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E. COLI IN POULTRY PRODUCTION:

LABORATORY DIAGNOSTIC OF AVIAN PATHOGENIC STRAINS (APEC)

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Escherichia coli is probably the most famous bacteria in broiler production …. Along with Mycoplasma, it is responsible for many economical losses (meat produced of antibiotics spent to fight against). Colibacillosis is indeed the most frequently reported disease in surveys of poultry diseases or condemnations at processing. Yogaratnam in 1995, for instance, showed that 43% of broiler carcasses condemned for disease at processing had lesions consistent with colisepticemia.
Moreover, *E. coli* infection can have a significance for public health. First, even though most of APEC isolated from poultry are pathogenic only for birds, chickens are susceptible to colonisation with *E. coli* O157:H7 (an important Shigatoxin-producing, enterohemorragic pathogen of human). And characteristics of virulent *E. coli* in birds and other "animals" are shared, so that some studies are already suggesting that avian strains can be a source of genes and plasmids that encode for antimicrobial resistance and virulence factors.
The disease itself in chicken may occur as colisepticemia, which typically leads to death. Even though some birds can fully recover from colisepticemia, some others may recover with sequelae. Colibacillosis may also be a localized infection, manifesting as omphalitis, yolk sac infection, cellulites, swollen head syndrome, enteritis, acute vaginitis, salpingitis, or peritonitis.

It is interesting to stress that 10-15% of the E.coli normally present in the chicken intestine are potentially pathogenic strain. For *E. coli* infections to become apparent, adverse environmental factors or other infectious agents are nevertheless usually required : in farms, the stress of aggressive Newcastle vaccine strains, the Drinking Water vaccination itself or immunosuppressive disease management (like IBD) are the most common factors triggering secondary infections like colibacillosis. This issue of HEO will thus discuss about laboratory diagnostic of this important disease for poultry producers ….

COMMON CHARACTERISTICS OF *E. COLI* STRAINS AND BASIC BACTERIOLOGIC IDENTIFICATION

Belonging to the family of Enterobacteriaceae, *E. coli* is a coccobacillus GRAM negative (2-3 x 0.6 µm), non-spore-forming and able to grow in aerobic and non-aerobic condition. It can have a capsule. Most strains are motile and have peritrichous flagella. The basic metabolic characteristics of this enterobacteria are to be catalase + and oxydase –, as well as to ferment glucose and reduce nitrate into nitrites. The genus *E. coli* is closely related to the genus Shigella.

First of all, **a good sampling** is necessary to avoid confusing results:
Only internal organs or blood, not feces or intestine, are useful samples. Because normal intestinal flora *E. coli* readily invade other tissues after death, specimens from fresh carcasses are necessary. When acute colisepticemia is suspected, heart blood and liver should be sampled aseptically. One mL of blood collected by needle and serynge can be used to inoculate broth media (1:10), which is used to streak agar plates.

Sterile culture swabs or inoculation loops can be stabbed into:

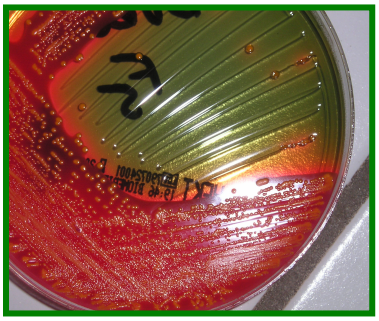
- the liver parenchyma after searing the capsule with a flamed scalpel or spatula
- or into the pericardial sac, air sac, joints when fibrinopurulent lesions suggest subacute colibacillosis. Nevertheless, lesions present more than 1 week are often sterile.
- or into the bone marrow when postmortem changes are obvious, suggesting dissemination of intestinal *E. coli* in liver and others.

Even if *E. coli* isolates can survive well on sealed agar slants for **storage and shipping**, it is adviced for long-term storage to mix broth culture with sterile glycerol 1:1 (-20° to -60°C).

This bacteria is very **easy to grow on ordinary nutrient** media at temperatures of 18 to 44°C or even lower.

The selective media can be used for identification:

- Tergitol gelose (yellow colonies)
- EMB medium (dark colonies, metallic aspect)
- Mac Conkey gelose (pink colonies). The sorbitol MacConkey agar is useful for distinguishing *E. coli* O157H7 from other *E. coli* because it does not ferment sorbitol.
- Drigalzski, Hektoen (cf. picture) …



The optimal condition for growth is 37°C, and at this temperature, the generation time being 20 min, it is possible to isolate *E. coli* colonies after 24H. For information, the generation time and number of organisms during a specific time period are related to temperature in accordance with the following table.

TEMPERATURE	GENERATION TIME	Number of *E. coli* in 24 hours
0	20 hours	2
4.4	6 hours	8
10	3 hours	128
15.6	2 hours	2048
21.1	1 hour	8388608
26.7	45 min	3435973800
32.2	30 min	24073749000000
37.8	20 min	236118320000000000000

Regarding the **identification of the colonies** grown, the differential characteristics (commonly used by API 20 E system or automatic identification machines) are the following:

- Indole production
- β -galactosidase often present
- NEGATIVE for H2S (different from Salmonella), Citrate, Urease (different from Klebsiella)

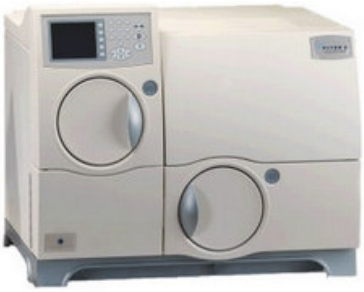
A table is placed as appendix with more details on diagnostic characteristics of *E. coli*.

Once a culture is established, and the identification is clear, there are two possible ways to know the **antibio-sensitivity of the strain isolated (antibiogram):**

- a quantitative way based on **dilution**: a dilution series of antibiotics is established (this is a series of reaction vials with progressively lower concentrations of antibiotic substance). The last vial in which no bacteria grow contains the antibiotic at the Minimal Inhibiting Concentration.

- a semi-quantitative way based on **diffusion** (Kirby-Bauer method); small discs containing different antibiotics, or impregnated paper discs, are dropped in different zones of the culture on an agar plate, which is a nutrient-rich environment in which bacteria can grow. The antibiotic will diffuse in the area surrounding each tablet, and a disc of bacterial lysis will become visible. Since the concentration of the antibiotic was the highest at the centre, and the lowest at the edge of this zone, the diameter is suggestive for the Minimum Inhibitory Concentration, or MIC, (conversion of the diameter in millimeter to the MIC, in µg/ml, is based on known linear regression curves).

Nowadays, quicker and more practical methods are available on the market. Automatic machines (same day identification and antibiogram) and E-test (*cf. pictures – Biomerieux / AB Biodisk*) are affordable for routine analysis. Nevertheless, the cost-benefit of these methods is depending on the volume and frequency of analysis to be done.



DIVERSITY OF *E. COLI* STRAINS AND CHARACTERIZATION OF THE STRAINS ISOLATED

Escherichia coli is an heterogen bacterial species, not only by its genome size: 4.6 to 5.3 Mpb but as well by its surface antigens and, even more important, its pathogenicity. It is thus possible to have a more precise identification of the strains isolated, based mainly on the surface antigens and virulence factors. This can be of interest to know the origin of the infection and to get epidemiologic data to analyse in the poultry industry.

Diversity of Surface Antigens

We can distinguish mainly 5 groups of antigens, among which many various forms exist.

- Ag O : LPS, lipopolysaccharide – *173 different ones*
  They are polysaccharidic chains based in the outer bacterial membrane thanks to the lipid A, so called “endotoxin”. It protects the bacteria from the COMPLEMENT system (innate immunity).

- Ag K : surface polysaccharides (CAPSULAR) - *# 80 different*

  These very hydrated polysaccharides allow this bacterial genus to show a big diversity of capsules. The capsule is aimed at avoiding the immune system, and the very low immunogen ones will be the more pathogenic (like K1, K5). Specific antisera exist to serotype them.

- Ag H : flagellin (protein) - *# 56 different* (no pathogenic effect).

Ag F : Fimbriae or Pili (proteins) - *# 17 different*

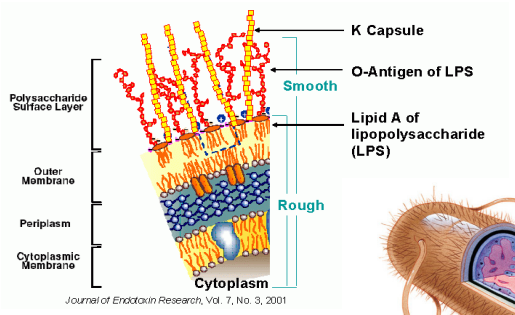
Their expression is linked with the growth medium, and they are involved in the attachment to cells. Many tests have been developed for detecting them, especially for human (F1,F2,F3), swine (F4) and calves (F5). They can be classified in mannose sensitive/resistant depending on inhibition of their agglutination.

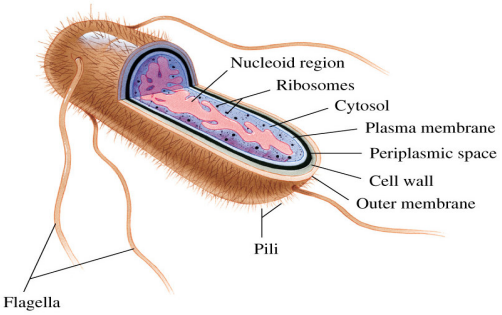
OMP : Outer Membran Proteins (canals)

Currently, the classification of Kaufmann (1944) based on surface antigens is still used, following the model:

Ox : Ky : Hz

Since there are some preferential association between O, K and H, the total number of isolated serotypes is much less than the total number of possible combinations.





Diversity in Virulence

There is a big heterogeneity in the virulence shown, according to the strains. There are purely apathogenic E.coli (e.g. K12) and … on the other hand SEVERAL PATHOTYPES.

One pathotype is a collection of strains showing:

- a particular profile of virulence
- associated to a particular kind of infection
- and very often with host-specificity

As a reminder, the following table is showing the two big groups of pathotypes, where the pathotype APEC (Avian Pathogenic Escherichia Coli) is figured.

INTESTINAL		EXTRA-INTESTINAL	
ETEC	Entero-Toxinogen	**UPEC**	Urinary tractus infection
EPEC	Entero-Pathogen	**MENEC**	Meningitis
EIEC	Entero-Invasive	**SEPEC**	Scepticemia
EHEC (VTEC)	Entero-Hemorragic (Vero-Toxinogen)	**APEC**	**Avian colibacillosis**
EAggEC	Entero-aggregative	***(*) Remark**: Til now, no E.coli is demonstrated to be primarily pathogen for Avian Digestive Tract.*	
AIEC	Associated to Crohn disease		

There are different ways to determine the pathogenicity of a particular strain of *E. coli* isolated, among which are the following (detailed in the diagram enclosed, appendix 2):

- of course, **experimental challenge** on day-old-chicks
- **genetic** characterization
- **biotypes** thanks to API 20 E system for instance
- **serogroups** based on the surface antigens aforementioned.

➔ Certain pathotypes are associated to particular serotypes, which can help in the routine epidemiology / diagnosis.

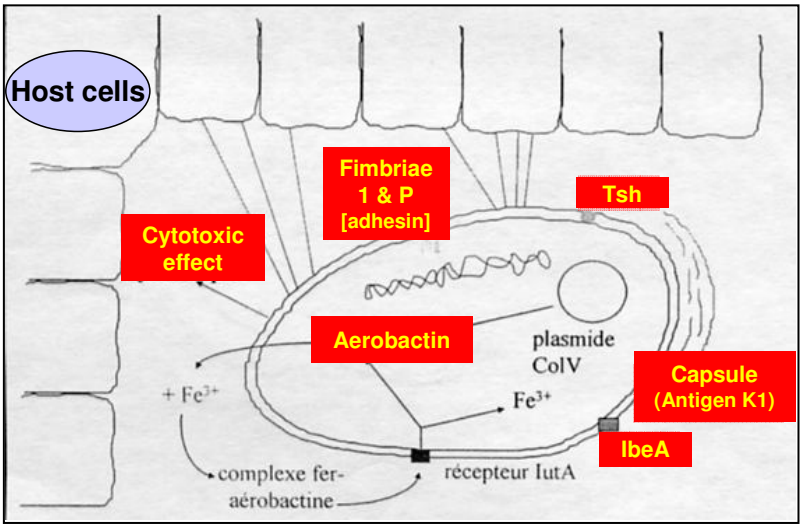
Nevertheless, new ways to explore pathogenicity of APEC strains are found in the VIRULENCE factors. Indeed, during infection, some properties are helping the bacteria to perform the following steps:

- Colonisation of the respiratory tract
- Penetration of the bacterias inside the blood stream
- Resistance to the host Immune Response
- Multiplication in the blood
- Dissemination in the internal organs
- Induction of lesions

Virulence factor	Remark
Adhesins ?	*Mostly fimbriae*
IbeA protein	*Colonization of tissues ?*
Capsule	*Inhibit Complement, low immunogenicity*
Tsh protein	
Aerobactin	*Bacterial siderophor, feeding bacteria even if low IRON concentration*
Cytotoxins ?	*Chick-Lethal-Toxin, …*

In an European project on APEC strains conducted between 1999 and 2002, on 560 strains confirmed as pathogens (lethality test), the frequency of virulence genes was the following:

F1 *(fimA, fimH)*	**90%**
P *(papC, felA, papG)*	**28%**
S/F1C	**11%**
F17	**6%**
Eae	**< 2%**
Afa	**5%**
Tsh	**55%**
Capsule K1 *(neuB, neuC)*	**27%**
Aerobactin system *(iutA)*	**80%**
Toxin CDT	**7%**
Toxin CNF	**< 2%**
Toxins SLT, ST, LT	**0 %**



Even if so far, serogrouping is the easiest and cheapest way to characterize the pathogenicity of strains isolated, some factors look like major and can help for characterizing pathogenicity of strains:

- some specialized poultry laboratory, after classical steps of isolation and serogrouping, will use monoclonal specific antiserum of IutA protein to show the presence of **AEROBACTIN** system on bacterial colony (DOT-BLOT method, *INRA, France*).
- Some applications can also be foreseen for a cost-effective and time-saving **multiplex PCR**, for diagnosis or epidemiologic purposes.

APPLICATIONS : EPIDEMIOLOGY STUDIES AND MANAGEMENT OF CONTAMINATION.

E.coli infection is a balance between the predisposing factors and the pathogenicity of the strain. Consequently, when colibacillosis is a strong concern in an area or in a company, it is interesting to:

- Take measures in order to reduce its infection, clinical expression and amplification in the sick birds, by removal of stress, adaptation of vaccination program and protect as well as possible against immunodepressive disease ;
- know the source of contamination to target as much as possible the field exposure (biosecurity, decontamination of water, egg infection and others).

Indeed, *E. coli* is a common inhabitant in the intestinal tracts of poultry at concentrations up to 10^6/g. Higher numbers are found in younger birds, birds without an established normal flora, and in the lower intestinal tract. Coliform bacteria can be found in litter and fecal matter, and dust in poultry houses may contain 10^5—10^6 *E. coli*/g. These bacteria persist for long periods, particularly under dry conditions.

Its presence in drinking water is considered indicative of fecal contamination. Among normal chickens, 10—15% of intestinal coliforms belong to potentially pathogenic serotypes. Pathogenic serotypes can indeed be introduced into poultry flocks through contaminated well water and remain in the pipes stuck to the biofilm, contaminating flock after flock the newly arrived day-old-chicks.

Egg transmission of pathogenic *E. coli* is common and can be responsible for high chick mortality. Pathogenic coliforms are more frequent in the gut of newly hatched chicks than in the eggs from which they hatched, suggesting rapid spread after hatching. The most important source of egg infection seems thus to be fecal contamination of the egg surface with subsequent penetration of the shell and membranes.

Feed is often contaminated with pathogenic coliforms, but hot pelleting processes can destroy these. Rodent droppings frequently contain pathogenic coliforms.

CONCLUSION

In order to manage the multiple variety of colibacillosis in poultry industry, a clear and organized approach should be established. The cost of resistances acquired (new antibiotic rotations,…) as well as the always increasing concern on public health are leading the industries in this way. It is commonly acknowledged that an effective control of colibacillosis depends on identifying and eliminating the predisposing causes of the disease at each stage of the broiler production:

- Breeder stage
- Hatchery
- Growing period in farms.

A good diagnostic of the epidemiologic situation is then compulsory to focus on the right targets. The reduction of the contamination by pathogenic strains is nevertheless efficient if in the same time, most of the predisposing factors are removed.

Thus, the preventive program for the other diseases should be closely observed. Every stress in the farm can enhance their expression and should be avoided. As well, the best management of immunosuppressive diseases is one major factor to reduce the incidence of this disease.

APPENDIX: *identification of E. coli.*

CHARACTERISTIC	RESULT FOR *E. COLI*
Catalase	+
Oxydase	-
Indole	+
Hydrogen sulfide (H2S)	-
Citrate (Simmons)	-
Urease	-
Glucose	+
Lactose / β-galactosidase	+
Sucrose	+ / -
Mannitol	+
Dulcitol	+ / -
Adonitol	-
Inositol	-
Reduction of nitrates (into nitrites)	+
Gelatin	-
Indole	+
Methyl red	+
Voges-Proskauer	-
KCN medium	-
Lysine decarboxylase	+
Ornithin decarboxylase	+ / -
Phenylalanine decarboxylase	-
Salicin	+ / -