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Escherichia coli: an infectious or a factorial pathogen?

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Esherichia coli **(**rus.; **кишечная палочка [kishechnaya palochka]- "intestinal rod")** – is a well-known and widely studied ubiquitous Gram-negative, facultative anaerobic, non-sporulating rod-shaped bacterium, isolated it 1885 by a German-Austrian pediatrician from the feces of healthy individuals and originally named *Bacterium (bacillus) coli communis*.

As natural inhabitants of the lower intestine of warm-blooded organisms, most of more than 700 known E.coli serotypes behave like harmless commensals, but some (EHEC, ETEC, EIEC, EaggEC, EPEC and DAEC) can cause serious illness in humans (e.g. O104:H4, O121, O104:H21, O157:H7 etc.) including bloody diarrhea, stomach cramps, vomiting and occasionally fever. The bacteria can also cause pneumonia, neonatal meningitis, hemolytic-uremic syndrome, peritonitis, mastitis, septicemia, and there is evidence of their involvement in cancer. Strains of *E. Coli* that cause disease outside the intestinal tract of any species share common characteristics and are called Extraintestinal pathogenic *E. Coli* (ExPEC). Research into ExPEC has shown that its potential transmission from food animal sources is likely to be implicated in human infections and that chickens, together with beef and pork, are a major reservoir. Nowadays the most dangerous are so called ESBL E.coli, which produce an enzyme called extended-spectrum beta lactamase (ESBL). The Infectious Diseases Society of America (IDSA) included ESBL E.coli into the group of multi-drug resistant "ESKAPE bacteria" ("Bad Bugs–No Drugs"), which includes Enterococcus faecium (VRE), Staphylococcus aureus (MRSA), Klebsiella pneumoniae (ESBL), Acinetobacter baumanii, Pseudomonas aeruginosa, and Enterobacter species.

The occurrence of ESBL *E. Coli* in chicken meat increases worldwide. According to the Technical University of Denmark (2012) in Danish chicken meat it increased from 8.6% in 2010 to 44% 2011 while the occurrence in imported chicken meat was 50% in 2010 and 48% in 2011. Colibacillosis was first described in chickens in 1894. Since then, there have been numerous reports on colibacillosis in poultry and considerable research on the disease has been completed. Many investigators doubt that *E. Coli* is a primary pathogen. Others are convinced that certain serotypes are primary pathogens and their opinion seems to prevail. When in mammals it is mostly a primary enteric or urinary tract disease, colibacillosis in poultry is typically a localized or systemic infection occurring secondarily when host defenses have been impaired or overwhelmed by virulent *E. Coli* strains - avian pathogenic Escherichia coli (APEC). Most APEC are ExPEC and share characteristics with mammalian ExPEC. The O (somatic) antigen serotypes most commonly associated with disease outbreaks in poultry are O1, O2, O35, and O78. The K (capsular) antigens most commonly associated with virulence are K1, K80, K88, K99. In the intestinal tract of normal poultry, nonpathogenic serotypes far outnumber pathogenic serotypes, with 10-15% of intestinal coliforms being potential pathogens.

Birds are continuously exposed to the bacteria through contaminated feces, water, dust, and environment. Poultry colibacillosis has many "faces": colisepticemia, coligranulomatosis (Hjarre's disease), omphalitis and yolk sac infection, aerosacculitis (chronic respiratory disease, CRD), swollen-head syndrome (SHS), panophthalmitis, conjunctivitis, pericarditis, pneumonia, perihepatitis, splenitis, salpingitis, "egg peritonitis" (in layers, breeders), cellulitis, osteomyelitis/artritis/tenosynovitis, femoral head necrosis (FHN), food pad dermatitis (FPD), enteritis etc. However, lesions alone do not allow concluding about *E. Coli* infection, because other opportunistic bacteria (Aerobacter, Proteus, Klebsiella, Pseudomonas, Salmonella, Bacillus, Staphylococcus, enteric Streptococcus, Clostridia, ORT, MG, MS, MM, Pasteurella, Bordetella, etc.) can behave similarly to *E. Coli*, as secondary infections.

Among the conditions, predisposing to the development of *E.Coli* infection in poultry there are numerous external and internal factors, such as:

i. **Bad biosecurity and poor hygiene:** Old, multiage farms, improper house cleaning and disinfection, poor control of vectors (rodents, synanthropic birds, insects), litter quality etc.

ii. **Incubation:** Dirty, cracked, exploded eggs, overheated/dehydrated underdeveloped day old chickens with open navels-an easy target for any enterobacteria and other pathogens from the surrounding.

iii. **Management disturbances:** High stocking density, low feeding/drinking space, high growth speed, weighing, selection, beak trimming, fasting, thirst, onset of lay, catching, transportation, spiking, trauma etc.

iv. **Psychological:** Hierarchy, competition, fear, aggression, feather pecking, cannibalism etc.

v. **Environmental:** Physical (temperature, humidity, draught, dust, light, noise) and chemical (CO_2, NH_3, CO).

vi. **Nutrition:** Raw materials/feed quality and contamination, starvation, overfeeding, nutrients/minerals/vitamins excess/deficiencies, poor feed structure, weed seeds, (myco)toxins, poisoning, etc.

vii. **Water quality:** High bacterial count, high pH, low ORP, biofilm, Iron, pesticides etc.

viii. **Subclinical intestinal/respiratory infections/invasions:** coccidiosis, disbacteriosis, helminthes, MG/MS/MM, ORT etc.

ix. **Vaccine reactions:** some strains of IB, ND, ILT, TRT viruses can produce tissue reactions of the respiratory organs and mucous membranes, which may develop into a chronic respiratory disease. Live vaccines may have even more generalized detrimental effects depending on the flock's health status, the vaccine strain, the application method (rolling infections), interactions with other vaccines, environmental stress and combinations of these points.

x. **Immunosuppressive field/circulating viruses:** Infectious Bursal Disease Virus (IBDV) Marek's Disease (MD), Chicken Anemia Virus (CAV), turkey Hemorrhagic Enteritis Virus (HEV) – by damaging lymphocytes or their precursor cells, they can trigger chronic respiratory disease.

xi. **Respiratory field/circulating viruses:** Low pathogenic Avian Influenza (LPAI), Newcastle Disease (NCD), Infectious Laringotracheitis (ILT), Infectious Bronchitis Virus (IBV), Avian Metapneumovirus (Turkey Rinotrecheitits) – by damaging the respiratory tract cilia they open a passage for numerous bacteria.

xii. **Unjustified usage of broad-spectrum antibiotics (cephalosporins, fluoroquinolones etc.):** without having a preliminary sensitivity test done; incorrect doses and duration of antibiotic administration (h/day, consecutive days); combination of several BSA on one farm.

xiii. **Hormonal responses to stress:** Corticosterone is the main hormone associated with stress in chickens. Its concentration in plasma rises under stressful conditions. Changes in corticosterone have secondary effects on other hormone systems, such as the conversion of noradrenaline into adrenaline or the production of thyroid hormones. Elevated corticosterone levels in response to a chronic stress, irrespective to its nature, transform major metabolic processes of the organism into a catabolic rout with an irreversible effect, ending up with a severe immunosuppression, thus opening the gates for any secondary bacterial infection via intestinal (due to reduction of mucus production by goblet cells, damage to the villi, enterocytes, MALT/GALT elements, and tight junctions functionality) and/or respiratory tract.

E. Coli status in Russian poultry industry (2005-2013)

Despite the tremendous increase in poultry meat production in Russian Federation during 2000–2012 (~500%) the average broiler performance results are rather moderate: DWG 50g, FCR 1,83, mortality 7% at 40days, which roughly corresponds to the genetic potential of the main broiler breeds (Ross, Cobb, Hubbard) in 2003. At the same time the Min-Max spread of the results is very big: the EPEF (European Production Efficiency Factor) varies from 230 to 390 points, sometimes even on the farms of the same integrator. The age/size of the farms, the quality/cost of the constructions/equipment/insulation, cleaning/disinfection procedures, the farm management/operation style as well as the personnel's experience/attitude to the work determine the final results. Even some old but very well managed production units can show outstanding performance (close to EPEF 400), whereas the other new farms can hardly reach 2/3 of those results. According to the official statistics[1] the dominating infectious poultry pathology in Russia has bacterial origin with Colibacteriosis on the top of the list (48.5%). Poultry manure composition confirms the prevalence of *E. Coli*.[2] A similar proportion was found on the surface of broiler carcasses.[1]

The most epidemiologically important HUS-associated E.coli serotype isolated from chicken in Russia is EHEC O157:H7. During the period of 2005-2013 we have accomplished 462 technical visits to 73 broiler farms in Russia (including the old and newly built) , with a complete analysis of all production chain parameters from broiler breeders rearing, hatching eggs production and incubation - to broilers slaughter, with instrumental assessment of microclimate parameters through all growing phases (T, RH, CO/CO_2, NH_3 air flow visualization), performed 329 autopsies on more than 10 000 birds, followed by serological, bacteriological examinations (when necessary); performed more than 2500 laboratory tests of raw materials and feed samples. The preliminary analysis of the work results demonstrated prevailing presence of E.coli as local and systemic infections in 48% of the cases, leg problems–22%, metabolic and enteric disorders–14% each.[3,4]

Conclusion

Such a high level of respiratory infections with the presence of *E. Coli* (omphalitis, CRD aerosacculitis, SHS (ART), coliperitonitis, pericarditis, perihepatitis) as well as leg problems (FPD, varus/valgus, rickets, TD, FHN, spondylolisthesis, arthritis, tenosinovitis) on Russian broiler farms can be explained by a combination of several major factors: the farm size (250.000-1.8mn broilers) combined with the negative influence of suboptimal environmental conditions (heat, cold, high NH_3, CO_2, draught, dust, high litter humidity) throughout the year (with higher incidence in transitional periods - spring, autumn), often poor DOC quality compromised by overheating, dehydration during incubation, generally low level of biosecurity (multiage, "rolling" infections), often application of "hot" immunosuppressive vaccines (IBD), wrong vaccination timing and technique (too fine spray IBV, TRT) and unreasonable usage of broad-spectrum antibiotics, sometimes without sensitivity test, wrong doses and duration. We frequently registered overstocking (up to 25 birds (60kg meat)/m^2), foot pad lesions and digestive problems (disbacteriosis, necrotic enteritis, subclinical coccidiosis) especially on the old integrated complexes, combining for decades all the production chain elements (breeders, incubation, feed production, slaughter house) on one site or in an immediate vicinity. The culling in some the flocks with non-starters, MAS-chickens, femur head necrosis could reach 10-15% with a total loss up to 20-25%. Typical for such farms was poor flock uniformity from the first week and high carcass condemnation in the slaughterhouse.

a. ***E. coli* control:** For treatment narrow-spectrum **a**ntibiotics should be applied based on proper sensitivity test, administered with the right dose and duration. Air fumigation with Iodine monochloride is very popular and effective against CRD in Russia.

b. Prevention: Assumes strict biosecurity rules: hatchery, barns, vehicles, equipment thorough disinfection, hatching eggs quality control (dirty, cracked, exploded), incubation process control (egg shell temperature, "hatch window), water, feed sanitation (disinfectants, organic acids, pelletizing), monitoring and prevention of immunosuppressive viral infections, mycoplasma, salmonella, mycotoxins, stocking density control, environmental stress management, competitive exclusion (pre/probiotics), immunostimulants. The existing commercial (killed) vaccines have not gained popularity. Some farms tried autovaccines based on local APEC serotypes.

c. There are a few vaccines available in Russia: killed associated AVIVAC «Salmo-Coli-Pastovac» (*E. Coli*+*S. Enteritidis* C-5-A-

T+*P. multocida*) VNIVIP, killed (Stavropol), killed (VNIVIP, St. Petersburg, experimental), killed associated *E. coli* + Pasteurella (VNIVIP), killed for ducks O78, Live E.coli «Б-5» (Saratov, experimental).

d. Targets for future: The major improvements can be achieved by changing the old farms to "all in – all out" system, better understanding and controlling the environmental stress factors, using reliable laboratory diagnostics, antibiotics sensitivity test, more selective antibiotics application focused on narrow-spectrum drugs, application of new generation vaccines against APEC/ESBL E.coli.

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Conflict of interest

Author declares that there is no conflict of interest.

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