ESCHERICHIA COLI INFECTIONS IN BROILERS A REVIEW THE ORGANISM, PATHOGENICITY & PATHOLOGY

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INTRODUCTION:

Escherichaia coli infections in broiler chickens include colibacillosis [1], colisepticemia [1,2,3], airsacculitis [4], swollen head syndrome [5,6], synovitis, salpingitis, peritonitis [1], yolk sac infection and omphalitis [7], scabby-hip [8] and cellulitis [9].

Colibacillosis is an infectious disease in which *E. coli* is the primary or secondary pathogen [10]. When *E.coli* is the secondary pathogen, other pathogenic agents such as mycoplasma, infectious bronchitis virus (IBV), and Newcastle disease virus (NDV) are considered the primary pathogens [11,12,13,14].

Colisepticemia is a disease characterized by bacteraemia and pericarditis which primarily affects broiler chickens between three and eight weeks of age, though cases also occur in chicks [15]. Mortality in most cases is less than 2.5% and morbidity is usually above 10%[2].

Airsacculitis is a chronic respiratory disease characterized by thickening of the air sacs with fibrinous exudate, fibrinous pericarditis and perihepatitis [16]. The disease can occur in broiler chickens at any time during their growing period, but is most common between three and five weeks of age [10].

Swollen head syndrome is a disease of broiler chickens characterized by swelling and cellulitis of the head in which *E. coli* is involved [5]. The disease affects chickens between four to six weeks of age usually with high mortality and a morbidity rate of 5 to 10% [6].

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Yolk sac infection and omphalitis are generally associated with contaminated hatching eggs and/or poor sanitation in the hatchery [17]. Fecal contamination of eggs is the most important source of infection [11]. Omphalitis occurs when the organisms reach the unhealed navel and cause a purulent inflammation [17,18]. Yolk sac infection and omphalitis affect chicks during the first week of age with a mortality of 5-10% and 10-50% of the affected birds respectively [19].

Cellulitis is a recently recognized pathological condition in broiler chickens characterized by discoloration and thickening of the skin and inflammation of the subcutaneous tissues with the formation of fibrinous plaques [9]. *Escherichia coli* is the most frequent bacterium isolated from the lesions [20,21,22]. No clinical signs are observed in affected birds and the lesions are usually detected during processing. The condition was first reported in Britain in 1984 and was based on reports from processing plants dating back to 1976 [21]. Since then, cellulitis has been reported in Germany [22], U.S.A. [23], and Canada [24,25,7,26]. The condemnation rate due to celulitis has been increasing each year and it is now 7.5 times more prevalent than it was in 1986 [16,25]. Cellulitis ranks among the top three categories of condemnation for chickens in Canada [25].

مقدمة:

الإصابة بالاشريكية القولونية في الدجاج للاحم تتضمن مرض الكولوباسلوسس (Colibaillosis) [1] والكولوبيتسيميا (Colisepticemia) [1,2,3] والايرساكيولايتس (Airsaulitis) [4] ومرض ورم الرأس (Swollen head Syndrome) [5,6] والسينوفيتس (Synovitis) والسالبنجايتس (Salpingitis) والبيريتونايتس (Peritonitis) [1] وإصابة مح البيض (Yolk sac Infection) واصابة الحبل السري [7] (Omphalitis) وقشور الحوض (Scabby-hip) [8] والسليولايتس (Cellulitis) [9].

الكولوباسلوسس هو المرض البكتيري الذي تكون فيه الاشريكية القولونية المسبب الرئيس أو الثانوي [10]. عندما تكون الأشريكية القولونية هي المسبب الثانوي تكون هنالك جراثيم أخرى مثل المايكوبلازما (Mycoplasma) وفيروس التهاب الشعب

الهوائية (Infectious Bronchitis Virus) (IBV) وفيروس النيوكاسل (NDV) (Newcastle Virus) تعتبر المسبب الرئيس [14,13,12,11].

الكوليستيميا مرض يتميز بوجود الأشريكية القولونية وسمومها في الدم وإصابة جدار القلب في الدجاج اللاحم في العمر بين ثلاثة الى ثمانية أسابيع وقد تحدث الإصابة في الكتاكيت [15] . نسبة الموت في هذا المرض أقل من 2.5% بينما نسبة الإصابة أكثر من 10% [2].

الأيرساكيولايتس مرض مزمن يتميز بتورم الأكياس الهوائية وإفراز سوائل في الأكياس واصابة الأغشية المحيطة بالقلب والكبد [16]. تحدث الإصابة في أي عمر خاصة بين الأسبوع الثالث والخامس [10].

مرض تورم الرأس يتميز بوجود تورم في الرأس وتقرحات تحت الجلد. عالميا تعزل الاشريكية القولونية من هذا المرض [5]. يحدث المرض في الدجاج اللاحم في عمر 4-6 أسابيع . تتراوح نسبة الأصابة بين 5 إلى 10%.

إصابة مح البيض والحبل السرى دائما يحدثان أثناء عملية فقس البيض [17]. تلوث البيض بالبراز عامل مهم في هذين المرضين [11]. الاشريكية القولونية ما تعزل من هذين المرضين [7]. اصابة الحبل السري تحدث بعد وصول البكتيريا إلى السرة قبل اندمالها وتسبب اصابة متقيحة [17,18]. تتراوح نسبة الموت في اصابة مح البيض بين 5 إلى 10% وفي إصابة الحبل السري بين 10 إلى 50% [19] .

مرض السليولايتس عرف حديثا ويتميز بتغير لون الجلد وتورم في موضع الإصابة ووجود التهاب تحت الجلد [9]. الاشريكية القولونية دائما تعزل من موضع الإصابة [20,21,22]. دائما يكتشف المرض أثناء الكشف في السلخانة. عرف المرض في كل من بريطانيا [21] وألمانيا [22] والولايات المتحدة [23] وكندا [7,24,25,26]. إعدام الدجاج اللاحم بسبب أصابته بهذا المرض في تصاعد مستمر [14,25].

THE ORGANISM:

Escherichia coli is a Gram-negative, non-spore-forming bacillus, that is cytochrome oxidase negative and ferments glucose. The organism may be variable in size and shape. Many strains are motile and have peririchous flagella [11]. *Escherichia coli* grows on ordinary nutrient media at temperatures of 18-44 °C or lower. On agar plates incubated for 24 hr at 27 °C colonies are low, convex, smooth and colourless. On MacConkey agar incubated for 24 hr at 37 °C colonies are pink, shiny and convex [1,20,26].

A number of schemes have been developed to characterize isolates of *E. coli* and to identify pathogenic strains including: serotyping, biotyping, colicing-typing and testing for virulence factors [27].

Several studies have used serotyping to characterize *E. coli* isolated from broiler chickens[24,26]. Serotyping is the most widely used because an international scheme has been established on the basis of O (cell wall), K (capsular), and H (flagellar) antigens [28]. The O (somatic) antigen is a somatic factor liberated on autolysis of smooth cells and implicated in virulence [1]. It is composed of a polysaccharide-phospholipid complex resistant to boiling and it is used to define the specificity of various O antigens [29]. K (capsular) antigens are polymeric acids containing 2% reducing sugars and are associated with virulence [1]. They are divided into three classes: L,A and B. L type K antigens can be removed by heating to 100 °C for one hour; however, A type K antigens require heating for two and half hours at 121 °C [29]. H(flagellar) antigens are not correlated with pathogenicity. They are proteins that are destroyed at 100 °C[11]. H (flagellar) antigens, however, are sometimes important markers of pathogenicity among the same O group. Strains of serotype O157:H7 produce a cytotoxin and are associated with hemorrhagic colitis in humans, whereas some strains of serotype O157:NM(nonmotile) or O157 (with an H antigen other than 7) do not produce the cytotoxin but produce enterotoxins and are implicated in diarrheal diseases in pigs [27].

The most common O serogroups implicated in chickens are O78, O2, O1 and less frequently O35[22,24,26,30]. Other serogroups

such as O127, O9, O14O[22],O33[30], O45 and O119[31] have been isolated from broiler chickens.

In addition to serotyping, drug resistance pattern has been used to characterize *E. coli* isolates. Barbour et al. [30] isolated *E. coli* serotypes from colibacillosis which were resistant to furazolidone-streptomycin-sulphatthiazole and streptomycin-sulphathiazole-tetracycline. Premkumar et al. [32] identified 20 islolates of *E. coli* from birds with colibacillosis which were all resistant to metronidazole and rifampicin while 95% were resistant to chlortetracycline, doxycycline, and tetracycline. Heller and Drabkin [33] noted multiple resistance to sulphonamide, streptomycin, oxytetracycline and chloramphenicol among *E.coli* serotypes isolated from infected birds. In another study, Cloud et al.[34] isolated 177 *E. coli* from 145 field-reared broiler flocks between two and eight weeks of age. The majority of the isolates were sensitive to chloramphenicol, gentamicin, nalidixic acid, trimethoprim, sulphadimethoxine, spectinomycin, neomycin and ampicillin. While about half of the isolates were sensitive to nitrofurantoin, less than 25% of the isolates were sensitive to streptomycin, tetracyclines, novobiocin, and lincomycin [34].

Biotyping is another scheme which can be used to identify and differentiate pathogenic *E. coli*. It is based on the pattern of reaction in selected biochemical tests. This scheme is not widely used for characterization of isolates of pathogenic *E. coli* because of the absence of a universal biotyping scheme [27]. Fermentation of sugars, however, is variable. In a study using biotyping to identify 20 *E. coli* isolates from poultry with colisepticemia, the sugars galactose, trehalose, xylose and melibiose were fermented by all isolates, arabinose by 95% raffinose by 85%, ducitol by 65%, ribose by 45%, adonitol by 20% and salicin by 5% [32]. Another study reported variable rates of carbohydrate fermentation and amino acid decarboxylation. Raffinose was fermented by 89.8% of the isloates, adonitol by 15.2%, sorbitol by 87.3%, sucrose by 78.2%, dulcitol by 20.3, salicin by 0%, lysine was anaerobically decarboxylated by 83.2% of the isolates, ornithine by 55.3%, and argininine by 0% [34]. *Escherichia coli* produces positive methyl red and negative

Voges-Proskauer reactions. Hydrogen sulphide is not produced on Klighers medium[1]. Barbour et al.[30] studiesd 11 *E. coli* isolates from chickens with colisepticemia for their ability to ferment nine sugars(fructose, glucose, lactose, maltose, manitol, saccharose, sorbitol, trehalose an xylose). All sugars were fermented by all *E. coli* isolates. Other positive biohemical reactions included production of indole and hyrogen sulphide, nitrate reduction, methyl red, and Voges-Proskauer reactions. There was no utilization of citrate or malonate, lysing decarboxylation or production of urease or hemolysin, and no motility observed.

Phage-typing is a scheme used to determine the susceptibility of *E. coli* isolates to a standard set of bacteriophages[27]. A major advantage of phage-typing is its ability to differentiate between strains of the same species, serotype and biotype[29]. Colicin-typing is another scheme carried out by detecting colicin(s) produced by an isolate then determining the effect of the colicin(s) on a standard set of indicator strains of *E. coli*. These two schemes are not in common use for identification of pathogenic isolates of *E. coli* [27].

PATHOGENICITY

Pathogenic and non-pathogenic serotypes of *E. coli* are common gut inhabitants of poultry. The possibility of the intestine being a reservoir of pathogenic strains of *E. coli* has been suggested [16]. Among normal chickens, 10-15% of intestinal coliforms belonged to potentially pathogenic serotypes [3]. The extent to which the chicken environment is contaminated may depend on the degree of concentration of *E. coli* per cm^2 of intestine[1,35,36]. A study conducted to investigate the degree of contamination of the chicken environment, has shown that dust in poultry houses may contain up to one million *E. coli*/gram and these bacteria persist for long periods[35].

Although the pathogenesis of *E.coli* infection is poorly understood, there is general agreement that bacteraemia is an essential element of the clinical disease [1]. A study conducted to investigate the colonizatin of *E. coli* in the intestine of turkeys, has shown that oral inoculation with *E. coli* resulted in extensive colonization of the

caecum and colon with fewer bacteria in the lungs and liver [36]. Infection of the liver is believed to be due to translocation of bacteria from the intestine. A recent study to examine the possibility of pathogenic *E. coli* invading the blood stream via the intestine, has shown that stressing of exposed turkeys and chicken results in isolation of pathogenic bacteria from the blood and spleen of turkeys and in bacteraemia and mortality in chickens [37].

The presence of pathogenic *E. coli* serotypes in the upper respiratory tract of healthy chickens has been demonstrated [3,13]. It has been suggested that colonization of the respiratory tract of healthy chickens by virulent strains may be a preliminary step for the development of *E. coli* infection. Considerable colonization of the trachea of healthty chickens following oral inoculation with *E. coli* strains has been experimentally documented [38].

It is generally thought that the natural route of *E. coli* infection is via the respiratory route following inhalation of dust contaminated with *E. coli* [39,40]. The mechanism by which *E. coli* pass from the mucosal surfaces of the respiratory tract to the systemic circulation is not well understood. However, damage to the epithelial surfaces by other infectious agents or toxic gases has been suggested as predisposing factors [40]. In chickens, bacteraemia commonly occurs following primary infection of the upper respiratory tract with subsequent extension into the lower respiratory tract [38].

An important initial step in the pathogenesis of infectious disease involving mucosal surfaces is the adherence of the organism to the mucosa [41]. Several pathogenic strains of *E. coli* isolated from severe infections of colisepticemia in chickens and turkeys posssess pili and these pili facilitate adherence of the bacteria to chick tracheal epithelial cells both *in vitro* and *in vivo* [42]. The presence of adherence pili on infecting bacteria affect both the prevalence and severity of the disease [43]. Fimbriae have been reported to be involved in adherence of pathogenic *E. coli* to the epithelial cells [44]. In a study to investigate the prevalence of adhesiveness to host cells and iron-sequestering abilities of virulent an avirulent avian *E. coli* strains, the adhesive properties were demonstrated in 64% of lethal strains and in only 23% of nonlethal strains [38]. Another

recent study, however, failed to detect the presence of adherence pili by mannose-resistant hemagglutation and adherence to Hela cells and chicken fibroblasts[45]. In order to determine the mechanism of attachment of pathogenic *E. coli* to respiratory epithelial cells of chicken, Gyimah and Panigrahy [46] used adherence-inhibition procedures and succeeded in blocking adherence by antipilus antibodies and monosaccharide, indicating that there is a role for monosaccharides in host cell receptor.

In addition to adhesion to respiratory epithelial cells, iron acquisition and production of K1 polysaccharide capsules have been proposed as potential virulence factor of avian *E. coli*. It has been experimentally documented that *E. coli* strains with virulence factors result in the most severe pathological effects measured by mortality, lesions in the air sacs, heart and liver, weight gain and reisolation of *E. coli* from internal organs, while *E. coli* devoid of virulence factors are able only to induce mild pathological effects restricted to the respiratory tract [47].

Iron is an essential element for bacterial growth, but its availability as a free ion in the body is very low, owing to strong binding by physiological carrier molecules such as transferrin. The strong affinity of aerobactin and other bacterial siderophores for iron, enables producer strains to remove iron from transferrin and to grow in the iron-limiting conditions of the internal organs and body fluids [48]. In another study investigating iron-sequestering abilities of virulent and avirulent avian *E. coli* strains, it has been found that the ability to grow in limited iron conditions is strongly correlated with lethality [38]. It has been found that most of the pathogenic *E. coli* isolated from chickens with colisepticemia produce erobactin [49,45].

A link between Colicin V (Col V) plasmids and invasive strains of *E. coli* has been established [50]. Under experimental conditions, the posssession of the plasmid markedly enhances the virulence of *E. coli* strains in comparison with plasmid free strains [45,51]. It has been noted that the availability of iron in the body of an infected host is crucial in determining the ability of an invading bacterial species to proliferate in tissues and body fluids [52]. Therefore, Col V plasmids

of bacteremic *E. coli* strains are involved in iron uptake. The possibility of an efficient plasmid-specified iron-sequestering system which would significantly enhance the virulence of *E. coli* strains by facilitating survival in conditions of iron stress has been suggested [53]. In spite of the high frequency of Col V producing strains among the pathogenic *E. coli* strains studied, Colicin V itself is not essential to virulence [54]. It is the plasmid Col V which carries genes that are responsible for the invasion and pathogenicity [50,51]. The aerobactin system comprises genes responsible for the synthesis of the hydroxamate siderophore aerobactin and for ferric aerobactin uptake [55]. This system is present and expressed in virulent avian isolates of *E. coli* and absent from most nonvirulent avian isolates [26,56]. Hence, it has been suggested that the production of aerobactin is involved in the virulence of avian septicemic *E. coli*. The importance of aerobactin-mediated iron uptake as a bacterial virulence determinant in animal infection has been indicated by the high incidence of the aerobactin system among septicemic isolates of *E. coli* an observation that strongly suggests an important role for this mechanism of iron assimilation in pathogenesis [26,49]. Serum resistance is another characteristic of pathogenic *E. coli* thought to be associated with Col V plasmids and implicated as a virulence factor [45].

It has been observed that a wild type of *E. coli* absorbed Congo red that was incorporated into the medium. This observation provided the basis to possibly distinguish invasive from noninvasive *E. coli* isolated from chickens. A direct correlation between the ability of clinical isolates of *E. coli* to bind Congo red dye and their ability to cause septicemic infection has been demonstrated in chickens[57]. These findings are also supported by the results of another experimental study which succeeded in reproducing colisepticemia in 4-6 week old chickens by inoculation of Congo red positive *E. coli* through the abdominal sac [58]. Although findings only indicate that Congo red dye was identifying virulent *E. coli* in poultry, the epidemiologic association between Congo red *E. coli* and avian colisepticemia has been established [59]. In a recent prospective cohort study designed to confirm the association

between Congo red binding *E. coli* and airsacculitis in vroilers, congo red *E. coli* have been identified as an important airsacculitis risk factor [60]. However, cultures of *E. coli* regardless of their pathogenic history, rarely produced red colonies on the Congo red medium without added bile salts [61].

PATHOLOGY

Colibacillosis is seen clincally as acute colisepticemia, as subacute fibropurulent serositis and as chronic granulomatous disease of the viscera. At necropsy, the viscera are hyperaemic and swollen, and hemorrhages are present in livers, subcutis and joints. Edema is usually present in tiussues of the respiratory tract, with ascites and inflammatory exudate in the pericardial cavity. Air sacs are generally cloudy but no exudates are seen [18,62]. Green livers, congested pectoral muscles and small white foci on the liver have been described [1]. Hiostologically, acute coliseptiemia is characterized by hyperemia, reticuloendothelial hyperplasia and small foci of necrosis in the liver, spleen and lymphoid tissues [18]. Necrosis with fibrinous exudate in the ellipsoids and lymphoid follicles of the spleen, and fibrinous thrombi in sinusoids of the liver with occasional necrosis of hepatic cells have been observed in cases of acute septicemia [13].

Subacute fibrinous serositis is described as the most common form of colibacillosis characterized by lesions of fibrinopurulent pericarditis, pleuritis, peritonitis, airsaccuitis and pneumonia. Infected air sacs are thickened and have caseous exudate on the respiratory surface. The pericardial sacs are edematous, cloudy and filled with a light yellow fibrinous exudate [1,18]. The myocardium is congested, edematous and degenerated [31]. Fibrinopurulent inflammation with granulmatous changes in the epicardium and hepatic peritoneal sacs accompanied by septicemic lesions in the spleen and liver are also common in cases of subacute serositis [13]. Histologically, edema an heterophil infiltration have been seen as the earliest changes followed by mononuclear phagocytes and giant cells around the necrotic foci [1]. Plaques of fibrin on serosal surfaces which progressively expand to become organized, purulent and necrotic, vascular and fibrinous material are common in the air sacs

[18]. Lesions of catarrhal tracheitis, multifocal pneumonia, hepatitis an splenitis with heterophil infiltrates have been noticed [63].

In chronic granulomatous infection, lungs, livers and spleens are markeldy fibrotic and contained large amounts of inspissated plasma and fibrin among the granulomatous tissue. Histologically, lesions are characterized by focal or multifocal granulomas surrounded by giant cells [18]. However, it is suspected that *E. coli* may induce transient lymphocytic depletion of the bursa of fabricius and the thymus in chickens [13,64].

Necrotic dermatitis in broiler chickens has been described in a few studies. The colour of the affected skin varies between bright yellow, dull yellow or reddish-brown. Skin is swollen at the sites of inflammation and there is a fibrinous scab which could be lifted from the skin surface in many of the affected birds. Fibrinous plaque between muscle and subcutis is the most characteristic feature of the lesion [22]. Occasionally, other organs such as heart muscles, liver tissues and kindeys are affected by infiltration with heterophil granulocytes. The alteration of the skin and subcutis shows inflammatory debris, infiltrates of heterophilic cells and lymphocytes in the cutis and subcutis, and lumps of bacteria within the epithelium of some skin sections [22]. The main features of the ulcerated lesions include congeston of dermal capillaries and hyperplasia of the epidermis at the outer edged of the lesion, and complete destruction of the normal epidermis which is replaced by an eosinophilic mass containing basophilic nuclear debris in the centre of the lesion [65].

Cellutitis as a separate identity has also been described in a few studies. It is well documented that the location of cellulitis lesion is primarily abdominal below the vent, with the tendency to be unilateral [9,24]. The skin is discoloured, thickened and there are scratches on the skin overlying the lesions [9,24,66]. On opening the skin there were varying degrees of subcutaneos edema, muscle hemorrhage, and subcutaneous exudate which can be localized or extended under the thigh over the breast and back. The size of the lesion varied betweeen 1 to 10 cm in diameter [9,24]. Histopathology of cellulitis lesion is charateried by dissecting tracts of inflammation originating at the epidermis and extending through

the dermis to the subcutis, and by ulcers and crusts on the epidermis [24]. Moderate hyperkeratosis and hyperemia of the epidermis, marked fibrous thickening of the dermis with evidence of neovascularization, and diffuse infiltration of mononuclear cells and heterophils have been observed [24]. The presence of coalescing granulomas in the subcutaneous tissues is considered a characteristic of the cellulitis lesion. These granulomas are characterized by the accumulation of fibrinocaseous exudate surrounded by a thin layer of epithelioid and multinucleated giant cells and few layers of mononuclear cells mixed with fibroblasts and collagen fibres. Numberous clumps of Gram-negative rods. There is also evidence of the involvement of feather follicles characterized by local loss of follicular epidermis and marked distortion due to the accumulation of an exudate similar to the one observed in the dermal and subcutaneous granulomas [24].

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