Department for Farm Animals and Veterinary Public Health
University of Veterinary Medicine Vienna

University Clinic for Poultry and Fish Medicine
Unit of Poultry Medicine
(Head: Univ.-Prof. Dr. vet. med. Dr. h. c. Michael Hess)

Aligning the pathogenicity of *Escherichia coli* isolates in embryos and young chickens

Diploma thesis

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Submitted by
Richard Friedrich Liermann

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Supervisor and reviewer

Supervisor:

Dr. Surya Paudel

Clinic for Poultry and Fish Medicine

University of Veterinary Medicine Vienna

Reviewer:

Priv.-Doz. Dr.med.vet. Dipl.ECVM Joachim Spergser

Institute of Microbiology

University of Veterinary Medicine Vienna

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Contents

1. Introduction

Escherichia coli is a gram-negative bacterium and a member of the family *Enterobacteriaceae.* It is part of the microflora in the lower intestinal tract of chickens with essential roles in normal physiological functioning (Abdelhamid et al., 2021; Dho-Moulin & Morris Fairbrother, 1999; Guabiraba & Schouler, 2015). These intestinal isolates from healthy birds are described as non-pathogenic commensals. But *E. coli* is also the causative agent of colibacillosis, which is one of the major infectious diseases in poultry worldwide. The most common lesions associated with colibacillosis are the subacute form of pericarditis or airsacculitis beside the acute form of septicaemia (Dho-Moulin & Morris Fairbrother, 1999; Dziva & Stevens, 2008; Kabir, 2010). An infection with avian pathogenic *E. coli* (APEC) is also characterized with other lesions like peritonitis or perihepatitis (Lister & Barrow, 2008). It is present either as the primary pathogen or only as a secondary agent (Kabir, 2010; Nolan et al., 2019). In poultry, multiple serotypes of *E. coli* are reported (Dziva & Stevens, 2008). The serotype classification of *E. coli* is based on somatic (O), flagellar (H), and capsular (K) antigens (Panth, 2019). In the field, serotypes O1, O2 and O78 are mostly prevalent. The severity of colibacillosis might vary due to differences in the virulence of isolates or the presence of predisposing factors (Dho-Moulin & Morris Fairbrother, 1999; Dziva & Stevens, 2008).

It is also suggested that *E. coli* can penetrate through the shell if the eggs are in contact with feces (Panth, 2019). This might be one of the factors for the spread of *E. coli* in chickens during hatching, resulting into omphalitis or early mortalities (Kabir, 2010; Rezaee et al., 2020).

The APEC strains are also *in vitro* characterized based on the presence of different virulence-associated genes or phylogenetic groups. APEC in general are suggested to have essential virulence factors in contrast to the avian non-pathogenic strains. These are for example different fimbrias, toxins or plasmid bound factors like hemolysine (Kathayat et al., 2021). The virulence-associated genes (VAGs) and the different combinations of the genes are also useful approaches for the APEC identification. However, it is known that no single gene is exclusively associated with the avian pathogenic *E. coli* (Kemmett et al., 2013).

The Embryo Lethality Assay (ELA) is common for determining pathogenicity. This test has been used to differentiate between non-pathogenic and pathogenic APEC isolates. The ELA is a sensitive and specific assay for the virulence evaluation of *E. coli* isolates in laboratories (Oh et al., 2012).

The objectives of the thesis are:

- to investigate the differences in pathogenicity of *E. coli* isolates belonging to the O78 serogroup in embryos
- to investigate the macroscopic and microscopic lesions, as well as tissue colonization of three selected strains, in young chicks
- to investigate the correlation between the pathogenicity of *E. coli* isolates with different VAGs in embryos and in young layer chicks.

2. Material and Methods

2.1 Embryo lethality test

The embryo lethality test was performed with 15 *E. coli* isolates. Details of the isolates used are provided in Table 1. All the isolates were collected from the femur of broilers showing different severity of femoral head necrosis (Gaußmann et al., 2018).

2.1.1 Bacterial isolates

The stock culture of *E. coli* was grown on MacConkey agar plates overnight at 37 °C. Then on the next day, two to four colonies of *E. coli* were grown in 25 ml of freshly prepared lysogeny broth (LB) medium. After ten hours of incubation at 37 °C and 150 rpm, the colony-forming units (CFU) were counted from the culture.

For this, 10 fold dilution series with 900 µl phosphate buffered saline (PBS) and 100 µl of the culture was used until 10^{10} and the CFU from 10^{6}, 10^{8} and 10^{10} were counted by spreading 100 µl of each dilution in duplicates in MacConkey agar plates, and visible colonies were counted. After the count of the colonies, the cultures were diluted to prepare 10^{2} CFU/ml. For the infection 100 µl of 1-8.2 x 10^{2} CFU/ml per egg was used.

Table 1: Isolates from broilers that were used for embryo lethality assay

PA-number	Agglutination	sumVAGs[1]	Lesion score[2]	Antibiotic sensitivity test[3]			
				Number sensitive	Number intermediate	Number resistant	Classes resistant
16/02376-3	O78:K80	5	2	4	0	10	5
15/22748-1	O78:K80	5	0	12	0	2	1
15/19103-3[4]	**O78:K80**	**5**	**1**	**4**	**0**	**10**	**5**
16/02596-3	O78:K80	3	2	9	1	4	1
15/24960-2	**O78:K80**	**3**	**1**	**13**	**0**	**1**	**1**
16/003317-1	O78:K80	3	0	12	1	1	1
16/003317-3	O78:K80	3	2	12	1	1	1
16/02374-1	O78:K80	3	2	12	1	1	1
15/19580-2	O78:K80	3	2	13	0	1	1
15/24599-3	O78:K80	3	0	11	1	1	1
15/25396-3	**O78:K80**	**3**	**1**	**13**	**0**	**1**	**1**
15/19579-2	O78:K80	3	0	12	0	2	2
16/04316-1	O78:K80	2	2	12	0	2	2
16/00910-3	O78:K80	1	1	13	0	1	1
15/25396-3	O78:K80	1	0	12	1	1	1

[1] eight different VAGs were investigated
[2] lesion scoring system of the femurs that were sampled: 0 – no gross pathological lesions of the femoral head, 1 – degeneration with separation of the cartilage of femoral head, 2 – necrosis and/or rupture of femoral head (Gaußmann et al., 2018)
[3] 14 different antibiotics were investigated (Gaußmann et al., 2018)
[4] strains used in animal trail are in bold

2.1.2 Embryo inoculation

Specific pathogen free (SPF) eggs were candled before the infection and infected according to Spackman & Stephens, (2016) with some modifications. Based on candling of eggs, live embryos were selected and the chorio-allantoic sac (CAS) 2-hole method was used for infection.

After selection of twelve live eggs per group the edge of the air cells and a second spot for inoculation were marked with a pencil. The optimal place for this second spot is about 1-2 cm from the bottom of the air-cell, and free of blood vessels.

After disinfection of the surface of the eggs, a small hole was made at the spot and on top of the air-cell. Then 0.1 ml of the inoculum material was inoculated into the allantoic cavity via the lower hole by using a syringe and a needle (23 g).

The fluid was carefully and slowly inoculated so that the fluid does not flow out of the hole due to the back pressure. For the negative control group the same amount of PBS through the same route was inoculated. Before the eggs were placed in the incubator, the holes were closed with wax.

The optimal temperature in the incubator was maintained as 37.5-37.8 °C with 60-65 % relative humidity. The eggs were candled daily until 4 days post infection (dpi) and dead embryos were recorded and were used for the necropsy.

2.1.3 Necropsy and Sampling

Necropsy was done in dead embryos immediately after daily candling, and at 4 dpi, the remaining embryos were killed for sampling. The eggs were disinfected with 70 % alcohol and opened with forceps for the necropsy.

Changes in embryos and yolk sacs were recorded. Yolk samples from all embryos were processed for a bacteriological detection of *E. coli* with direct plating. The bacteriological samples were important for the detection if the embryos are infected with *E. coli* or not (positive/negative control).

2.2 Animal trial

The pathogenicity of three selected *E. coli* isolates was also studied by performing an animal trial in chickens. The following *E. coli* strains were chosen based on the mortality rate in embryos: PA15/24960-2, PA15/19103-3 and PA15/25396-3 right.

2.2.1 Growth of bacterial isolates

Selected fresh colonies of the bacterial isolates were incubated overnight in 25 ml of LB broth for six hours in agitation. Before infection, the bacterial cultures were washed three times for ten minutes at 5000 rpm and 4 °C. Afterwards the bacterial culture was resuspended in PBS. The CFU count was made before and after the infection steps.

2.2.2 Birds and Housing

For the animal trial, 48 young SPF layer chicks were randomly divided into four groups (Table 2). Each group of chicks was housed separately in isolators with controlled temperature, light and air flow. Feed and water were supplied *ad libitum*.
All birds were tagged with a Swifttag (an individual number tag). Figure 1 shows the birds inside the isolator before infection.

Figure 1: Fourteen-day-old chicks inside the isolator before the infection

Table 2: Experimental design of the animal trial

Group	Number of birds	Strain used for infection (at 14 days of life)	Killing and sampling
1	12	PA15/19103-3	4 birds at weekly intervals
2	12	PA15/25396-3 right	4 birds at weekly intervals
3	12	PA15/24960-2	4 birds at weekly intervals
4	12	no	4 birds at weekly intervals

2.2.3 Infection

The infection was made at 14 days of age. Birds were infected by aerosolization of the inoculum by a nebulizer inside a box placed in each isolator (Figure 2). The procedure of aerosol infection lasted for 30 minutes altogether. After nebulization of the inoculum the birds were released out of the box. Birds in groups 1 to 3 were infected with 1 ml/bird of 2.95-4.35 x 10^8 CFU/ml of *E.coli* suspensions in PBS. Sterile PBS was used for the birds in the negative control group (group 4) to mimic the infection procedure.

The birds were controlled at least twice a day for clinical scores following a scoring scheme (Table 3). For sampling and necropsy four birds were euthanized from each group at 7, 14 and 21 dpi.

Table 3: Clinical scoring scheme

Score	Clinical signs
0	Animal active with no clinical symptoms
1	Slightly weak, dropping wings and depressed
2	Weak and ruffled feathers, reluctant to move, apathy
3	Animal unable to move or stand, eyes closed and intensified breathing

Figure 2: Infection of fourteen-day-old chicks inside a box by a nebulizer

2.2.4 Body Weight and Necropsy

For killing, birds were injected with 1.5 ml Ketamin and Xylazine intramuscularly. Body weight of birds was recorded during the necropsy before bleeding. After anaesthesia, chickens were bleed out by cutting the jugular vein. The necropsies were done following a standard protocol, macroscopic lesions were recorded and samples were collected.

The lesions in organs were analysed used the lesion scoring system according to Antao et al., (2011) with some modifications. Four target organs (lung, air sac, heart, liver) were scored from 0 to 2. Only the air sac had a three-part scoring system. A detail description of different scores is shown in Table 4.

Table 4: Macroscopic lesion score system

Organ	Score	Lesion
Air sac	0	Normal
	1	Slight opaque and/or thickened membranes
	2	Moderate opaque and/or thickened air sac with small amounts of fibrin
	3	Moderate opaque and/or thickened air sac with severe amount of fibrin
Heart	0	Normal
	1	Opacity with lack of transparency
	2	Thickened pericardium with marked pericarditis
Liver	0	Normal
	1	Slight amount of fibrin
	2	Marked perihepatitis with massive amount of fibrin
Lung	0	Normal
	1	Lesions are ≤ 1/2 of the lung (uni- or bi-lateral)
	2	Lesions are ≥ 1/2 of the lung (uni- or bi-lateral)

2.2.5 Blood collection

Blood samples were collected by cutting of the jugular vein. The collected blood was centrifuged at 4000 rpm for twelve minutes at room temperature for extraction of serum. The samples were stored in the fridge at -20 °C.

2.2.6 Histopathology

The following samples were collected for the histopathology: air sac, lung, liver, heart and spleen. Tissue samples were fixed in neutral buffered formalin (10 %), were embedded in

paraffin and sectioned into 5 μm slices. The slices were strained with hematoxylin and eosin following a routine procedure.

The lesions in air sac, lung, liver, heart and spleen were examined based on a scoring system from 0 to 2 under a light microscope (Paudel et al., 2021). A detail description of the scoring system is shown in Table 5.

Table 5: Microscopic lesion score system

Score	Lung	Air sac	Liver	Spleen[1]	Heart
0	normal	normal	normal	normal (n=0-5)	normal
1	Inflammatory cell infiltration into secondary bronchi or in lung tissue	Focal to multifocal thickening with infiltration of inflammatory cells and/or edema	1 to 2 focal of mononuclear cell infiltration	Heterophilic infiltration (n=5-14)	Infiltration of inflammatory cells into epicardium
2	Inflammatory cell infiltration into secondary bronchi and in lung tissue	Diffuse thickness with sever inflammatory cells infiltration	Multifocal infiltration of inflammatory cells with hepatocellular necrosis	Heterophilic infiltration (n≥15)	Infiltration of inflammatory cells into epicardium extended to myocardium

[1]In 40 objective, average of 8 random images

2.2.7 Bacteriology

The following samples were collected for bacteriology: air sac, lung, liver, heart and spleen. Organ samples were plated on MacConkey agar plates and incubated overnight. These samplings were necessary for understanding the pattern of *E. coli* distribution in organs.

3. Results

3.1 Embryo lethality test

3.1.1 Embryo mortality

The Kaplan-Meier survival test was used to show the survival of embryos following infection with different *E. coli* strains (Figure 3).

No mortality was recorded in the negative control group, the survivability was 100 %. Among infected groups, varying mortalities were observed ranging from 33 % to 100 %.

During four days observation time in groups 1 to 15 the total survival rates were between 66 % for the isolates 16/00910-3 or 15/19580-2 and 0 % for the bacterial strains 15/25396-3 right or 15/19103-1.

The highest mortality rate was recorded at 2 dpi, irrespective of the isolate used for infection. On this day, for example, 6/12 embryos infected with 15/19103-3, 5/12 embryos infected with 15/24960-2 or 4/12 embryos infected with the isolate 15/25396-3 right died. At 4 dpi, least mortality rates were observed.

The average mortality rate was 68 %, which means that on average 9 of 12 birds died. The majority of the mortality rates ranged between 50 % and 83 % but in two cases the mortality rate was either lower with 33 % or higher with 100 %.

Following infection with isolates 15/19103-3 and 15/25396-3 right all twelve embryos died whereas 10/12 embryos died by the isolate 15/24960-2. These three isolates showed the highest mortality rates and they were used subsequently for the *in vivo* pathogenicity test.

3.1.2 Macroscopic lesions in embryos

In infected dead embryos, the yolk was very congested and turbid. Strong and intensive smell was typical for the infected embryos. The dead embryos also showed reddened skin on the neck and the head. The yolk sacs showed pathological signs of damaged blood vessels and were hyperemic.

The negative control embryos had no changes or lesions in the yolk sac or yolk contents. All the infected embryos were positive for *E. coli*.

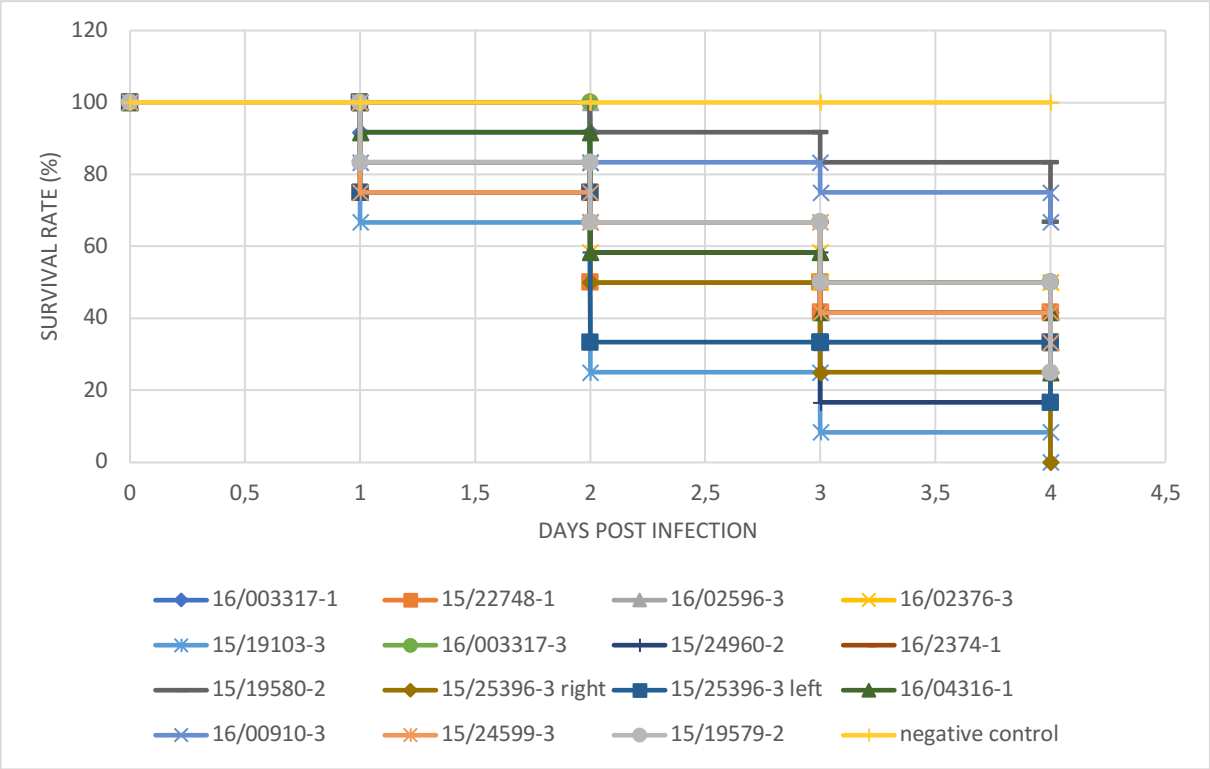


Figure 3: Survival rate of the embryos

Table 6: Mortality rates of embryos and re-isolation of *E. coli* after infection with different isolates

Strains	dpi				Total dead embryos	Mortality	Re-isolation of *E.coli* from yolk
	1	2	3	4			
15/19103-3	**3**	**6**	**2**	**1**	**12/12**	**100 %**	**12/12**
15/25396-3 right	**2**	**4**	**3**	**3**	**12/12**	**100 %**	**12/12**
15/24960-2	**0**	**5**	**5**	**0**	**10/12**	**83%**	**12/12**
15/25396-3 left	3	5	0	2	10/12	83 %	12/12
16/003317-1	1	3	3	2	9/12	75 %	12/12
16/04316-1	1	4	2	2	9/12	75 %	12/12
15/19579-2	2	2	2	3	9/12	75 %	12/12
15/22748-1	3	3	1	1	8/12	67 %	12/12
16/02596-3	0	5	3	0	8/12	67 %	12/12
16/2374-1	2	2	2	2	8/12	67 %	12/12
15/24599-3	3	1	3	1	8/12	67 %	12/12
16/02376-3	3	2	1	0	6/12	50 %	12/12
16/003317-3	0	4	2	0	6/12	50 %	12/12
15/19580-2	0	1	1	2	4/12	33 %	12/12
16/00910-3	2	0	1	1	4/12	33 %	12/12

3.2 Animal trial

3.2.1 Clinical signs

Details of the clinical score are provided in the Table 7. The clinical scoring of the chickens showed that in the first week post infection two birds had a score of 2, one in group 1 (isolate 15/19103-3) and the other one in group 3 (isolate 15/24960-2).

Group 1 had the highest number of birds with a clinical score 1 (5/12) and also in both other groups there were some birds which were slightly weak with dropped wings and depression. In group 2 (isolate 15/25396-3 right) 10/12 had a score 0 but only two other birds had the clinical score 1.

Figure 4 is an illustration for score 2 in comparison to a healthy bird which had no clinical signs. In the second and last week there were no birds with score 2 anymore. In week 2 especially birds from group 1 (3/8) showed a score 1 and in the last week only one bird from group 2 had a score 1. No clinical signs were seen in the negative control group (group 4) for the whole time.

Figure 4: One chicken with a score 2 in comparison to a clinical healthy chicken (left bird)

Table 7: Clinical signs scored in birds after infection with *E. coli*

Isolate	Clinical score			
	Score 0	Score 1	Score 2	Score 3
	Week 1 post infection			
15/19103-3	6 /12	5/12	1/12	0/12
15/25396-3 right	10/12	2/12	0/12	0/12
15/24960-2	8/12	3/12	1/12	0/12
no	12/12	0/12	0/12	0/12
	Week 2 post infection			
15/19103-3	5/8	3/8	0/8	0/8
15/25396-3 right	7/8	1/8	0/8	0/8
15/24960-2	6/8	2/8	0/8	0/8
no	8/8	0/8	0/8	0/8
	Week 3 post infection			
15/19103-3	4/4	0/4	0/4	0/4
15/25396-3 right	3/4	1/4	0/4	0/4
15/24960-2	4/4	0/4	0/4	0/4
no	4/4	0/4	0/4	0/4

3.2.2 Macroscopic lesions in animals

In lungs, seven birds from groups 1 and 2 had a score 1 and two birds had a score 2 (Table 8). In group 3 there were five chickens with score 1 and three chickens with score 2.

In the air sac a certain variation was noticed between the groups. Especially in group 1 two birds showed score 2 and two birds had a score of 3. In the other groups most of the chickens had slight opaque and/or thickened membranes (score 1). Only one chicken in group 3 showed organ lesion score of 3 (severe airsaculitis).

Five birds in groups 1 and 3 and six in group 2 had opacity with lack of transparency on the hearts and only in the first and third group some birds had thickened pericardium with marked pericarditis.

In each group, 2-4 birds per killing time point had a pathological score of 1 in the liver. Marked perihepatitis was observed in one bird each from groups 1 and 3 (score 2). No pathological lesions were observed in the negative control group.

Table 8: Macroscopic lesion score in birds after infection with *E. coli*

Group	Lungs			Air sac				Heart			Liver	
	LS 0	LS 1	LS 2	LS 0	LS 1	LS 2	LS 3	LS 0	LS 1	LS 2	LS 0	LS 1	LS 2
1	3	7	2	4	4	2	2	4	5	3	9	2	1
2	3	7	2	6	6	0	0	6	6	0	9	3	0
3	4	5	3	4	7	0	1	5	5	2	7	4	1
4	0	0	0	0	0	0	0	0	0	0	0	0	0

3.2.3 Histopathology

The mean histological lesion scores in lung, air sac, heart and spleen are shown in Figure 5 A-D. Figure 6 A-D show histological lesion score 2 in the different target organs.

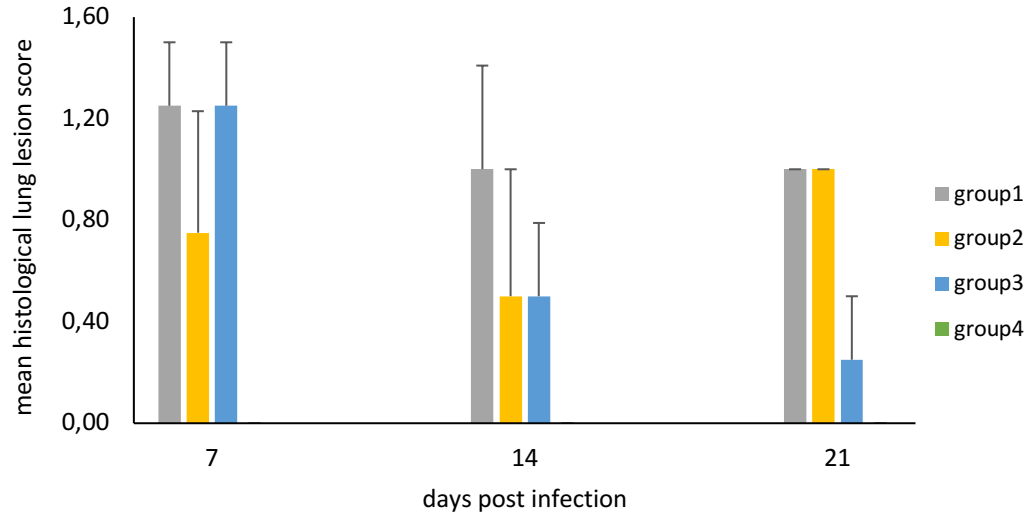
Average lesion scores in lungs were between 1-1.25. Comparing groups, the score of group 2 is below 1 (0.5-0.75) at 7 and 14 dpi but not at 21 dpi. At 7 and 14 dpi, average lesion scores in groups 1 and 3 were similar but at 21 dpi group 3 has less than in group 1.

In air sac, in group 1, the highest lesion score was noticed at 7 and 14 dpi. The lesion score in group 2 was lower (0.5) or there were no histological changes at 21 dpi. The scoring for group 3 varied between 0.5 at 14 dpi and 1.0 at 7 and 21 dpi.

The mean histological lesion scoring for the heart of the chicks was lower than in the other target organs. For instance, the lesion score was 0.25 in group 2 or there were only minimal changes (group 3).

The scoring of the spleens is also different to the other organs especially at 7 dpi. The highest lesion scores of all target organs were observed in groups 1 and 3.

A



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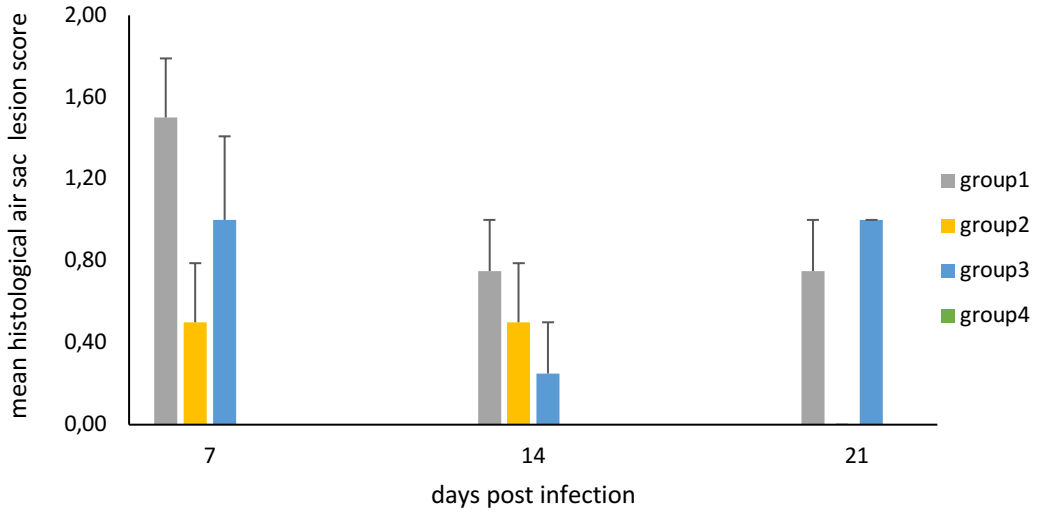

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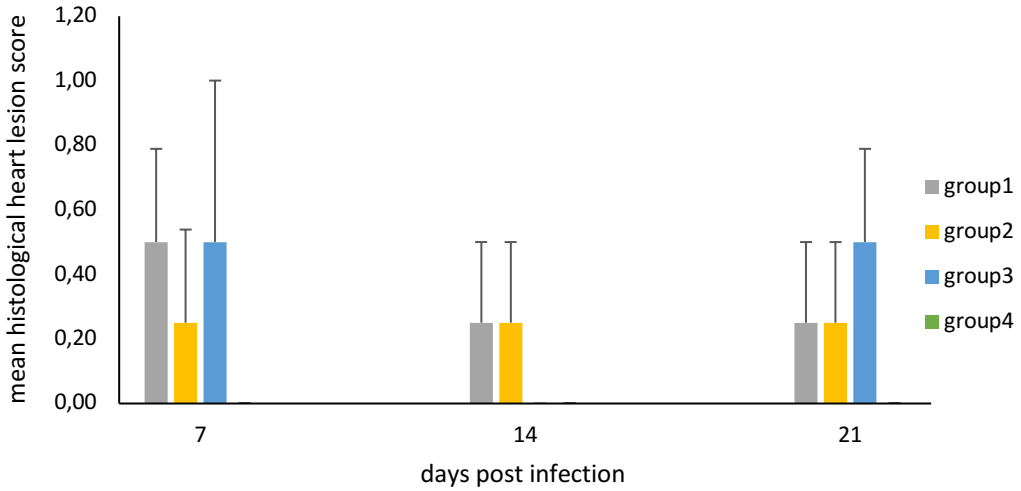

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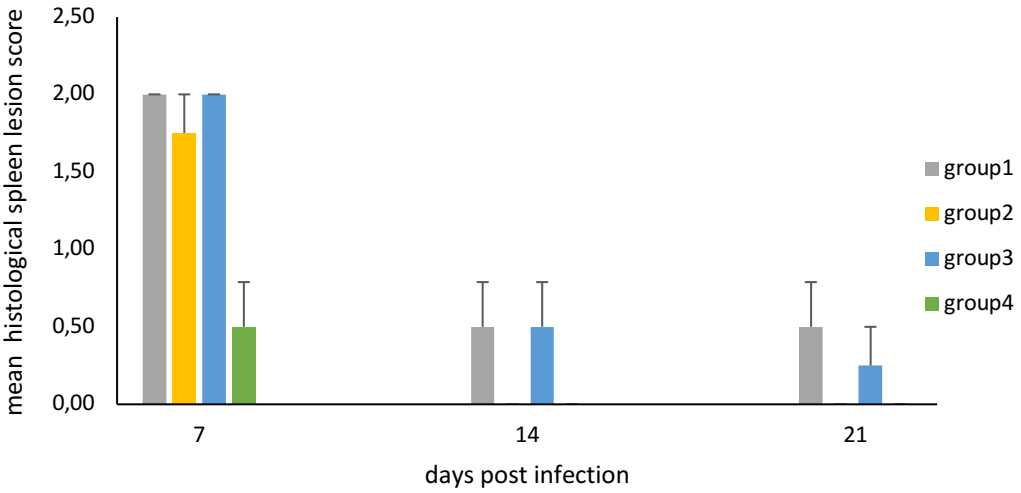

Figure 5: Mean histological lesion scores A: lung B: air sac C: heart D: spleen

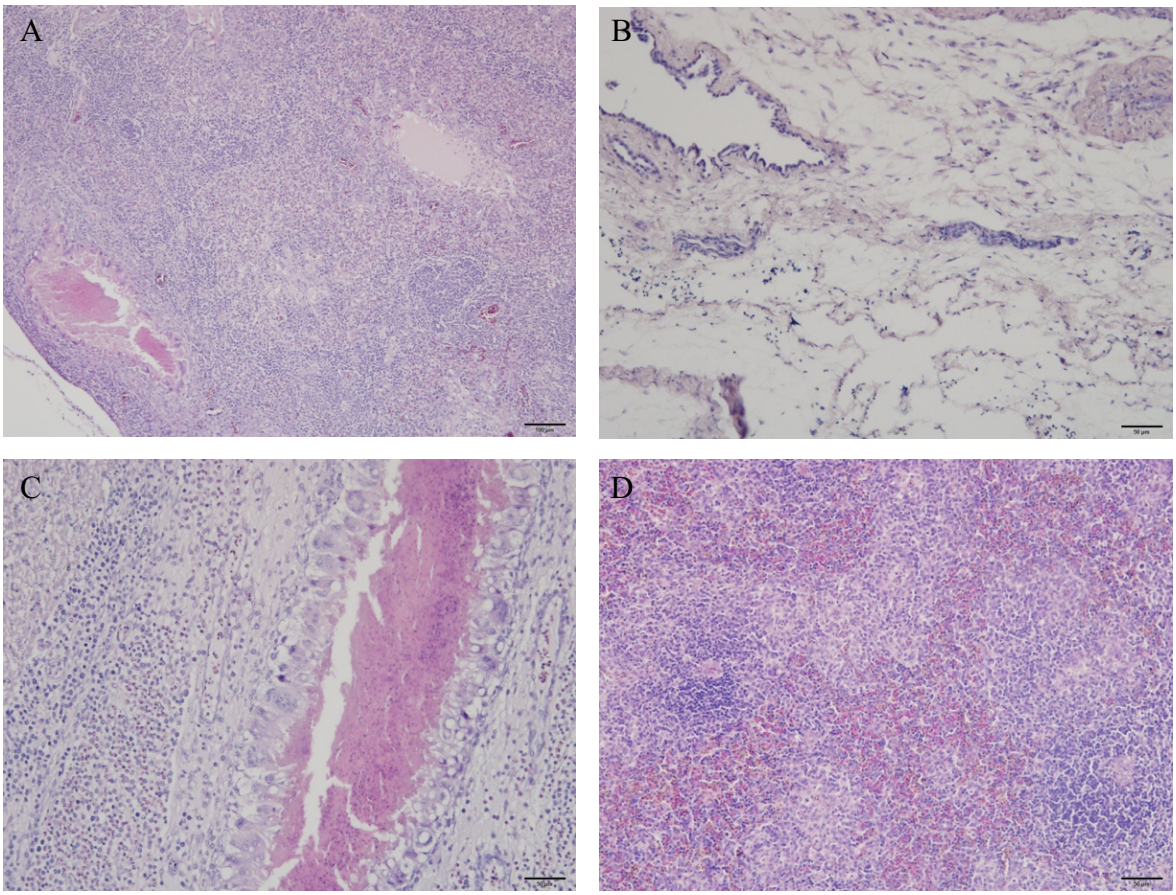

Figure 6: Histological lesion score 2 A: lung B: air sac C: heart D: spleen (in 20x objective)

3.2.4 Bacteriology

The results from direct plating of the *E. coli* isolates are shown in Table 9. The highest number of positive birds were recorded from group 1 followed by groups 2 and 3.
There were no positive bacteriological culture from birds of the negative control group.

Table 9: Number of birds positive for *E.coli* isolates in direct plating

Group	Lung	Air sac	Heart	Liver	Spleen
1	3[2]	4	2	2	3
2	2	4	1	1	1
3	1	1	2	1	1
4	0	0	0	0	0

[2] total number of birds positive for *E. coli* in direct plating

4. Discussion

Infections with *E. coli* in hatching eggs are often reported from hatcheries and are also used in experimental trials. The biggest problem can be due to a contamination in the hatcheries or the transmission of infected embryos from the parent chickens resulting into dead embryos, sick chickens or a lower hatchability. Virulence of *E. coli* isolates are often tested in embryos. An important principle for embryo lethality assays is the mortality pattern, which might show that different isolates of bacteria might differ in embryo mortality (Rezaee et al., 2020; Siegmann & Niemann, 2012).

In the present study, the pathogenicity of isolates was determined in two separate experiments i) in embryos and ii) subsequently in young chicks. The Kaplan-Meier survival test in embryos showed the great differences in the survival rates of embryos after infection with *E. coli* isolates. This is an interesting observation as all the isolates were collected from femurs of broilers and can be considered as systemic isolates. The mortality pattern in embryos did not correlated with the severity of lesions found in birds. For instance, it was observed that some isolates like 15/25396-3 right or 15/19103-3 were able to kill all embryos. On the other side strains 15/19580-2 or 16/00910-3 were less pathogenic although all the embryos were infected as demonstrated by the direct plating on MacConkey agar. The mortality ranged between 33 % to 100 %. This was the first sign for determining of the difference between the pathogenicity and also shows the differences in the virulence potential of 15 selected *E. coli* isolates. But, remarkably, it was observed that three isolates with the highest mortality rate in embryos differed in their pathogenicity in chicks evaluated on the basis of macroscopic and microscopic lesions, clinical signs as well as pattern of bacterial distribution in organs. This revealed that there can be a discrepancy between the pathogenicity of the *E. coli* strains in embryos and in young chicks.

Important for the direct plating of the bacterial isolates was that *E. coli* was found in every target organ. This also revealed the possibility of *E. coli* to colonize different tissues in diverse target organs of young chicks. Most of the positive cases in direct plating for the *E. coli* isolates were found in the air sac of the birds. This could be due to aerosol infection but also supports that the respiratory route is an optimum route of infection to induce colibacillosis as demonstrated earlier (Paudel et al., 2021). A typical way for the normal infection with *E. coli*

in the field can be through the ventilators or the air conditioners in poultry farms (Siegmann & Niemann, 2012).

All the isolates included in the present study were *in vitro* characterized especially for the presence of VAGs and for antibiotic resistance (Gaußmann et al., 2018). These isolates were characterized as O78 serogroup. The isolate 15/19103-3 that showed the highest pathogenic potential in embryos and in chickens had in total 5 VAGs, which was two more than others selected for the *in vivo* study. This isolate was sensitive only against the four antibiotics.

In contrast, 15/24960-2 and 15/25396-3 were characterized with three different virulence-associated genes and were sensitive for most of the antibiotics with the exclusion of the antibiotic nalidixic acid. A few 15/25396-3 strains are intermediate for some antibiotics like ciprofloxacin or cefotaxime. However, there was no direct correlation between the pathogenicity of the other strains and the antibiotic sensitivity. Some strains, like 15/19103-3, showed high pathogenicity and a low antibiotic sensitivity but isolates, like 16/02376-3 or 15/22748-1, were with a lower pathogenicity and a varied antibiotic sensitivity.

There was a difference in the pathogenicity in embryos and in young chicks. For example, the isolate 15/19103-3 revealed a high number of VAGs in combination with a 100 % mortality in embryos and high lesions scores in young laying chicks. In contrary, isolates 16/02376-3 and 15/22748-1 showed a mortality of 50 % respectively 67 % harbouring the same number of VAGs like 15/19103-3.

The important difference could rely on the combinations of the virulence-associated genes and not on the numbers of the different VAGs per se. Such differences might well be the determining factor for varying pathogenicity in embryos and chickens which was not the scope of the study. Thus, it needs to be investigated in future studies whether the pattern of antibiotic resistance is associated with the higher pathogenicity of isolates in birds.

Differences in virulence potential of *E. coli* isolates in embryos and chickens showed that it is essential to perform *in vivo* studies in target animals to confirm the pathogenicity of *E. coli*. It would also be worth to perform similar studies in adult chickens as well as to investigate if isolates from broilers can cause colibacillosis in layers.

Multiresistance in *E. coli* isolates from poultry is a growing concern in recent years (Bartel, 2020). It might be interesting to note that the 15/19103-3 isolate used in the present study was the most pathogenic but with multi-resistance. This might lead to limited treatment options in

affected flocks. Vaccination could be an alternative against colibacillosis (Kathayat et al., 2021) but limited vaccine candidates are available on the market.

In conclusion, it was shown that the pathogenicity of the *Escherichia coli* in chicken embryos and in young chickens differ.

The embryo lethality assay demonstrated great differences between the examined isolates although all the isolates were obtained from a systemic organ – the femur - of broilers. Further, the animal trial revealed differences in the macroscopic and microscopic lesions induced by the three selected isolates, without correlating with embryo lethality assay.

5. Summary

Escherichia coli is the causative agent of colibacillosis, which is known as one of the major infectious diseases in poultry worldwide. For the determining pathogenicity of *E. coli* isolates embryo lethality essay (ELA) and an animal trial was performed. In the ELA, groups of twelve-day-old embryos were infected with 15 different isolates of avian pathogenic *E.coli* via the allantoic sac and were investigate for the differences in mortality of the strains for 4 dpi. It was observed that isolates greatly differ in terms of pathogenicity showing embryo mortality from 33% to 100%. Based on the results of the ELA, three O78:K80 isolates with the highest mortality rate were used for infection of young laying chicks. The results showed great differences between the used isolates in the ELA and also for the three selected strains in the animal trial. The isolates differ in pathogenicity for the embryos and in macroscopic and microscopic lesions in the young laying chickens. The isolate with the highest number of virulence-associated genes was the most pathogenic in chickens but this correlation could not be justified in ELA which indicated certain discrepancy in pathogenicity between embryos and chickens. There was no correlation between the resistance against certain antibiotics and the pathogenicity of the three selected strains.

6. Zusammenfassung

Escherichia coli ist der Erreger der Kolibazillose, die weltweit als eine der wichtigsten Infektionskrankheiten bei Geflügel gilt. Zur Bestimmung der Pathogenität von *E.coli* Isolaten wurde ein Embryo-Lethalitäts-Test und ein Tierversuch durchgeführt. Im Embryo-Lethalitäts-Test wurden Gruppen von zwölf Tage alten Embryonen mit 15 verschiedenen Isolaten von APEC über den Allantoissack infiziert und für 4 Tage auf den Unterschied in der Mortalität der Stämme untersucht. Es wurde beobachtet, dass sich die Isolate hinsichtlich der Pathogenität stark unterscheiden und eine Mortalität bei den Embryonen von 33 % bis 100 % zeigen. Basierend auf den Ergebnissen des ELA wurden drei O78:K80 Isolate mit der höchsten Sterblichkeitsrate für die Infektion junger Legeküken verwendet. Die Ergebnisse zeigten große Unterschiede zwischen den eingesetzten Isolaten im ELA und auch für die drei ausgewählten Stämme im Tierversuch. Die Isolate unterscheiden sich in der Pathogenität für die Embryonen und in den makroskopischen und in den mikroskopischen Läsionen bei den jungen Legehennen. Das Isolat mit der höchsten Anzahl an virulenz-assoziierten Genen war bei den Hühnern am stärksten pathogen, aber diese Korrelation konnte beim ELA nicht gefunden werden, was auf eine gewisse Diskrepanz in der Pathogenität zwischen Embryonen und Hühnern hinweist. Es gab keine Korrelation zwischen der Resistenz gegen bestimmte Antibiotika und der Pathogenität der drei ausgewählten Stämme.

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8. Abbreviation

APEC: Avian pathogenic *E. coli*

CFU: Colony-forming unit

dpi: Days post infection

E. coli: *Escherichia coli*

ELA: Embryo lethality assay

PBS: Phosphate buffered saline

SPF: Specific pathogen free

VAG: Virulence-associated gene

9. List of tables

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