Deciphering the infection biology of avian pathogenic *Escherichia coli*: role of experimental infection models

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Avian pathogenic *Escherichia coli* (APEC) is a well-recognised pathotype that causes localised and systemic infections in avian species of all ages including eggs. To develop successful intervention strategies, a deep understanding of bacterial and host factors mediating crucial processes in pathogenesis is required. Such information can only be gathered through approaches that embrace infection models using the target hosts. Existing infection models for APEC include: intra-tracheal inoculation, intra-airsac inoculation, intravenous injection, intraperitoneal inoculation, intranasal inoculation, aerosol exposure, subcutaneous injection, intramuscular injection and intra-allantoic inoculation. Describing these models in detail, this chapter highlights salient information on how each of these infection models has contributed to our understanding of the pathogenesis, host responses and testing of control methods for APEC infections.

Keywords APEC; pathogen; infection model; avian; host

1. Introduction

Avian pathogenic *Escherichia coli* (APEC) represent a pathotype of enteric bacteria that affects avian species causing colibacillosis, a severe and most common infectious disease of farmed poultry. Colibacillosis exerts a substantial economic and welfare burden to all poultry producers worldwide where losses are realised through increased mortality, condemnation of carcasses at slaughter, decreased egg production and costs associated with treatment and prophylaxis. Sustainable poultry farming is vital to the supply and safety of food derived from such species. Depending on the virulence of the strain and host status, the infection initiates as a septicaemia which either results in sudden death or in recovered birds is followed by localised inflammation in multiple organs. The classical disease is defined by perihepatitis, airsacculitis and pericarditis, though other syndromes; coligranuloma, peritonitis, salpingitis, omphlitis, cellulitis and arthritis are also commonly encountered. Only in turkeys, osteomyelitis complex (TOC) affects young rapidly growing male poults, a disease characterised by lesions including green discoloration of the liver, arthritis/synovitis, soft-tissue abscesses, and osteomyelitis of the proximal tibia in normal-appearing processed carcasses. The avian intestinal tract harbours both potentially pathogenic and commensal *E. coli* strains [1] and infections generally arise from inhalation of contaminated dust particles in poultry houses. Experimental infection by the oral route rarely leads to systemic pathology despite extensive colonisation of the caecum and colon [2]. Systemic disease seems to occur in the presence of stressful environmental conditions including concurrent viral or *Mycoplasma* infections and inappropriate husbandry practices. In hens reared under free range systems, injury associated with formation of a social hierarchy and onset of lay often predispose to colibacillosis. Although the mode and genetics of systemic translocation of APEC from the intestinal tract are ill-defined, the respiratory tract is considered important in the pathogenesis of systemic infections. Avian airsacs are relatively avascular structures lacking effective resident defense mechanisms hence rely on recruited inflammatory heterophils. The precise site and mechanism of bacterial entry into the bloodstream has remained elusive, though entry via pulmonary lymphatics has been suggested [3]. Control by antibiotics in food-producing stock is prohibited to prevent residues from entering the food chain. Moreover, the existence of multiple antibiotic resistance traits that are readily transferrable between pathogenic strains further complicates this treatment option for colibacillosis. Of the available control options, vaccination is considered the most viable, but existing vaccines only confer serotype-specific protection in the presence of multiple pathogenic serotypes. In this context, selective breeding may be a sensible option. Differential resistance to APEC exists in commercial lines of poultry. Therefore, targeting the selective breeding route offers prospects of long-term control of this recalcitrant infection of poultry hampering profitable productivity.

The value of infection models in enhancing our understanding of host-pathogen interactions cannot be over-emphasised. To gain a better understanding of diverse aspects of avian host-APEC interactions, various infection models involving either chicken or turkey, the predominant food-producing species, have been described over years. One such aspect attracting a huge interest over several decades is the desire to elucidate the pathogenesis of colibacillosis in poultry. Furthermore, the search for better intervention strategies also drove the development of APEC infection models to unprecedented levels. Broadly, these infection models can be grouped according to the body system targeted; respiratory, gastrointestinal, musculoskeletal, dermatological, vascular system and chicken embryo system. Here, I examine how each of these various avian infection models has contributed to our understanding of; APEC virulence mechanisms, clinico-pathological processes, host immunological responses, host genetic resistance and intervention approaches to control colibacillosis.

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2. Experimental models of infection

2.1 Intra-tracheal inoculation with prior exposure to virus

Colibacillosis has been known for over a century and early workers believed the disease rarely occurred under field conditions without a predisposing infection or an environmentally stressful factor implying that APEC were either opportunistic or secondary invaders. Cognisant of this fact, the intra-tracheal infection model uses prior exposure or co-infection with an upper respiratory tract virus. In chickens, Infectious Bronchitis Virus (IBV is consistently used for prior infection before APEC challenge. Typically, this involves an initial oculonasal inoculation of 14 day-old chicks with 10^5 ciliostatic units of IBV strain in phosphate-buffered saline. Depending on the preference of research workers, mean embryonic infectious dose (EID), mean ciliostatic doses or even mean cytotoxic doses (CD_{50}) of IBV have been used. Pre-infection with IBV generally results in transient cloudiness of the airsacs with no mortality or lesions on the liver, pericardium or peritoneum. Three days later, IBV-predisposed birds are inoculated via the intra-tracheal route with up to 10^8 colony forming units (CFU) of a log-phase of statically-grown culture of an APEC strain. Cultivation of bacteria under static conditions in liquid medium is believed to preserve surface adhesins required for colonisation of the upper respiratory tract, but the advantages are not that obvious. However, the age at which birds are infected can vary depending on the objective of the study. Significantly, this model has facilitated the testing of the protective efficacy of live-attenuated vaccine candidates [4] and therapeutic efficacy and administration regimes of newly developed antibiotics [5]. In all these instances, the defining parameter was the development of pathological lesions. Apart from studying the pathological processes induced by APEC in the target host, the model has enabled assessment of roles of APEC genes in the disease process [6].

Turkey Rhinotracheitis Virus (TRTV), also referred to as Avian MetaPneumovirus (APV) is the standard predisposing virus routinely used for pre-infection in the induction of colibacillosis in turkeys [7]. This model closely resembles the technique employed in chickens. Initially, 10^5 mean ciliostatic units of TRTV strain in phosphate-buffered saline is given by intra-nasal inoculation and/or via the conjunctival route. TRTV causes a mild respiratory infection characterized by sneezing, tracheal rales and swollen infraorbital sinuses with no mortality or lesions on the pericardium or peritoneum. Three days later the TRTV-predisposed birds are inoculated via the intra-tracheal route with a log-phase of statically grown APEC culture. And as previously stated, the advantages of using a statically grown culture are not very obvious as the colibacillosis can still be induced with cultures grown under agitated conditions or washed bacterial suspensions. TRTV enhances colonisation and invasion of the respiratory tract by APEC increasing the severity of systemic infections, though in some cases that might not be clearly evident (Fig. 1a). Consistent with this description, clinical, pathological and microbiological dynamics have been described following a dual infection in turkeys [7]. Whilst TRTV could be expected to be specific for turkeys, similar clinico-pathological manifestations were described in chickens using a dual TRTV/APEC infection model [8]. Despite this, the model provides a sound platform for evaluating the efficacy of APV vaccines in an APV/APEC dual infection [9] or testing the efficacy of newly developed antimicrobials against APEC in turkeys [10].

Intra-tracheal inoculation of APEC offers an advantage of ensuring that a standard dose is administered at the perceived site of initial colonisation though this is not fully representative of a natural infection process. Under field conditions, it is very unlikely a bolus of infection will be deposited in the trachea and moreover, this inoculum bypasses the nasal and laryngeal mucosal defences which may be crucial in controlling the infection. Tracheal defences include mucus that trap bacteria and dust particles, which are propelled proximally by ciliary activity and subsequently coughed out or swallowed. As a consequence, APEC often finds it difficult to colonise healthy tracheal mucosa. Pre-infection with a ciliostatic virus removes this barrier allowing colonisation and subsequent invasion of the lower respiratory tract. Though IBV or APV predominantly induce ciliostasis, how other predisposing factors contribute to the severity of colibacillosis may not be directly related to ciliostasis. Nevertheless, this infection model in the target food-producing birds has provided an excellent opportunity to identify colonisation and invasive factors of APEC. The simplicity and relative ease of adaptability of this model is evidenced by extensive use in many different laboratories [6].

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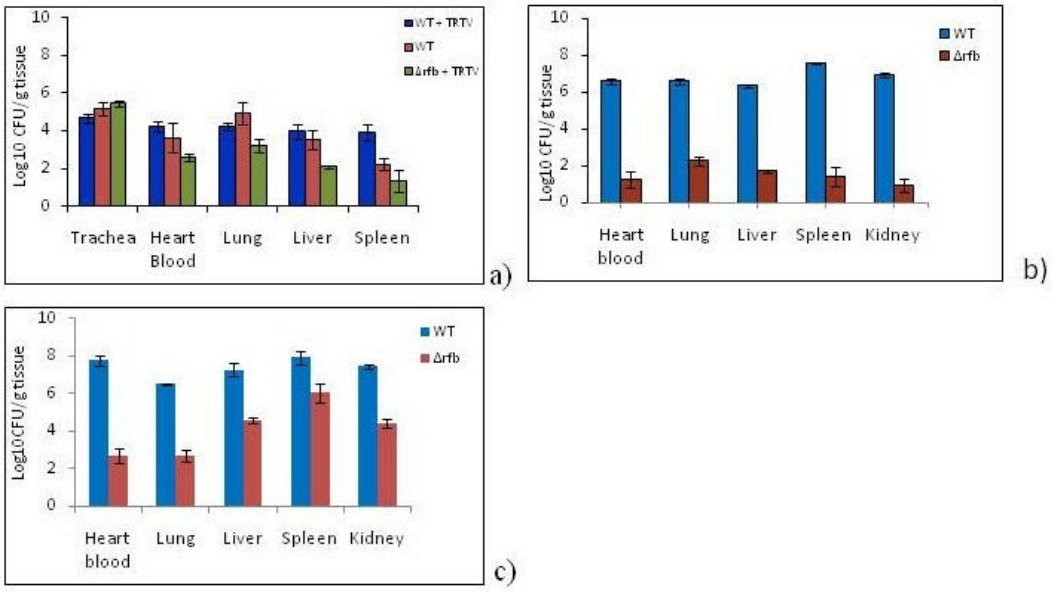

Fig. 1 Comparative assessment of colonisation of internal organs by avian pathogenic *Escherichia coli* serotype O78:K80:H9 strain χ7122 and its isogenic *Δrfb* mutant using 3 different infection models in conventional turkeys: a) = intra-tracheal inoculation with prior virus infection; b) = intra-airsac inoculation; c) = intra-venous inoculation. WT = wild type, TRTV = Turkey Rhinotracheitis Virus

2.2 Intra-tracheal infection with no virus

The inclusion of a pre-infection stage with an upper respiratory virus in an infection model is tantamount to relegating APEC to an opportunistic pathogen or secondary invader. Indeed, upper respiratory viruses like IBV and TRTV cause complete ciliostasis which a predominant inherent respiratory defence strategy in all mammals. Undoubtedly, this severe compromise of avian URT defences clearly exacerbates colonisation and invasion by APEC, but this should not overshadow the fact that APEC is capable of inducing colibacillosis in its own right. In this context, direct inoculation of APEC onto normal tracheal epithelial leading to induction of colibacillosis disproves this notion and confirms the virulence of APEC in its entirety. Cognisant of this fact, a model has successfully been developed and used to identify APEC genes mediating the development of septicaemia by signature-tagged transposon mutagenesis (STM) approach [11]. Up to 10^8 CFU of bacteria is deposited on the tracheal mucosa of healthy 5 week-old chickens using a special gavage needle and syringe. It is noteworthy that bacterial inocula of 10^9 CFU and above results in sudden death in 5 week-old birds. However, intra-tracheal inoculation with serial 10-fold dilution led to the identification of bacterial numbers that only colonises the trachea without invasion – referred to as a lung colonisation model [12] This model ensures bacteria are recovered from the lungs only but not in the bloodstream or distal organs. How APEC counteracts trapping by mucus and associated ciliary activity and also the mechanisms by which APEC is restricted to the lungs without systemic invasion merit further investigation. Despite all these, a novel APEC fimbrial adhesion (Yqi) termed exPEC adhesin 1 [13] is among some of the significant data derived from use of this model.

2.3 Intranasal inoculation

Intranasal inoculation addresses the fact intra-tracheal inoculation bypasses important upper respiratory tract defences of the nasal and laryngeal mucosa. The model involves deposition of bacteria onto anterior nares singly or in combination with IBV. A mixture of 5.5 units mean cytotoxic dose (CD_{50}) with $10^{8.3}$ CFU APEC are adequate for the induction of colibacillosis in chickens [14]. Significantly, this model has revealed differences in the genetic susceptibility of inbred lines of chickens to IBV, APEC or in combination [15]. Though the model appears to closely resemble the natural route of infection, issues surrounding correlating inoculum dose and clinical disease need to be seriously considered as these are likely to affect reproducibility. Deposition of bacteria and viruses on anterior nares does not guarantee that all infectious particles are inhaled to reach deeper tissues. The possibility of losing part of the bacterial inoculums through dripping off the nostrils is undeniable. As such, this model would be unsuitable for screening random mutant libraries or for obtaining conclusive evidence on the virulence status of defined mutants.

2.4 Intra-airsac inoculation

As stated in section 2.2, the inclusion of a predisposing step masks the potential virulence of APEC in its own entirety. As such models that bypass the upper respiratory tract successfully prove that APEC is the primary aetiology of

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colibacillosis. The intra-airsac (IAS) inoculation model brings bacteria into direct contact with the respiratory mucosa. A log- or stationary phase grown *E. coli* is injected into posterior thoracic or abdominal air sac and bacteria are detectable in the bloodstream as early as 3 hours and the peak reached in 24 hours. Indeed, up to 10^{10} CFU can be recovered from the kidney 24 hours after IAS inoculation of 10^8 CFU, thus suggesting this to be the likely site for systemic translocation. The early invasion occurring after IAS challenge clearly indicating that the innate defence mechanisms at this site are overwhelmed. This short incubation from artificial infection to onset of bacteraemia allows us to study early innate events from colonisation of the airsac mucosa, invasion and survival in the bloodstream. Innate responses in the lungs and airsacs are mediated by resident macrophages and recruited heterophils, which are the key cellular mediators of the innate system. This model provides an excellent opportunity to evaluate vaccine candidates and more importantly to conclusively assess the virulence of defined APEC mutants (Fig. 1b). It is clear the isogenic Δrfb mutant of APEC was recovered in far much less numbers when compared to the intra-tracheal and intravenous routes. Moreover, bacteria can consistently be recovered in the bloodstream and systemic organs, thus positioning this model at a better advantage in as far screening random libraries is concerned. In this respect, a comparative assessment of recoveries from the lung and distal organs can easily be undertaken. Indeed, we have embraced this approach to identify APEC genes required for lung colonisation and those required for systemic translocation and survival in turkeys [16]. Distinct advantages over the intratracheal-based models include; i) no requirement for a predisposing factor, ii) short incubation period and iii) a standard dose is be administered directly at the site with lower risk of being coughed out. More importantly, it also confirms APEC is the primary aetiology of colibacillosis since relatively higher numbers of bacteria can be recovered from internal organs. Using the same strains, bacteria numbers as high as 10^7 CFU/g of tissue were recovered following IAS instillation of 10^7 CFU, whilst 10^2 CFU/g of tissue was recovered from a dose of 10^8 CFU given via the intra-tracheal route [17]. Evidently, the IAS model ably provides a relatively quick assessment of roles of defined APEC genes in systemic infection. Other classic examples where the model has been successfully employed include; i) testing the protective efficacy of vaccines, where immunity is assessed by the levels of bacterial colonisation in internal organs, lesions or mortality, ii) the role of passive immunisation in protection against APEC invasion and iii) evaluating the dynamics of APEC in the bloodstream and internal organs over a defined duration of time.

Growing interest to better understand the pathogenesis and epidemiology of an atypical turkey syndrome, TOC, led to the development of a relevant infection model in this host. The model takes advantage of dexamethasone-induced immunosuppression or transportation-induced stress to compromise the body's immune competency. Typically, dexamethasone is injected into the thigh muscle at 2 mg/kg body weight as serial injections on 3 alternate days followed by a single IAS challenge with 100-200 CFU. The model is highly reliable and reproducible. The induction and magnitude of immune parameters, effects of feed supplementation, host genetic susceptibility and treatment regimen are among the studies carried out using this model. Dexamethasone induces cell-mediated immunosuppression in a manner similar to transportation stress. But in the transportation model, birds are given 10^4 CFU of APEC, then 8 days later transported for 3 hours and held in the transport vehicle for a further 9 hours [18]. And since TOC is a chronic infection, its incidence increases with a reduced dose of *E. coli* because high doses tend to cause rapid death.

Whilst this model is extensively used in many laboratories around the world, it is widely acknowledged it does not reflect the natural infection process. As such, the infection so obtained could be described as opportunistic since the bacteria are placed in direct contact with an internal organ bypassing the natural defence mechanisms. However, chickens are more susceptible to APEC when inoculated via air-sacs than intravenous injection [19] suggesting the virulence of APEC is route-dependent.

2.5Aerosol inoculation

Perhaps the best alternative for mimicking natural field conditions is the aerosol administration technique. APEC is exposed to all defence mechanisms of the host lining both the upper and lower respiratory tract. In this context, it is only virulent strains that will reach the deeper tissues and cause colibacillosis. Consequently, various aerosol-based models exist and these have enhanced our understanding of the pathogenesis of colibacillosis. Typically, the model recognises the difficulty of inducing colibacillosis in normal healthy birds, and hence involves prior intranasal inoculation of IBV 2-4 days prior to exposing the birds to an aerosol exposure of an APEC culture which comes with different techniques of administration. Exposing birds for 40 mins to an *E. coli* aerosol delivered by a nebulizer under a cone-shaped aerosol chamber appeared to reliably induce colibacillosis [20], whilst repeated exposure birds to aerosols is a better way of mimicking field conditions [21]. Repeated exposure closely resembles what happens in birds housed in a heavily contaminated dusty environment. Whilst earlier workers carried the notion that colibacillosis could not be induced in chicks of less than 2 weeks old owing to high levels of maternal antibodies, this has since been disproved. The role of a conjugative plasmid in upper respiratory colonisation was confirmed using a day-old chick model whereby 1-day-old specific pathogen free chicks were predisposed with 10 times the immunizing dose of the IBV vaccine (Webster's VicS) prior to a 20 min aerosol exposure of *E. coli* at days 1, 4 and 7 [21]. This model offers a distinct advantage in that bacteria are inhaled in during normal breathing process resembling natural inhalation of contaminated poultry house dust. A relationship between contaminated dust in poultry houses and induction of colibacillosis exists. Although birds develop lesions and mortality is recorded, the model falls short of addressing standardisation of the

APEC dose. It is difficult to estimate the CFU of inhaled by the bird and also the numbers of bacteria required for the induction of colibacillosis. However, this infection model appears appropriate for testing vaccine candidates and assessing the virulence of mutant strains in isolation. And significantly, the role of a conjugative virulence plasmid carrying genes for hydroxymate siderophore in colonisation of the respiratory tract of the chicken was defined using this model [21].

2.6 Oral infection

In healthy birds, APEC carried in the intestinal tract is unable to translocate across the mucosa into the bloodstream to cause colibacillosis despite extensive colonisation of the caecum and colon. Up to 10^9 CFU can be given by oral gavage in birds of varying ages without the development of clinical disease. However, systemic disease occurs when APEC is given in the presence of physiological stressors [2] or concurrent enteric viral infections. The mechanisms by which systemic disease arises from the intestinal tract carriage have remained ill-defined. It is unclear whether stress predisposes to direct invasion of the intestinal mucosa or simply increases bacterial faecal shedding leading to higher levels of contamination in the poultry house dust that is subsequently inhaled. As such, this model does not reliably induce colibacillosis. However, studies aiming to understand mechanisms of intestinal carriage and persistence of APEC in the avian host would considerably benefit from this model. Indeed, the chicken intestine and its environment are reservoirs of extra-intestinal pathogenic *Escherichia coli* (exPEC) strains with zoonotic potential [22]. Such a model may also be beneficial to studies investigating the effect of stress responses on intestinal carriage and the development of systemic APEC infections.

2.7 Subcutaneous injection

APEC may manifest as cellulitis in chickens, which is a localised inflammatory condition predisposed by trauma to the skin and often this leads to condemnation of carcasses at slaughter. Strains recovered from these sites are capable of causing septicaemia and typical colibacillosis lesions when inoculated by other routes. To gain more insights into the pathogenesis of this form of colibacillosis, the disease can be reproduced by dermal scarification and local deposition of APEC or by a subcutaneous injection. In some instances, feathers may have to be plucked off, but in the majority of cases, the abdominal region is scratched with a 22-gauge needle and then contaminated with a swab impregnated with an APEC broth culture. Lesions are observed in 24-48 hours post-infection. As expected, such a procedure does not guarantee application of a standard dose of APEC. But, an invasive subcutaneous injection technique addresses this shortfall and more importantly, systemic infections can arise from this procedure. When applied to one day-old chicks, this model becomes referred to as the chicken mortality assay. Subcutaneous injection of APEC in day-old chicks is a gold standard for assessing the virulence status of APEC isolates [23]. Typically, day-old chicks are inoculated subcutaneously in the back of the neck with a defined APEC dose. The virulence is assessed by mortality initially at 6 and 12 hours post-challenge and thereafter at daily intervals for up to 8 days. Macroscopic lesions in survivals are recorded, scored and a comparative analysis between the causative strains undertaken.

2.8 Intravenous inoculation

The intravenous (IV) injection model fulfils the fact that bacteraemia is a significant early feature in the pathogenesis of colibacillosis. This infection model bypasses all external defence mechanisms to subject bacteria directly to systemic innate defence challenges. Up to $10^{9.5}$ CFU can be injected intravenously into the wing vein to assess the; i) interaction of virulent and non-virulent blood-borne bacteria with macrophages of the spleen and liver [24], ii) the virulence of different APEC serotypes [3] and iii) clearance kinetics of APEC from the bloodstream in the presence or absence homologous antibodies [25]. The major advantages of this protocol are; i) a standard dose of APEC can be administered and dynamics within the bloodstream monitored over a defined period of time, ii) the virulence status of the strains can be determined by ability to survive within the bloodstream in the presence of complement, phagocytes and defensins, and notably, iii) there is no requirement for a predisposing factor. For further practical relevance, screening of a random transposon mutant library of APEC by this route can generate a portfolio of bacterial genes required for survival in the bloodstream. However, a major drawback of this procedure lies in that it is not representative of the natural route of infection. In this context, mutants or strains unable to invade the respiratory or intestinal mucosa can be fully virulent when delivered into the bloodstream. In addition, it is difficult to address issues related to bacterial mechanisms required for respiratory tract colonisation and systemic translocation, which are vital processes in the pathogenesis of colibacillosis. In our model development, up to 10^6 CFU of the *Δrfb* mutant bacteria were recovered from the spleen following IV injection (Fig. 1c), which might be interpreted as being virulent. In this respect, attenuation of APEC mutants is route-dependent which is consistent with earlier observations [19].

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2.9 Intramuscular injection

Colibacillosis often peaks between onset of lay and 30 weeks of age. Induction of the disease can be accomplished by a single intramuscular injection of with up to $10^{8.3}$ CFU of APEC. This model allowed a correct assessment of the relative susceptibility of inbred lines of chickens to APEC [15] and also testing the protective efficacy of a spontaneously non-pathogenic, piliated strain of *E. coli* (BT-7) [26] including inactivated vaccines [27]. Like the intravenous and subcutaneous routes of infection, the potential sites of host resistance which are mucosal defences of the intestinal or respiratory tract are excluded by this model. Although the model ensures administration of a standard dose, it is unclear why it is not widely used for studying host-pathogen interactions.

2.10 Intra-allantoic route (Embryo lethality assay)

Like other previously described models, the chicken embryo lethality assay (ELA) has the capability to delineate virulent from non-virulent strains of APEC. Typically, embryonated eggs from specific pathogen-free hens are set in a humidified egg incubator at 37 ºC and held for 12 days before a standard APEC inoculum (up to 10^3 CFU) is deposited onto the chorioallantoic sac. Eggs are sealed, returned to incubation and examined for embryo mortality by candling daily for 7 days, though a maximum of 4 days has recently been recommended [28]. Days to death and percentage of mortality are significant discrimination factors. Inoculation of bacteria into the yolk sac route has a poor discriminatory power; both virulent and non-virulent strains kill embryos [28]. Apart from being simple and inexpensive, this model is capable of identifying APEC genes with attenuating effects and such data correlates very well with the IV challenge model [29] and subcutaneous [30] hence has the potential to substitute the adult or growing bird infection models.

2.11 Intraperitoneal injection

Another characteristic feature of colibacillosis is peritonitis. This model of infection involves injection into the peritoneum of day-old chicks with up to 10^4 CFU of APEC. Birds are monitored for mortality up to 8 days and bacteria are subsequently recovered from the spleen and bone marrow. Like the subcutaneous infection model, the substantial severity banding is adopted. This model has confirmed attenuation of a degenerate Type III secretion system from APEC O78 strain [31] and the protective efficacy of inactivated vaccines [27]. The increased risk of inadvertent injury to internal organs during injection seems to be a major drawback.

2.12 Cellular and explants models

To comply with the Replacement, Refinement and Reduction (3Rs) agenda, ex vivo assays have also been used to assess virulence of APEC strains. Tracheal and gut explants are typically derived from day-old chicks and maintained under *in vitro* organ culture conditions [32] and cell populations, mainly heterophils and macrophages are purified using established protocols and maintained under standard cell culture conditions. These have predominantly been used to assess the magnitude of expression of mRNA of pro-inflammatory chemokines and cytokines, and in some cases to validate *in vivo* observations.

3. Concluding remarks

Avian pathogenic *Escherichia coli* are a complex group of pathogens causing several clinical syndromes in poultry. The desire to understand virulence mechanisms and genetic factors predisposing to these APEC syndromes has driven the development of infection models to unexpected levels. Based on the number of infection models involving the respiratory tract, it is evident this system plays a significant role in the pathogenesis of systemic APEC infections. Mimicking the natural infection process and refining some experimental procedures have enhanced our understanding of a wide range of aspects of APEC in its target hosts. Models characterised by inclusion of a predisposing factor in the form of a virus, exposure to cold or injection of immunosuppressive doses of dexamethasone, generally assert the notion that APEC is an opportunistic or secondary invader in a mixed infection process. This may be likely since the clear description of what constitutes the APEC pathotype continues to be a challenging aspect in *E. coli* phylogeny. APEC is a heterogenous group characterised by varying combinations of virulence genes. It is still unknown which combination of genes is necessary to induce colibacillosis. In this context, some strains may be truly opportunistic pathogens since the virulence of APEC has been explicitly shown route-dependent. It is therefore reasonable to suppose that the choice of an infection model influences the outcome of studies evaluating the virulent status of APEC strains. Thus, lack of a clear description of the APEC pathotype complicates the process of defining the infection biology of APEC. A wide range of molecular techniques exist to dissect the molecular basis of APEC virulence, but these become of limited value if not used in combination with a relevant infection model. It is clear the introduction of powerful genetic approaches combined with a relevant infection model continue to further our understanding of the APEC infection biology. Indeed, we have recently applied Transposon insertion-directed sequencing (TraDIS) to unlock

APEC genes involved in respiratory tract mucosa colonisation and those required for translocation across the mucosa and/or survival at systemic sites in the target host using the IAS model [16]. Such work has provided access to a wide selection of vaccine candidates whose protective efficacy can subsequently be assessed in the same infection model. Poultry is by far the most extensively adapted species offering a wide variety of infection models at the disposal of the avian research community. One would hope that by now, the infection biology of APEC and other related poultry pathogens would have been completely unravelled. Despite this setback, these models of infection have collectively played key roles in the assessment of the; i) virulence status of strains or mutants, ii) protective efficacy of vaccine candidates, iii) therapeutic potential of antimicrobials, iv) host genetic susceptibility and v) contributory roles of either extrinsic or intrinsic host factors to the induction of colibacillosis. And our understanding of how APEC interacts with its target host(s) continues to thrive owing to the existence of these infection models.

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