production. The determination of hemolysis and motility for the strain classification was done to identify possible relations. From the results obtained, it is evident that hemolysis as a pathogenicity factor in layers is of no importance. About two-third of the strains were motile. However, among the strains identified as most pathogenic in the embryo pathogenicity test we found both motile and non-motile isolates. Our conclusion is that motility is unsuitable for pathogenicity predictions as well.

The systematic evaluation of antibiotic resistance should help to estimate possibilities and limits of antibiotic treatments. With Colistine and Neomycine, which both can be used without withdrawal period on eggs in Germany, the situation is still good. In case other products with systemic activity are intended to be used e.g. in parents or broilers, sensitivity testing is strongly recommended as many combinations in resistance exist.

The serotypes O1, O2 and O78 are described as the most important strains in poultry (DHO-MOULIN and FAIRBROTHER, 1999). Our results confirm this finding for O2 and O78, whereas the prevalence of O1 was found to be quite low in our study.

The embryo pathogenicity test was described by WOOLEY (2000) and used with slight modifications. In this test, the interaction between a specific pathogen and the chicken embryo was tested, without the need to perform animal trials. Disadvantages are the high cost for labour and material and the relatively low reproducibility. One possible explanation may be that different lots of hatching eggs possess varying level of antibodies against *E. coli*. Test results should rather be considered as a trend and the inclusion of positive and negative control strains in the experiments is essential for their validity. An interesting finding is that the pathogenicity within a serotype may vary strongly. Furthermore, the data indicate that, although isolates of the serotype O2 are found most frequently in commercial layers, they seem to be less embryo pathogenic than O78 strains. Especially non-typable isolates showed often very high indices of 60 and higher. The evaluation of the serotype alone does not allow to draw conclusions on the actual pathogenicity of a given isolate.

Outlook

In future precise molecular biological methods should be used also in veterinary medicine for the identification of pathogenicity factors. But for this, it is essential that these pathogenicity factors for commercial layers are identified and characterised. Several groups are working on these topics and first results are available (CHAFFER et al., 1999; FOLEY et al., 2000; POURBAKSH et al., 1997). The results may attribute to the improvement of diagnostics and development of new vaccination concepts.

Due to the lack of better alternatives the embryo pathogenicity test in the meantime could be used to evaluate

the pathogenic potential of *E. coli* isolates which are intended to be used as antigens for vaccine production. In future, lab and field trials should be used to prove the efficacy of different vaccines and vaccination programs.

Summary

Pathogenicity evaluation in *E. coli* isolates by testing for hemolysis or motility is not useful. According to serotyping results a higher incidence of certain serotypes, especially O2, could be demonstrated. However, this does not allow any conclusion regarding the pathogenicity of the isolate. In tendency, isolates of the serotype O78 were more pathogenic than those of serotype O2. Of practical importance is the evaluation of phenotypic parameters in selecting strains for inclusion in autovaccines. We recommend to use strains of different serotypes and from those ones with the highest embryo pathogenicity.

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