Pathologic response of gnotobiotic turkeys following oral challenge with highly and weakly virulent strains of Escherichia coli

Mark Anthony Dominick

Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Animal Sciences Commons, and the Veterinary Medicine Commons

Recommended Citation
https://lib.dr.iastate.edu/rtd/7755

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
INFORMATION TO USERS

This reproduction was made from a copy of a document sent to us for microfilming. While the most advanced technology has been used to photograph and reproduce this document, the quality of the reproduction is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help clarify markings or notations which may appear on this reproduction.

1. The sign or “target” for pages apparently lacking from the document photographed is “Missing Page(s)”. If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure complete continuity.

2. When an image on the film is obliterated with a round black mark, it is an indication of either blurred copy because of movement during exposure, duplicate copy, or copyrighted materials that should not have been filmed. For blurred pages, a good image of the page can be found in the adjacent frame. If copyrighted materials were deleted, a target note will appear listing the pages in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed, a definite method of "sectioning" the material has been followed. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For illustrations that cannot be satisfactorily reproduced by xerographic means, photographic prints can be purchased at additional cost and inserted into your xerographic copy. These prints are available upon request from the Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases the best available copy has been filmed.
Dominick, Mark Anthony

PATHOLOGIC RESPONSE OF GNOTOBIOTIC TURKEYS FOLLOWING ORAL CHALLENGE WITH HIGHLY AND WEAKLY VIRULENT STRAINS OF ESCHERICHIA COLI

Iowa State University

University Microfilms International

300 N. Zeeb Road, Ann Arbor, MI 48106
PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark ✓.

1. Glossy photographs or pages ✓
2. Colored illustrations, paper or print
3. Photographs with dark background ✓
4. Illustrations are poor copy
5. Pages with black marks, not original copy
6. Print shows through as there is text on both sides of page
7. Indistinct, broken or small print on several pages
8. Print exceeds margin requirements
9. Tightly bound copy with print lost in spine
10. Computer printout pages with indistinct print
11. Page(s) _________ lacking when material received, and not available from school or author.
12. Page(s) _________ seem to be missing in numbering only as text follows.
13. Two pages numbered _________ . Text follows.
14. Curling and wrinkled pages
15. Other

University
Microfilms
International
Pathologic response of gnotobiotic turkeys following oral challenge with highly and weakly virulent strains of *Escherichia coli*

by

Mark Anthony Dominick

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Major: Veterinary Pathology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1984
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>Avian colibacillosis</td>
<td>3</td>
</tr>
<tr>
<td>Organism</td>
<td>4</td>
</tr>
<tr>
<td>Histology of the avian intestine</td>
<td>10</td>
</tr>
<tr>
<td>Intestinal colonization</td>
<td>11</td>
</tr>
<tr>
<td>Bursal anatomy</td>
<td>12</td>
</tr>
<tr>
<td>Bursal functions</td>
<td>13</td>
</tr>
<tr>
<td>Peripheral lymphoid tissue of the intestine</td>
<td>15</td>
</tr>
<tr>
<td><strong>COLONIZATION AND PERSISTENCE OF ESCHERICHIA COLI</strong></td>
<td>17</td>
</tr>
<tr>
<td>IN AXENIC AND MONOXENIC TURKEYS</td>
<td></td>
</tr>
<tr>
<td>SUMMARY</td>
<td>18</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>19</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>21</td>
</tr>
<tr>
<td>Axenic and monoxenic turkeys</td>
<td>21</td>
</tr>
<tr>
<td>Bacteria</td>
<td>21</td>
</tr>
<tr>
<td>Experimental design</td>
<td>22</td>
</tr>
<tr>
<td>Quantitation of bacteria</td>
<td>22</td>
</tr>
<tr>
<td>Histopathology</td>
<td>23</td>
</tr>
<tr>
<td>RESULTS</td>
<td>24</td>
</tr>
<tr>
<td>Tissue bacterial titers</td>
<td>24</td>
</tr>
</tbody>
</table>
**GENERAL INTRODUCTION**

*Escherichia coli* is a common cause of respiratory disease in poultry.\(^1\) Airsacculitis and septicemia occur after inoculation of virulent strains of *E. coli* into the respiratory tracts of susceptible chicks\(^2,3\) and pouls.\(^4\) Respiratory infection occurs naturally by inhalation of feces-contaminated dust into the lower respiratory tract.\(^1\) Colisepticemia likely develops by invasion of *E. coli* through pulmonary microvasculature.

Pathogenic serotypes of *E. coli* are part of the normal intestinal coliform population of poultry.\(^5\) Persistent undiminished colonization of the crop and intestine occurs in gnotobiotic and conventional turkeys inoculated orally with pathogenic strains.\(^6\) Lower numbers of *E. coli* are found in the intestine of gnotobiotic and conventional chicks and pouls that receive a 'protective' intestinal flora prior to challenge with *E. coli*.\(^6-8\)

In poultry, a relationship may exist between virulence and intestinal colonizing capacity of *E. coli*. Type 1 pili, which are expressed by virulent *E. coli* in *vitro*\(^9,10\) could function as bacterial adhesins *in vivo* and promote intestinal colonization of virulent strains. In addition, the response of gut-associated lymphoid tissue to intestinal colonization of *E. coli* is not clearly defined in the turkey.

The objectives of these studies were i) to compare highly and weakly virulent *E. coli* for their ability to colonize the intestinal tract of gnotobiotic turkeys, ii) to ascertain the role of type 1 pili
in intestinal colonization of *E. coli*, and iii) to characterize lesions in intestine and cloacal bursae after intestinal colonization by *E. coli*.

This dissertation is presented in the format of the American Journal of Veterinary Research and consists of 3 manuscripts submitted to refereed scientific journals. The review of the literature precedes the first manuscript. The first and second manuscripts have been accepted for publication in the American Journal of Veterinary Research. The third manuscript has been submitted to Veterinary Pathology. A general summary and discussion follows the last manuscript. A list of references appears at the end of each manuscript. Literature cited in the introduction, literature review and general summary and discussion appears at the end of the dissertation.

The Ph.D. candidate, Mark A. Dominick, was the principal investigator for each study. Allen Jensen, the coauthor on the first and second studies, was responsible for isolation and purification of type 1 pili. Norman F. Cheville, the coauthor on the final study assisted in interpretation of electron micrographs and in the preparation of the manuscript.

Gnotobiotic turkeys used in the studies were provided by Dr. Peter J. Matthews, Animal Supply Officer, National Animal Disease Center, Ames, Iowa. E. Deane Dennis, Roger J. Spaete, Ervin L. Zook, Martha A. Church, Cindy K. Blessing and Syd K. Hartman provided technical support in the care and management of the gnotobiotic turkeys during the course of the experimentation.
LITERATURE REVIEW

Avian colibacillosis Respiratory disease and colisepticemia account for the greatest incidence of morbidity and mortality observed in field outbreaks of colibacillosis. Three forms of respiratory infection have been described in naturally occurring disease. First, colisepticemia occurs primarily in young birds and causes lesions within the respiratory tract, heart, liver, spleen and lymph nodes. Secondly, subacute fibrinopurulent polyserositis is found as a sequelae to septicemia in surviving birds or as a result of infection with less virulent serotypes of *Escherichia coli* and represents the most common form of colibacillosis in birds. Lastly, a low incidence of chronic granulomatous disease is reported in flocks previously affected with respiratory infection or colisepticemia.

*Escherichia coli* septicemia has been produced experimentally following exposure to virulent serotypes via the air sac, intra-tracheal and aerosol routes. In poultry confinement housing operations, pathogenic serotypes of *E. coli* are commonly isolated from house dust and litter. The intestinal tract of birds likely serves as the major source of environmental contamination with *E. coli*. Up to 15% of the adult broiler population harbor pathogenic serotypes of *E. coli* in their intestinal tract. Natural infection is believed to arise following inhalation of dust and litter contaminated with *E. coli*. Although infections in birds most often occur secondarily to a predisposing viral, mycoplasmal or other bacterial infection, uncomplicated colibacillosis may occur under conditions of overcrowding.
and poor sanitation. Mycoplasma gallisepticum commonly complicates E. coli infections in chickens\textsuperscript{16-19} while M. meleagridis is often associated with E. coli infections in turkeys.\textsuperscript{20-21} Newcastle disease and infectious bronchitis also predispose birds to secondary E. coli infections.\textsuperscript{22,23}

Organism E. coli isolates are serotyped by identification of somatic or cell wall (O), capsular (K) and flagellar (H) antigens present on the bacterial surface.\textsuperscript{24,25} Pathogenic strains of E. coli isolated from birds with colibacillosis are represented primarily by serotypes 01, 02, 036 and 078.\textsuperscript{14,26-28} Other serotypes have been isolated in lower frequency from birds with colibacillosis.\textsuperscript{1,28} Virulence of many of these isolates has been experimentally confirmed by measuring mortality rates in 3-week-old poults and chicks following IV inoculation of broth cultures of virulent serotypes.\textsuperscript{1}

Characteristics of pathogenic E. coli in vitro have been investigated by examination of piliation, motility and generation time of serotypes virulent and avirulent to poults.\textsuperscript{9} Most pathogenic serotypes tested were found to be piliated and motile when cultured in nutrient broth.\textsuperscript{9,10} Surface pili were classified as type 1 pili on the basis of structural morphology and the tendency of whole organisms to cause mannose-sensitive hemagglutination of erythrocytes from various vertebrate species. Significant differences in generation time were not observed among virulent and avirulent strains examined.\textsuperscript{9}

Bacterial pili are filamentous surface structures that often facilitate adhesion of bacteria to cell surfaces.\textsuperscript{29-31} Since intestinal
and bacterial surfaces are negatively charged, the presence of pili promote closer proximation of these surfaces by minimizing repelling electrostatic charge. In addition, the hydrophobicity of bacterial surfaces promotes binding of bacteria to epithelial surfaces.

*E. coli* enteropathogens in man express CFA 1 and CFA 2 pilus antigens on their surface to facilitate colonization of the small intestine. The human enterotoxigenic *E. coli* strain H10407 expresses both type 1 and CFA 1 pili. When H10407 was grown under laboratory conditions that promote expression of only one pilus type, it was found that CFA 1 pili promoted *in vivo* adherence to human brush border cells whereas type 1 pili did not. In contrast, *E. coli* expressing only type 1 pili adhered to human intestinal cells *in vitro* but not as readily as strains expressing CFA 1 and CFA 2 pili. K88, K99, and 987P pilus antigens facilitate adherence of enteropathogenic serotypes of *E. coli* to pig intestinal epithelium. The pilus antigen K99 is also expressed by *E. coli* serotypes enteropathogenic to calves and lambs and confers passive protection of lambs against colibacillosis by colostral transfer of antibodies from K99 vaccinated ewes.

The significance of type 1 pili in the pathogenesis of colibacillosis in birds has not been determined. Type 1 pili bind mannose residues which are widely distributed on mucosal cell membranes and may therefore act as receptors for *E. coli* adherence. Inoculation of *E. coli* and a mannose analog into the mouse bladder resulted in a reduced incidence of bacterial cystitis when compared to mice inoculated with *E. coli* alone. Presumably, urinary tract colonization was
reduced by competitive binding of the mannose analog to specific ligands on the type 1 pili.

Studies with uropathogenic strains of E. coli suggest that type 1 pili may mediate bacterial adherence to urinary tract mucosa in vivo. Expression of type 1 pili by E. coli strains uropathogenic to humans and mice was not correlated with pyelonephritogenicity but promoted bacterial adherence to the bladder mucosa. Addition of type 1 pili by mutation of a murine strain improved bacterial recovery from the mouse bladder but not from the kidney. E. coli mutants deficient in type 1 pili failed to adhere to mouse uroepithelial cells in vitro and were recovered at 100-1000X lower numbers from the bladder walls 3 hours postinoculation when compared with parent strains expressing type 1 pili. Other investigators have proposed that type 1 pili promote trapping of E. coli in the mucus gel overlying the bladder epithelium and, therefore, do not mediate direct adherence to uroepithelial cells.

Susceptibility of gram negative bacteria to phagocytosis may be affected by expression of type 1 pili. Depiliation of type 1 gonococci resulted in loss of ability to adhere to cell surfaces and increased susceptibility to phagocytosis. In contrast, phagocytosis of heavily piliated E. coli was prevented by depiliation with ultraviolet irradiation. Incubation of piliated E. coli with neutrophils and/or macrophages in the presence of D-mannose and its analogs significantly reduced phagocytosis. The attachment of opsonized bacteria to leukocytes is not inhibited by D-mannose and α-D-mannopyranoside since
attachment is mediated by complement and Fc receptor/recognition mechanisms.

Granulocyte killing mechanisms may be stimulated by piliated \textit{E. coli}. In one study, protein iodination was stimulated by interaction of piliated \textit{E. coli} with human neutrophils whereas the non-piliated serotypes failed to stimulate the neutrophils.\textsuperscript{59} Opsonization of the piliated \textit{E. coli} was found to cause a 15-fold increase in granulocytic stimulation as measured by protein iodination. This stimulatory effect was dependent on anti-fimbrial antibody and was reduced by methyl-\(\alpha\)-D-mannoside.

\textit{K}-antigen, exopolysaccharide capsular material, has been associated with increased virulence of \textit{E. coli} because it inhibits ingestion of \textit{E. coli} by phagocytes.\textsuperscript{60,61} \textit{K}-antigen reportedly inhibits bacteriolysis by impeding attachment of antibody to the bacterium and interfering with antibody-mediated complement activity.\textsuperscript{62} Strains of \textit{E. coli} containing a large amount of \textit{K}-antigen were poorly phagocytosed and lysed in the presence of complement and antibody when given IV in mice.\textsuperscript{63} Phagocytosis was increased by anti-OK antiserum while anti-K antiserum had little effect on bactericidal activity.

\textit{E. coli} isolates from urinary tract infections in man have been shown to exhibit greater \textit{K}-antigen content than strains isolated from blood or feces.\textsuperscript{64} Strains isolated from feces and blood had similar \textit{K}-antigen content. No correlation was determined between the \textit{K}-antigen content of bacteremic strains and the severity or outcome of bacteremia. Bacteremic strains of \textit{E. coli} were also shown to possess more exposed
negative surface charges and this was hypothesized to be associated with
the K antigen. However, negatively charged surface antigens were
associated with O antigen lipopolysaccharide, K-antigen polysaccharide
or both and not associated exclusively with K-antigen in studies of
immunoelectrophoretic patterns of E. coli extracts.

The contribution of capsular and cell wall antigens to the virulence of
E. coli should be considered when measuring bacterial resistance to phagocytosis. Acapsular strains of E. coli with smooth hydrophilic surface O antigens were found to resist phagocytosis as readily as negatively charged K-antigen possessing strains. Mutational deletion of the saccharide component of cell wall lipopolysaccharide (LPS) renders E. coli more susceptible to phagocytosis and less virulent. When these mutants were allowed to synthesize complete LPS, a marked decrease in susceptibility to phagocytosis and restoration of virulence was observed. These results suggest the external carbohydrate moieties of LPS are important to the virulence and antiphagocytic potential of the organism.

Evidence for plasmid-mediated virulence properties of certain
strains of invasive E. coli came with the discovery of the Vir and Col V
transmissible plasmids. The Vir plasmid was found in an E. coli strain causing bacteremia in a lamb and induced production of a heat and acid labile, non-dializable toxin lethal to mice, rabbits and chickens. Later it was determined the Vir plasmid was responsible for conferring transmissible adhesive properties to Vir strains of E. coli and to code for the expression of surface pilus antigen.
Adhesion of Vir* and Vir recombinant strains to calf epithelial tissue was inhibited by glucosamine, mannosamine, N-acetyl derivatives and wheatgerm lectin. No adhesion occurred with Vir* strains grown at 18°C. The Vir plasmid is poorly distributed among invasive strains of E. coli, has not been demonstrated in septicemic E. coli of birds but has been identified in strains isolated from humans, calves and lambs.

The Col V plasmid, unlike the Vir plasmid is widely distributed among invasive strains of E. coli. Colicine V production has been demonstrated in strains isolated from calves, lambs, pigs, chickens and man. Treatment with sodium lauryl sulfate yields a Col V- strain of E. coli that is significantly less pathogenic to chickens than the Col V+ wild form as measured by mortality following IM inoculation. Presence of the Col V plasmid has been associated with an increased resistance to host defense mechanisms and increased pathogenicity and survivability in vivo. The Col V plasmid interfered with phagocytosis of E. coli K1 strains and complement attachment to bacterial cells. Col V specified aerobactin, a siderophore, is secreted by plasmid-bearing invasive E. coli strains during periods of iron stress and promotes efficient iron uptake independent of the enterobactin system for iron uptake. The plasmid specified iron chelator was chemically determined to be a hydroxamate compound. In another study, 83% of E. coli isolates from cases of bacteremia in poultry were positive for hydroxamate. It was postulated the presence of
hydroxamate positive *E. coli* was associated with active host defense mechanisms restricting iron availability.

**Histology of the avian intestine** The avian intestine is divided on the basis of size and function with functional regions maintaining similar basic structural features. The mucosa is formed from epithelial cells lining villi that decrease in length as one moves from the duodenum to the colon. The villous epithelium rests on a basement membrane supported by the subjacent lamina propria. The muscularis mucosae is poorly developed in the cecum and colon and only slightly more developed in the small intestine. The submucosa is poorly developed and separates the muscularis mucosae from the tunica muscularis. The latter contains a poorly developed outer longitudinal layer and a well-defined circular layer.

The intestinal villi and crypts are lined by a simple columnar epithelium composed of absorptive enterocytes, mucus-secreting goblet cells and enterochromaffin cells. All are produced by mitotic division in the crypts and migrate up the villi during maturation. The greatest concentration of goblet cells occurs within intestinal crypts and the ratio of goblet cells to absorptive cells increases distally in the intestine. Enterochromaffin cells are also more numerous in the crypts, and contain deeply eosinophilic cytoplasmic granules that are selectively stainable by silver methods and contain 5-hydroxytryptamine.

The diameter of the avian intestine varies little from duodenum to cloaca. At the junction of the ileum and colon, a pair of caeca
anastomose with the intestine. This pair of blind sacs extend anteriorly and attach to the ileum by mesenteric folds. The relatively short colon extends to the colorectum which terminates into the cloaca. The cloaca communicates anteriodorsal with the bursa of Fabricius via the bursal duct and terminates with the vent which opens to the external environment.

**Intestinal colonization**  Pathogenic serotypes of *E. coli* are commonly isolated from the intestinal tract of poultry. Persistent, undiminished colonization of all segments of the gastrointestinal tract was reported in monoxenic and holoxenic poults inoculated with pathogenic *E. coli* and salmonella. Bacterial persistence in highest titer occurred in crop, ceca, colon and cloaca. Bacteremia was detected for up to 35 days postinoculation but intestinal invasion of *E. coli* did not occur. Differences in intestinal colonizing capacity and persistence between virulent and avirulent serotypes of *E. coli* has not been established.

The effects of a normal indigenous microflora on intestinal colonization by pathogenic *E. coli* has been extensively investigated. Conventional one-day-old chicks treated with native intestinal microflora and challenged with nalidixic acid-resistant pathogenic *E. coli* at three days developed lower intestinal titers of *E. coli* than birds orally inoculated with *E. coli* at three days without receiving a 'protective' bacterial flora. Similar protection by intestinal microflora was observed in conventional chicks inoculated with salmonella and pathogenic *E. coli* together or gnotobiotic chicks and
poults inoculated with *E. coli* alone. The investigators concluded that the native intestinal microflora was protective by competing with the pathogens for nutrients and mucosal attachment sites. Cross-protection has been demonstrated with chicks and poults challenged with *E. coli* following treatment with the other species intestinal flora.

Bacterial invasion of the intestinal mucosa has not been established as an important mechanism in colibacillosis of poultry. In one study, translocation of bacteria from the intestinal lumen to the liver in newly hatched conventional and germ-free chicks was limited to the first four days of life. In addition, coliforms were not recovered from the liver of poults examined; however, staphylococci and streptococci were readily cultured. Inhibition of translocation of *E. coli* from the intestinal tract to mesenteric lymph nodes by bacterial antagonism has been demonstrated in mice. No evidence supports inhibition of translocation from the intestine to the liver by the normal enteric microflora of birds.

**Bursal anatomy** Normal gross, histologic and ultrastructural anatomical studies show the bursa of Fabricius of the turkey to be structurally similar to the cloacal bursa of the chicken. The bursa is a blind oval sac-like structure located dorsal to the proctodeal cloaca. The lumen is obscured by the presence of twelve mucosal plicae. Histologically the gland can be separated into three distinct areas: serosa, muscularis, and mucosa.

The mucosa contributes greatest to the mass of the organ and is divided into a connective tissue matrix, lymphoid follicles and
epithelium. The bursal lymphoid follicles are divided into a highly cellular cortex and a less cellular medulla separated by a single row of epithelium residing on a basement membrane. The medulla and cortex are populated by small to large lymphocytes, lymphoblasts, and macrophages with the greatest concentration of cells located in the cortex. The cortex and medulla contain a reticulo-epithelial supporting matrix that is continuous with the interfollicular stroma. A delicate capillary network subjacent to the epithelial basement membrane further separates the cortex from the medulla. The basement membrane underlies a single row of undifferentiated epithelium that represents the outermost component of the medulla. The undifferentiated epithelium is continuous with the overlying epithelium.

The epithelium is divided into follicular and interfollicular components. The follicular epithelium is stratified low columnar to polygonal without an underlying basement membrane. In contrast, the interfollicular epithelium in the turkey is pseudostratified tall columnar with an underlying basement membrane. Quantitative surface analysis of the bursal epithelium in four-week-old chicks demonstrated 10% of the bursal surface as follicular epithelium. In addition, it was estimated that 8,000 to 10,000 follicles were present in the bursa of a four-week-old chick.

**Bursal functions** Central to the function of the bursa of Fabricius is the differentiation and maturation of lymphoid cells into immunoglobulin bearing B-lymphocytes. Migration of progenitor stem cells from the yolk sac of the developing chick to the bursa begins by
six days incubation.\textsuperscript{90} Lymphocytes with surface IgM can be found along the bursal epithelium by 14 days incubation.\textsuperscript{91} A clonal switch from IgM to IgG and IgA production occurs within the embryonic bursa as part of the normal maturation cycle.\textsuperscript{92} Once the mature immunoglobulin secreting lymphocyte migrates from the bursa to the peripheral pool, it is committed to production of a specific immunoglobulin isotype.\textsuperscript{93}

In addition to its role as a primary reticuloepithelial organ, the bursa functions in the chicken as a gut-associated lymphoid organ responsive to antigen. If the bursal duct is clipped on the 19th day of incubation, retardation of bursal development (organ weight and follicular size) and immunologic reactivity as measured by the Jerne plaque assay are observed.\textsuperscript{94} The bursal duct allows for continuous exchange between bursal and cloacal contents. Exchange of bursal and cloacal contents occurs with the rhythmic compression of the bursa caused by skeletal muscle contraction during respiration.\textsuperscript{95} In addition, the bursal muscularis undergoes slow, peristaltic activity.\textsuperscript{95} Contact of feces with the anus elicits an anal sphincter reflex that provides an additional mechanism of exchange between bursal and cloacal contents.

Tracer studies have demonstrated uptake of inert and radioactive particles by the follicular epithelial tufts and movement of these particles into the follicular medulla and cortex.\textsuperscript{96-102} Endocytosis of colloidal carbon by follicular epithelium in the chicken bursa of Fabricius occurred within five minutes of anal lip application.\textsuperscript{101} Phagosomes fused forming vesicles containing the colloidal carbon and
were exocytosed into the intercellular space. Residual bodies were also formed by combination of vesicles with lysosomes. Within 12 hours of application, the majority of tracer had passed through the epithelium into the follicular medulla.

Uptake of viable bacteria by the follicular epithelium has not been demonstrated. No bacteria were demonstrated in follicles after introduction of $5 \times 10^8$ E. coli into the bursal duct. Rarely organisms were observed by light microscopy associated with the follicular epithelium. Application of Brucella abortus to anal lips of one-day-old, four-week-old and ten-week-old chicks for five consecutive days did not result in localization of organisms within follicles.

The response of follicular lymphocytes to antigenic stimulation has also been measured. Immunization of 16-week-old chicks with human serum albumin elicited at 24 hours a significant bursal follicular lymphocyte response as measured by $^3$H-thymidine incorporation into DNA. By 72 hours post-immunization, no differences in $^3$H-thymidine uptake was present between immunized and control chicks suggesting an early transient increase in follicular lymphoid cell proliferation in response to antigen.

Peripheral lymphoid tissue of the intestine

Diffuse lymphoid tissue occurs within the lamina propria and submucosa along the entire intestinal tract. Germinal centers are commonly observed histologically within the lymphoid tissue along the intestinal tract. The largest gut-associated peripheral lymphoid tissues in the bird are the caecal tonsils. The structures form grossly visible enlargements of the caudal
ends of the caeca near the ileocaeco-colic junction and contain
diffusely dense masses of lymphocytes and scattered low numbers of
macrophages. The lymphoid tissue of the caecal tonsils often extends
from the lamina propria through the submucosa and into the muscular
layers of the caeca.\textsuperscript{103} Plasma cells are not present within the caecal
tonsils until after hatching and germinal centers appear in the chick by
ten days of age.\textsuperscript{104}

Epithelial lymphocytes are present within intercellular spaces
along the entire intestinal tract.\textsuperscript{103} They are particularly abundant
along the crypts and villous epithelium overlying the caecal tonsils.\textsuperscript{103}
Globular leukocytes, located along the basal one-half of the epithelium
within intercellular spaces, are also present along the entire intes­
tinal mucosa. These cells derive their name because of characteristic
globular, eosinophilic, PAS-positive, toluidine blue metachromatic
inclusions located within their cytoplasm.\textsuperscript{105} The cellular derivation
and function of these cells has not been determined.
COLONIZATION AND PERSISTENCE OF *ESCHERICHIA COLI*
IN AXENIC AND MONOXENIC TURKEYS

M. A. Dominick and A. E. Jensen

Manuscript accepted for publication in the
American Journal of Veterinary Research

From the National Animal Disease Center, Agricultural Research
Service, U.S. Department of Agriculture, P. O. Box 70, Ames, IA 50010.
Presented in part at the 1983 North Central Avian Disease
Conference, Ames, IA.

No product endorsements are implied herein.
SUMMARY

Sites of colonization and persistence of virulent (078) and avirulent (02) *Escherichia coli* were determined in 3-week-old axenic turkeys. Colonization of 078 in turkeys infected with *Streptococcus faecalis* was also determined. Colonization and persistence of *E. coli* in the intestinal tract and lung were not correlated with virulence. Both serotypes persisted in high titer in crop, ileum, ceca, colon, and lung. Persistence of *E. coli* in the liver was positively correlated with virulence, although both serotypes were recovered from the liver in the absence of bacteremia suggesting intestinal translocation of *E. coli* to the liver. A reduction in intestinal colonization and translocation of 078 was determined in monoxenic turkeys infected with *S. faecalis*. In contrast, *S. faecalis* had no effect on lung titers of 078. Air sacculitis, pericarditis and pneumonia were observed in axenic and monoxenic turkeys infected with 078. Bacteremia was detected in one turkey exposed to 078.
INTRODUCTION

The intestinal tract of domestic birds is a source of continuous environmental contamination by coliform bacteria. Nearly 15% of normal, mature broiler chickens harbor pathogenic serotypes of *E. coli* within their intestinal tract. In addition, approximately 50% of birds with colisepticemia were found to harbor within their intestinal tracts pathogenic serotypes of *E. coli* other than the septicemic strain isolated. Coliform counts of approximately $10^6$/gm have been recorded in broiler house dust samples in which *E. coli* was the major coliform isolated.

Uncomplicated colibacillosis may occur in birds housed under conditions of overcrowding, poor sanitation, and inadequate ventilation. Feces-contaminated dust harboring pathogenic *E. coli* is inhaled into terminal airways and air sacs where the pathogens may elicit a local inflammatory response and/or cause septicemia. Often, however, natural infection occurs secondary to a predisposing mycoplasma or viral infection.

The native intestinal microflora of birds limits colonization by pathogenic *E. coli* and salmonellae. Pathogenic *E. coli* readily colonize the gastrointestinal tract of gnotobiotic chicks and turkeys. However, the correlation between virulence and intestinal colonization and persistence of *E. coli* has not been determined. These experiments were conducted to determine the distribution and sites of persistence of virulent and avirulent *E. coli* in axenic turkeys. In addition, the
effect of prior infection with \textit{S. faecalis} on the subsequent colonization of monoxenic turkeys by \textit{E. coli} was tested.
MATERIALS AND METHODS

Axenic and monoxenic turkeys  Broad-breasted white turkey eggs were obtained from a commercial source\(^a\) and temporarily housed in conventional incubators. At 25 days incubation, eggs were passed into positive-pressure flexible plastic isolators\(^b\) as previously described.\(^9\) Briefly, unscrubbed clean eggs were passed through a 1.0% mercuric chloride solution and into isolators. One week after hatching and immediately prior to inoculation, the cloacae of birds in each isolator were swabbed and swabs were cultured for aerobic and anaerobic bacteria. In addition, samples of litter were cultured for fungal organisms. *Streptococcus faecalis* was isolated from newly hatched turkeys in two isolators.

The turkeys were fed a commercial turkey starter\(^c\) sterilized at 5.3 Mrads. Prior to use, a sterile vitamin concentrate\(^d\) was passed through a 0.22 μm filter and the water supply was autoclaved for 1 hour at 120°C and 15 psi pressure.

Bacteria  A virulent serotype of *E. coli* (078:K80:H9) and an avirulent serotype (02;K1:H6) previously isolated from turkeys with colisepticemia were used in the experiment. Virulence was previously determined by mortality in 3-week-old turkeys following IV inoculation

\(^a\)Jerome Foods, Barron, WI.
\(^b\)Standard Safety Equipment Co., Palatine, IL.
\(^c\)United Suppliers, Inc., Eldora, IA.
\(^d\)Vet-A-Mix, Inc., Shenandoah, IA.
of bacteria. Serotype 078:K80:H9 (078) is heavily piliated (in vitro), motile, and causes mannose-sensitive hemagglutination of turkey erythrocytes. Serotype 02:K1:H6 (02) is non-piliated to weakly piliated (in vitro), motile and non to weakly hemagglutinating. Bacteria were grown in trypticase-soy broth (TSB) for 24 hours and resuspended in .04 M phosphate buffered saline solution (PBSS, pH 7.2). Ten-fold serial dilutions of the suspension were cultured on blood agar and colony-forming units (CFU) were counted to determine the numbers of organisms/ml PBSS.

Experimental design Thirty-eight gnotobiotic turkeys were separated into 3 treatment groups containing 10 birds each and 1 uninoculated control group containing 8 birds. At 3 weeks of age, one group was orally inoculated with a 1.0 ml PBSS suspension containing $10^{8-8.5}$ CFU of 078. The second group, infected with S. faecalis at hatching, was inoculated similarly with 078. Pouls in the last treatment group were orally inoculated with $10^{8-8.5}$ CFU of 02 at 3 weeks. Two birds from each treatment group were killed and necropsied at post-inoculation day (PID) 1, 2, 5, 8, and 12. Two uninoculated controls were killed and necropsied at PID 0, 2, 8, and 12.

Quantitation of bacteria Biologic samples were triturated and serially diluted (10-fold) in cold (4°C) trypticase soy broth (TSB). Numbers of E. coli were determined by pipetting onto blood agar 0.5 ml of serially diluted samples and counting CFU after 24-hours incubation at 37°C. One centimeter segments of duodenum, ileum, cecum, and colon were opened longitudinally and washed free of contents in cold TSB prior
to trituration. Bacterial titers/gm were determined for liver, lung, crop contents, cecal contents, and colon contents. Bacterial titers/ml were determined for blood samples collected from the wing vein and bile samples aspirated from the gall bladder.

**Histopathology**  Samples of small and large intestine, lung, liver, heart, and abdominal air sacs were collected, fixed in 10% neutral buffered formalin or Bouin's solution, dehydrated in graded alcohols, processed by routine paraffin technique, and sectioned at 5 μm. Sections were stained with hematoxylin and eosin. Selected tissues were stained with Warthin-Starry silver and Gram stains.
RESULTS

**Tissue bacterial titers**  Virulent and avirulent *E. coli* readily colonized and persisted in the intestinal tract of axenic turkeys (Table 1). The number of epithelial-associated bacteria in the proximal small intestine ranged between $10^{3.5-7.0}/cm$ tissue throughout the period of exposure. Duodenal titers of 078 peaked at PID 5 and exceeded titers of 02 by 1 log. Titers of both serotypes declined to $10^{4.5}/cm$ by PID 12.

The number of epithelial-associated 078 in the ileum exceeded $10^8/cm$ at PID 1 and declined to $10^7/cm$ by PID 12. In contrast, the maximum ileal titer of 02 ($10^7/cm$) occurred at PID 1 and subsequently declined to $10^6/cm$ by PID 12. Each serotype readily colonized and persisted in high titer in the cecum and colon. Cecal 02 titers at PID 1, 2, and 5 exceeded 078 cecal titers by $10^{0.5-1}/cm$; however, titers at PID 8 and 12 were similar between serotypes. Titers of epithelial-associated bacteria within the colon were similar for each serotype and relatively constant throughout the experiment.

Titers of *E. coli* were similar in cecal and colonic contents of individual birds throughout the experimental period. In addition, no differences in bacterial titers in cecal and colonic contents were recorded among treatment groups. Titers exceeded $10^{10}/gm$ of intestinal contents at PID 1 and persisted $\geq 10^{10}/gm$ through PID 12. 078 and 02 persisted in crop contents at approximately $10^{8.5}/gm$. However, crop content titers of 078 declined $10^1/gm$ on PID 12 when compared to previous time periods.
Table 1. Epithelial-associated *E. coli* titers in the intestinal tract of axenic and monoxenic turkeys orally inoculated at 3 weeks

<table>
<thead>
<tr>
<th>Bacterial inoculum</th>
<th>Intestinal segment</th>
<th>Mean (SD) $\log_{10}$ CFU/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PID 1</td>
<td>2</td>
</tr>
<tr>
<td>02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>duodenum</td>
<td>5.3 (.1)</td>
<td>5.7 (.6)</td>
</tr>
<tr>
<td>ileum</td>
<td>7.2 (.1)</td>
<td>6.9 (.2)</td>
</tr>
<tr>
<td>cecum</td>
<td>8.4 (0)</td>
<td>8.7 (.6)</td>
</tr>
<tr>
<td>colon</td>
<td>8.1 (0)</td>
<td>8.2 (.3)</td>
</tr>
<tr>
<td>078</td>
<td></td>
<td></td>
</tr>
<tr>
<td>duodenum</td>
<td>5.6 (0)</td>
<td>6.1 (.4)</td>
</tr>
<tr>
<td>ileum</td>
<td>6.6 (.1)</td>
<td>8.0 (.8)</td>
</tr>
<tr>
<td>cecum</td>
<td>8.0 (0)</td>
<td>7.4 (.2)</td>
</tr>
<tr>
<td>colon</td>
<td>8.3 (.1)</td>
<td>7.9 (.7)</td>
</tr>
<tr>
<td>078 + streptococcus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>duodenum</td>
<td>6.2 (.5)</td>
<td>5.2 (.6)</td>
</tr>
<tr>
<td>ileum</td>
<td>5.4 (.3)</td>
<td>7.1 (.4)</td>
</tr>
<tr>
<td>cecum</td>
<td>8.0 (.7)</td>
<td>8.0 (.5)</td>
</tr>
<tr>
<td>colon</td>
<td>6.7 (.6)</td>
<td>7.1 (.1)</td>
</tr>
</tbody>
</table>
Liver titers of 078 exceeded $10^3$/gm of tissue in 7 of 10 birds necropsied (Fig. 1). Bacteria were cultured from liver in all 10 birds challenged with 078. In contrast, no bacteria were cultured from livers of 5 of 10 birds exposed to 02. Liver titers exceeded $10^3$/gm in 7 of 10 exposed to 078 but only 3 of 10 birds exposed to 02 (Fig. 1). Both E. coli serotypes readily colonized and persisted in the respiratory tract with lung titers of $10^4$-6/gm recorded in all birds exposed to 078 and 8 of 10 birds exposed to 02 (Fig. 2).

Intestinal titers of 078 were slightly lower in monoxenic turkeys infected with S. faecalis prior to 078 challenge when compared to birds infected with 078 alone (Table 1). Duodenal and cecal 078 titers were unaffected by S. faecalis early in the course of E. coli colonization. However, 078 titers in these tissues were reduced by $10^{0.5-1}$/cm at ≥ PID 2 when compared to the same tissues from turkeys challenged with 078 alone. Reduction in 078 colonization of ileum and colon was observed from PID 1 through PID 12. Exposure of turkeys to S. faecalis prior to 078 challenge was associated with reduced 078 titers in colonic, cecal, and crop contents by approximately $10^{0.5-1}$/gm.

Colonization and persistence of 078 in the lung was unaffected by concurrent S. faecalis infection. Lung titers of 078 remained $\geq 10^4$/gm in turkeys infected with 078 alone or following prior S. faecalis infection (Fig. 1). Liver 078 titers were $\geq 10^1$/gm in 8 of 10 birds exposed to S. faecalis prior to 078 challenge (Fig. 1). The remaining two birds had liver titers $\geq 10^3$/gm whereas 078 titers in axenic turkeys were $\geq 10^3$/gm in the majority of birds necropsied.
Fig. 1. *E. coli* titers/gm of liver in birds orally inoculated at 3 weeks with virulent (078) and avirulent (02) *E. coli*.

Fig. 2. Mean *E. coli* titers/gm of lung after oral inoculation of 3-week-old poults with *E. coli*. 
Bacteremia was detected in 1 of 10 turkeys exposed to 078 alone. No turkeys inoculated with 078 following *S. faecalis* infection or 02 alone had detectable bacteremia. *E. coli* was not cultured from bile.

**Gross and histologic lesions**  Airsacculitis developed early and persisted in axenic and monoxenic turkeys exposed to 078. In contrast, birds exposed to 02 did not develop respiratory disease. Early gross lesions in 078 birds were diffuse cloudiness of abdominal and thoracic air sacs and multiple 1 to 2 mm white to yellow caseous deposits on air sac membranes. The pericardium was slightly cloudy in birds exposed to 078 and necropsied at PID 2, 5. Early air sac lesions were characterized microscopically by diffuse edema, hyperemia, multifocal hemorrhage and heterophil and macrophage infiltration of the connective tissue stroma (Fig. 3). Abdominal air sacs in turkeys necropsied at > PID 5 were characterized by multifocal granulomas, multiple foci of fibroplasia, and epithelial hyperplasia and vacuolation (Fig. 4). In addition, the connective tissue stroma of air sacs contained moderate numbers of macrophages, lymphocytes, plasma cells, heterophils and lymphoid nodules. Subacute locally extensive suppurative pneumonitis with multifocal granulomas (Fig. 5) and peribronchial lymphoid cell hyperplasia were observed in one bird necropsied at PID 8. Multifocal fibrinopurulent to lymphohistiocytic pericarditis occurred in turkeys necropsied at PID 2, 5, 8. Myocardial lesions were not observed.

Histologic changes in the intestine were similar among treatment groups and consisted of multifocal infiltration of the lamina propria in the ileum, cecum, and colon by low numbers of heterophils, eosinophils,
Fig. 3. Infiltration of abdominal air sac by high numbers of heterophils 24 hours after oral inoculation of 078. Hematoxylin and eosin stain; x 945

Fig. 4. Abdominal air sac 5 days after oral inoculation of 078. The epithelium is hyperplastic, vacuolated and infiltrated by low numbers of heterophils and macrophages. Vacuolated spaces contain small amounts of fibrinous material and macrophages. Hematoxylin and eosin stain; x 945
Fig. 5. Locally extensive fibrinopurulent bronchopneumonia in bird necropsied at PID 8. Secondary bronchus is filled with fibrinopurulent exudate, the bronchial epithelium is focally necrotic and the lamina propria is edematous and infiltrated by inflammatory cells. Parabronchi and air capillaries are consolidated. Hematoxylin and eosin stain; x 54. Inset - Focus of epithelial necrosis and fibrinopurulent exudate in secondary bronchus. Hematoxylin and eosin stain; x 135
and macrophages. There was an increase in the number of lymphocytes and plasma cells within the lamina propria by PID 8. High numbers of bacteria were visible along the villous epithelium and within the mucus blanket of the ileum, ceca, and colon without producing epithelial alterations. Bacteria were associated primarily with the distal one-half of the villous epithelial border of the ileum; in contrast, bacteria were associated with the entire villous margin of the ceca and colon.

The livers of turkeys infected with 078 and 02 infrequently contained small lymphoid nodules. The presence of these structures was not correlated with persistence of the organism in the liver. No lesions were observed in sections of spleen. Bacteria were not observed in sections of spleen or liver.
DISCUSSION

The ability of *E. coli* to colonize and persist within the intestine of axenic turkeys did not correlate with virulence. The decline in titer of 078 and 02 in the duodenum and persistence of these organisms in the lower intestinal tract were expected since the rapid motility of the small intestine contributes to bacterial clearance whereas the sluggish motility of the cecum and colon would favor bacterial colonization. Persistence of *E. coli* in the cecum and colon in high titer is indirect evidence for a strong bacterial association with the mucosal surface in these regions since segments of intestine were washed in TSB prior to bacterial titration. The role of capsular, cell wall and pilus antigens in promoting *E. coli* colonization of the intestinal mucosa in birds is not understood; however, persistence of *E. coli* in washed intestinal segments likely involved surface receptor-bacterial ligand interactions.

Inhibition of colonization of *E. coli* and salmonellae by the normal intestinal microflora in birds is well documented.\(^5-8,11\) An established flora affects colonization of other bacteria by competing for nutrients and mucosal attachment sites.\(^11\) These direct microbial interactions are maximal in the lower intestine where the indigenous bacterial flora is stable and diverse. In this study, a concurrent *S. faecalis* infection was associated with reduced intestinal colonization of virulent *E. coli*.

078 and 02 colonized and persisted at similar titers in the lungs of infected turkeys. In contrast, previous work with the same strains demonstrated persistence of 02 and clearance of 078 15 days after
aerosol exposure of 2.5-week-old conventional turkeys. Streptococcus faecalis had no apparent influence on 078 titers in the lung although the former organism was also isolated from the lungs.

In the liver, bacterial persistence was positively correlated with virulence although both 078 and 02 were recovered from liver late in the course of infection. In the absence of concurrent bacteremia, high liver titers of E. coli early in the course of infection support translocation of these organisms from the intestine to the liver or inefficient bacterial clearance by hepatic Kupffer cells. Translocation of intestinal bacteria to the liver in germ-free and conventional chicks has been reported to occur primarily in the first 4 days of life. The mechanism operant in translocation inhibition after 4 days was postulated as an increased efficiency of the reticuloendothelial clearance system.

Since colonization of turkeys by S. faecalis reduced liver titers of 078, S. faecalis likely inhibited translocation of 078 from the intestine to the liver by an unexplained mechanism. Higher intestinal titers of E. coli occur in gnotobiotic chicks and turkeys than are found in birds with a normal indigenous microflora. A high population of E. coli within the intestine may be critical to bacterial translocation to the liver from the intestine. Inhibition of translocation of E. coli from the gastrointestinal tract to the mesenteric lymph nodes by a normal cecal flora has been demonstrated in gnotobiotic mice with reduction in the incidence of bacterial translocation from 100% to 0%. The normal intestinal microflora of birds likely inhibits translocation
of pathogenic *E. coli* to the liver; therefore, *E. coli* invasion through the intestinal barrier to the liver may not represent an important mechanism in the pathogenesis of colisepticemia in conventional turkeys.

The lack of a predisposing viral or mycoplasmal infection and environmental stresses may have contributed to the low incidence of bacteremia observed in turkeys challenged with virulent *E. coli*. In addition, the route of exposure has a significant effect on the onset and incidence of bacteremia. In previous work, bacteremia was observed within 3 hours following air sac inoculation of broiler chickens with *E. coli*; however, oral inoculation produced no effect. 15

Respiratory lesions characteristic of uncomplicated colibacillosis and similar to lesions observed in this study have been described in conventional turkeys. 16 In the absence of concurrent viral, mycoplasmal or other bacterial infections, respiratory disease associated with *E. coli* occurs under conditions of overcrowding and poor sanitation where inhalation of pathogenic *E. coli* is promoted. In positive-pressure germ-free isolators, aerosolization of bacteria may be promoted and thus facilitate bacterial invasion of the respiratory tract and resultant respiratory disease. However, oral exposure of germ-free and specific-pathogen-free turkeys to a similar serotype (078:K--H9) and under similar conditions to those used in this experiment failed to produce airsacculitis in the majority of birds challenged. 3,17

Virulence of *E. coli* for turkeys has been associated with in vitro bacterial expression of type 1 pili. 18,19 Type 1 pili have been shown to promote in vitro adherence of *E. coli* to mouse 20,21 and human 22,23
uroepithelial cells. However, type I pili have not been shown to promote intestinal or respiratory tract colonization in poultry or mammals. In this study, no differences in intestinal colonizing capacity were observed between piliated and non-piliated *E. coli* serotypes. This observation does not negate a role for type I pili in adherence of *E. coli* to avian mucosal surfaces. Further work is needed to elucidate the significance of type I pili to the pathogenicity of *E. coli* in poultry.
REFERENCES


10. Arp, L. H.; Jensen, A. E. Piliation, hemaglutination, motility, and generation time of *Escherichia coli* that are virulent or avirulent for turkeys. Avian Dis. 24: 153-161; 1980.


EXPRESSION OF TYPE 1 PILI BY \textit{ESCHERICHIA COLI} STRAINS OF HIGH AND LOW VIRULENCE IN THE INTESTINAL TRACT OF GNOTOBIOTIC TURKEYS

M. A. Dominick and A. E. Jensen

Manuscript accepted for publication in the American Journal of Veterinary Research

From the National Animal Disease Center, U.S. Department of Agriculture, P. O. Box 70, Ames, IA 50010.

No product endorsements are implied herein.
SUMMARY

Highly virulent (strain 1) and weakly virulent (strain 3) *Escherichia coli* were examined by immunofluorescent and electron microscopic techniques for their ability to express type 1 pili in the intestinal tract of 3-week-old gnotobiotic turkeys. Turkeys were necropsied on post-inoculation day (PID) 1, 2, 5, 8, and 12. Non-piliated forms of strain 1 and 3 were more numerous than piliated forms in cecal and colon contents examined by negative staining electron microscopy. A piliated form of strain 1 was seen in intestinal contents on each PID and was more numerous in cecal contents than in colon contents. The mucus blanket of the cecum and colon contained large numbers of bacteria although organisms were seldom closely associated with the intestinal epithelium. Large numbers of piliated forms of strains 1 and 3 were demonstrated by immunofluorescent staining within the mucus blanket of the cecum and colon on PID 2, 5, 8, and 12. Piliated bacteria were seldom seen in the ileal mucus blanket. Serum antibody titers to type 1 pili increased markedly by PID 5 and persisted in turkeys inoculated with strain 1. In contrast, antibody titers in turkeys exposed to strain 3 rose gradually and varied markedly among birds at each PID.
INTRODUCTION

The intestinal tract of poultry serves as a major source of pathogenic *E. coli* capable of causing respiratory disease and colisepticemia. Bacteria persist at high titer in the cecum and colon of gnotobiotic poult's inoculated orally with pathogenic *E. coli*. However, the bacterial-mucosal association in the intestinal tract of turkeys infected with *E. coli* of varying virulence has not been thoroughly examined.

*Escherichia coli* serotypes that are virulent in turkeys express type 1 pili when cultured *in vitro*. Under the same conditions, avirulent or weakly virulent serotypes are sparsely or non-piliated. These bacterial properties *in vitro* may not have a correlate *in vivo* where conditions have a profound effect on bacterial phenotype.

Type 1 pili (fimbriae) are rigid, filamentous surface structures found on many strains of *E. coli*. They have a diameter of 7 nm, variable length of 0.5 to 2 μm and the ability to cause mannose-sensitive hemagglutination of erythrocytes from several vertebrate species. Although type 1 pili promote colonization and persistence of several uropathogenic strains of *E. coli* in the urinary tract of man and mice by facilitating attachment to epithelial cells and mucus, there is no evidence suggesting type 1 pili mediate adherence of *E. coli* to mucosal surfaces of domestic fowl.

The objectives of this study were twofold. First, to examine a highly virulent and weakly virulent strain of *E. coli* for expression of
type 1 pilin the intestinal tract of gnotobiotic turkeys. Secondly, to characterize, in part, the bacterial-mucosal association.
MATERIALS AND METHODS

**Bacteria**  
E. coli strains previously isolated at the National Animal Disease Center from turkeys with colibacillosis were used in this study (Table 1). All strains were cultured in brain-heart infusion broth (BHI) for 48 hours at 18°C and 37°C for slide agglutination tests. Strains 1 and 3 were also grown for 24 hours in trypticase soy broth (TSB), centrifuged at 1000 xg for 20 minutes at 5°C, washed twice in 0.01 M - phosphate-buffered saline solution (PBSS; pH 7.2) and resuspended to a final concentration of $10^{8.2}$ colony-forming units (CFU)/ml (standard suspension).

**Gnotobiotic turkeys**  
Eggs of Broad-breasted white turkeys were purchased from a commercial source, and were hatched in germ-free isolators as previously described. Control turkeys were contaminated with Bacillus brevis at hatching.

**Experimental design**  
Ninety-six gnotobiotic turkeys were divided into 2 treatment groups (36 turkeys each) and 1 uninoculated control group (24 turkeys). At 3 weeks of age, turkeys from the 2 treatment groups were orally inoculated with 1.0 ml of a standard suspension of either strain 1 or 3. Six turkeys from each treatment group and 4 control turkeys were killed and necropsied on post-inoculation day (PID) 1, 2, 5, 8, 12 and 16. A serum sample was collected from each turkey and tested for type 1 pilus antibody titer. Segments of ileum, cecum

---

*Jerome Foods, Barron, WI.*
Table 1. Slide agglutination reactions of *E. coli* strains mixed with rabbit anti-type 1 pilus IgG following bacterial growth in BHI for 48 hours at 18 or 37°C

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serotype</th>
<th>Agglutination Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG + 37°C culture</td>
</tr>
<tr>
<td>1</td>
<td>078:K80:H9:type 1 pili^+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>02a:K1:H6:type 1 pili^+/^-</td>
<td>+^†</td>
</tr>
<tr>
<td>68</td>
<td>078:K*:H9:type 1 pili^-</td>
<td>Neg</td>
</tr>
<tr>
<td>8</td>
<td>025:K*:H5:type 1 pili^+</td>
<td>+</td>
</tr>
<tr>
<td>40</td>
<td>02a:K2:H5:type 1 pili^+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>0138:K*:H^-:type 1 pili^+</td>
<td>+</td>
</tr>
</tbody>
</table>

^* Rabbit anti-pilus Ig absorbed against strain 1 or 8 prior to slide agglutination reaction.

^† Weak agglutination reaction at 1 minute.

^*K* - capsule present but not typeable.
and colon 1 cm in length were collected from 2 turkeys in each group on PID 1, 2, 5, 8 and 12. Sections were opened longitudinally, washed free of intestinal contents with cold (5°C) TSB, placed in O.C.T. compound, frozen over dry ice and stored at -70°C. Additional sections were collected from these turkeys for transmission electron microscopic examination. Cecal and colon contents were collected from two other turkeys in each treatment group on PID 1, 2, 5, 8 and 12 and examined for bacteria by negative staining electron microscopy.

**Negative staining electron microscopy**

One to two gram samples of cecal and colon contents were suspended in 15 ml of cold (5°C) distilled H₂O, allowed to stand 5 minutes in an ice bath and centrifuged at 1000 xg for 20 minutes at 5°C. The bacterial fraction was collected from the pellet surface, resuspended in 5 ml cold distilled H₂O and recentrifuged. The bacterial fraction of the pellet was resuspended in 1 ml cold distilled H₂O, passed through a Sepharose 2B column and 10 drop eluates were collected and stained using the following proportions: 1 drop of eluate, 1 drop of 1% bovine serum albumin, 4 drops of 4% phosphotungstic acid and 20 drops of distilled H₂O. The mixture was sprayed onto collodion-coated, carbon-strengthened copper grids with a glass nebulizer and examined with a Philips 200 electron microscope.

---

*aLab-Tek, Naperville, IL.
bIsolab, Inc., Akron, OH.*
Transmission electron microscopy One mm cubes of ileum, cecum, and colon were fixed in 2.5% glutaraldehyde for 2 hours, embedded in 2% agar, washed in sodium cacodylate buffer overnight, post-fixed in osmium tetroxide for 4 hours and washed again in sodium cacodylate buffer. Tissues were dehydrated in graded alcohol and propylene oxide and flat embedded in epoxy resin. Ultrathin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and examined with a Philips 410 electron microscope.

Pilus purification Type 1 pili were isolated and purified from strain 1 by the methods of Korhonen et al. after the following modifications of culture technique. Bacteria were passed 3 times within 72 hours by inoculating fresh BHI broth with organisms obtained from the surface pellicle of a stationary phase BHI broth culture. Bacteria from the pellicle in the final culture were passed into BHI and cultured for 4 hours to obtain a log phase inoculum. The inoculum was then cultured for 48 hours in BHI in 2L Erlenmeyer flasks mounted on a G10 Gyratory Shaker set at 280 rpm.

Purity of the pilus preparation was determined by negative staining electron microscopy and the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of Laemmli as modified by Dodd and Eisenstein.

\[\text{\textsuperscript{a}New Brunswick Scientific Co., Inc., Edison, NJ.}\]
Antisera New Zealand white rabbits were inoculated subcutaneously with 250 µg of the purified pili suspended in Freund's incomplete adjuvant. A second dose of 250 µg of pili suspended in Freund's incomplete adjuvant was given intramuscularly 28 days after the primary inoculation. Serum was harvested 7 to 10 days later. The IgG fraction of the serum was isolated by the method of Goudswaard et al. using protein A-Sepharose 4B. The following modifications were made: The column was equilibrated with 0.2 M phosphate-citrate buffer (pH 8.0), the sample applied, and the column was then washed with the same buffer. Rabbit IgG was eluted with 10 ml of 0.1M citric acid, pH 2.2. Whole serum and the IgG fraction were tested for reactivity with type 1 pili by an immunodiffusion and slide agglutination tests.

Immunofluorescent tests were done by the indirect method using type 1 pili antisera diluted 1:80 and fluorescein isothiocyanate-labeled goat anti-rabbit serum diluted 1:20. Sections of intestinal segments were also fixed in methanol, stained with toluidine blue and examined by light microscopy.

Passive hemagglutination assay Turkey blood samples collected by cardiac puncture were prepared for pilus adsorption by the methods of Onkelinx et al. Washed erythrocytes were standardized by adjusting

---

aDifco Laboratories, Detroit, MI.

bPharmacia Fine Chemicals, Uppsala, Sweden.

cMiles Laboratories, Inc., Elkhart, IN.
the concentration of lysed cells to 50% transmission at 520 nm.

Erythrocytes were coated with a subagglutinating concentration (3.02 µg/ml) of type 1 pili by gently mixing 1 ml of erythrocytes, 1 ml of purified pili and 0.15 ml of 1% glutaraldehyde for 30 minutes at room temperature. The suspension was centrifuged at 1800 rpm for 10 minutes and the supernatant was discarded. The sensitized erythrocytes were resuspended and washed in 2 ml of diluent (0.2% gelatin in 0.15 M NaCl), recentrifuged and resuspended in 1 ml of diluent.

Antisera were heat-inactivated at 56°C for 30 minutes and 2-fold serial dilutions were made in diluent. To 1.0 ml of antisera dilution, 50 µl of pili-sensitized turkey erythrocytes were added and the mixture was allowed to incubate at room temperature. Anti-pilus antibody titers, expressed as the reciprocal of the highest antiserum dilution giving an agglutination pattern, were determined at 2 and 18 hours incubation. Control tubes included tubes with diluent mixed with sensitized erythrocytes and heat-inactivated, serially diluted, positive control rabbit serum mixed with either sensitized or non-sensitized turkey erythrocytes.
RESULTS

Analyses of pilus purity A peptide migrating with an apparent molecular weight of 17,500 daltons was seen by SDS-PAGE of a solubilized sample of the pilus preparation (Fig. 1). The pilus preparation was devoid of membrane vesicles and flagella when negatively stained with phosphotungstic acid and examined by electron microscopy (Fig. 2).

Slide agglutination and immunodiffusion precipitation reactions Rabbit anti-pilus antisera and the isolated IgG fraction reacted with purified pili by immunodiffusion precipitation forming a line of complete identity (Fig 3). Multiple lines of precipitation were not seen. Absorption of the IgG fraction with strain 1 or with a heterologous, type 1 piliated strain (strain 8) resulted in loss of reactivity with type 1 pilus antigen by slide agglutination (Table 1) and immunodiffusion precipitation. The IgG fraction agglutinated broth cultures of strains 6, 8 and 40 grown at 37°C for 48 hours but did not agglutinate cultures of the same strains grown at 18°C for 48 hours (Table 1). A weak, delayed agglutination reaction occurred when strain 3 cells reacted with the IgG fraction.

Negative staining electron microscopy The percentage of E. coli that were piliated in samples of cecal and colonic contents from gnotobiotic turkeys given strains 1 or 3 are listed in Table 2. Piliated bacteria were found rarely in cecal or colon contents from turkeys exposed to strain 3. In contrast, marked variation in the percentage of piliated bacteria within cecal and colon contents was
Fig. 1. SDS-PAGE migration pattern of solubilized pilus preparation with a single wide band at approximately 17,500 daltons.

Fig. 2. Purified pili isolated from strain 1 and negatively stained with phosphotungstic acid. x 23,660
Fig. 3. Immunodiffusion precipitation reactions with type 1 pili (P).
1, Normal rabbit serum; 2, Rabbit antipilus serum; 3, Rabbit antipilus IgG; 4, Rabbit antipilus IgG absorbed against strain 1; 5, Rabbit antipilus IgG absorbed against strain 8; 6, Rabbit anti-078 serum
Table 2. Percent piliated bacteria observed in cecal and colonic contents by negative staining electron microscopy

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Bird/PID</th>
<th>Intestinal Contents</th>
<th>PID</th>
<th>1</th>
<th>2</th>
<th>5</th>
<th>8</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain 1</td>
<td>1</td>
<td>Cecum</td>
<td>PID</td>
<td>3.2</td>
<td>20.8</td>
<td>0.0</td>
<td>0.8</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colon</td>
<td>PID</td>
<td>1.0</td>
<td>4.5</td>
<td>2.9</td>
<td>0.0</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Cecum</td>
<td>PID</td>
<td>3.9</td>
<td>6.8</td>
<td>61.4</td>
<td>8.3</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colon</td>
<td>PID</td>
<td>0.0</td>
<td>2.0</td>
<td>22.2</td>
<td>3.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Strain 3</td>
<td>1</td>
<td>Cecum</td>
<td>PID</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colon</td>
<td>PID</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Cecum</td>
<td>PID</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colon</td>
<td>PID</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*Two birds necropsied from each treatment group on all specified PID.

†Percent piliated cells observed with a minimum of 100 bacteria examined from 3 eluates.
observed among turkeys exposed to strain 1. The percentage of piliated cells in cecal and colon contents from turkeys exposed to strain 1 ranged from 0 to 61.4% and 0 to 20.8%, respectively. The majority of strain 1 cells examined in cecal and colon contents were non-piliated. The only exception occurred with one turkey necropsied on PID 5 when 61.4% of the organisms in cecal contents were piliated. No correlation was found between percent piliation and period of exposure to cecal or colonic microenvironment. The greatest number of piliated forms was found in the cecal contents of birds necropsied at PID 2 and 5. Piliated cells were more numerous in cecal contents than in colon contents from the same bird.

The piliated form of strain 1 examined in intestinal contents was heavily piliated with ≥ 100 pili per cell (Fig. 4a). Pili exceeded 1 μm in length and were arranged peritrichously about the cell. The rare piliated form of strain 3 seen had few, short (< .5 μm) pili randomly arranged across the bacterial surface. Most of the strain 3 cells examined lacked pili (Fig. 4b). Although both strains readily produce flagella when grown in vitro, flagellated forms of either strain were not seen.

**Transmission electron microscopy** Intestinal infection with strains 1 and 3 was associated with mild, segmental blunting of microvilli of the cecal and colonic epithelium; however, microvilli of the ileal epithelium were unaffected by infection. Bacteria in the cecum and colon were observed primarily near villous tips, in spaces between microvillous borders of adjacent villi, rarely at the base of villi or
Fig. 4a. *Escherichia coli* strain 1 isolated from cecal contents and negatively stained with phosphotungstic acid. Bacterium with type 1 pili of variable length peritrichously arranged about the cell (Note the surface blebs). x 24,000

Fig. 4b. *Escherichia coli* strain 3 isolated from cecal contents and negatively stained with phosphotungstic acid. Bacterium lacks pili. x 24,000
within crypts. Scattered low numbers of bacteria were observed near villous tips and seldom along lateral borders of villi in the ileum.

Bacteria were seldom found in direct contact with microvilli but were found in a loose flocculent-to-granular material some distance from the mucosal surface (Fig. 5). The material had an electron density similar to mucus in goblet cells and was considered part of the mucus blanket. An electron lucent space often separated bacteria from this associated material. Type 1 pilus aggregates were not observed within the electron lucent area surrounding the bacteria examined.

**Immunofluorescence**  
Large numbers of bacteria were found associated with the mucus blanket of frozen sections of cecum and colon stained with toluidine blue and examined by light microscopy (Fig. 6). Bacteria were distributed diffusely along the villous borders with heaviest concentrations at villous tips and along the distal one third of the lateral villous margins. Piliated forms of strains 1 and 3 were demonstrated by immunofluorescence within the mucus blanket of the cecum and colon in moderate to high numbers in turkeys on PID 2, 5, 8, 12 (Table 3). Piliated bacteria were rarely found within the distal ileum of turkeys exposed to strain 3 but were found in moderate numbers in the ileum of turkeys exposed to strain 1 and necropsied on PID 2 and 8. Piliated forms of strain 1 and 3 were rarely seen in the cecum and colon at 24 hours post-exposure. Differences in the intensity of bacterial fluorescence occurred with respect to the strain inoculated. Piliated forms of strain 1 within the cecum and colon were intensely fluorescent compared to the weak fluorescence observed with piliated strain 3 cells.
Fig. 5. Colonic mucosa from turkey exposed to strain 1 and necropsied at PID 5. Bacteria are poorly associated with the microvillous border. Microvilli are intact. x 3300
Fig. 6. Association of *E. coli* with the colonic mucosa in a bird exposed to strain 1 and necropsied at PID 2. Bacteria associated with the mucus blanket with few organisms adjacent to the epithelium. Toluidine blue stain; x 525  Inset – Fluorescent bacteria within the mucus blanket overlying the colonic mucosa; x 1080
Table 3. Distribution of piliated E. coli along mucosal surfaces of intestinal segments examined by indirect fluorescent methods

<table>
<thead>
<tr>
<th>Intestinal Segment</th>
<th>Inoculum</th>
<th>PID</th>
<th>1</th>
<th>2</th>
<th>5</th>
<th>8</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ileum</td>
<td>Strain 1</td>
<td>-</td>
<td>++</td>
<td>+/-</td>
<td>++</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Cecum</td>
<td>Strain 1</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Colon</td>
<td>Strain 1</td>
<td>+/-</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

*Samples collected from two birds at each specified PID.

†+, low number of fluorescent bacteria, multifocal distribution; ++, moderate number of fluorescent bacteria, diffuse distribution; ++++, moderate to focally high number of fluorescent organisms, diffuse distribution; -, no fluorescence, +/-, low number of fluorescent bacteria seen in intestinal segment from one bird only.
Passive hemagglutination titers

Turkeys exposed to strains 1 or 3 had agglutinating titers to type 1 pili of \( \leq 40 \) on PID 1, 2. Major differences between groups occurred in the antibody response to type 1 pili by PID 5 (Fig. 7). Antibody titers in turkeys exposed to strain 1 were markedly higher on PID 5 when compared with titers from turkeys in the same group necropsied on PID 1 and 2 and turkeys exposed to strain 3 and necropsied on PID 5. Titers were high in strain 1 infected turkeys from PID 5 to 16 with peak mean titers (40,960) attained on PID 16. In contrast, serum agglutinating antibody titers to type 1 pili rose gradually over time in turkeys exposed to strain 3. The mean titer attained on PID 16 was approximately one-half the mean titer calculated from strain 1 infected poults necropsied on the same day.

Marked biologic variability occurred in serum agglutinating antibody titers among turkeys exposed to strain 3 and necropsied on the same PID. On PID 12, titers ranged from 160 to 10,240 among the 6 turkeys sampled. Titers among turkeys exposed to strain 1 and necropsied on the same day never varied by more than 2 dilutions. Serum antibody titers to type 1 pili remained \( \leq 40 \) in all control turkeys. Positive control rabbit sera had titers \( \geq 327,680 \).
Fig. 7. Means and ranges of hemagglutinating antibody titers to type 1 pili; ○, mean titers/PID of birds exposed to strain 1; 0, mean titers/PID of birds exposed to strain 3; ▲, mean titers in uninoculated controls. Lines represent linear regression of titers per group.
DISCUSSION

From this study, we have concluded type 1 pili do not mediate in vivo adherence of pathogenic E. coli to avian intestinal epithelium. Piliated organisms were not found adherent to intestinal epithelium from segments we had previously determined to be heavily colonized by the isolates. Evidence against type 1 pilus mediated adherence to human intestinal epithelium has been provided using E. coli strain H10407 that expresses both CFA I and type 1 pili. Organisms grown under conditions that stimulate expression of only type 1 pili failed to attach to human intestinal epithelium in vitro, whereas expression of CFA I alone resulted in adherence.

Although in vitro expression of type 1 pili by E. coli isolated from turkeys is positively correlated with virulence, a direct relationship between piliation and virulence was not clearly demonstrated in our in vivo model. The low number of piliated cells and sparse piliation per cell seen on strain 3 isolated from intestinal contents was similar to in vitro observations of this strain. Our demonstration by immunofluorescence of a piliated form of both strain 1 and 3 within the cecal and colonic mucus blanket, however, differed from in vitro findings where strain 1 was heavily piliated and strain 3 was sparsely to non-piliated when cultured in nutrient broth. High serum agglutinating antibody titers to type 1 pili in birds inoculated with strain 3 provides additional evidence for in vivo expression of type 1 pili by a weakly virulent strain.
The cecal microenvironment of the turkey appears to promote expression of a piliated (pil\(^+\)) phase more readily than the colonic microenvironment. The conditions within the cecal and colonic environments promoting the pilus phase variation observed are undefined. Phase variation between a pil\(^+\) and a non-piliated (pil\(^-\)) state is a common phenomenon observed with E. coli isolates grown under laboratory conditions.\(^{21}\) The percentage of piliated organisms within a particular microenvironment depends partially upon phase variation between a genotypically and phenotypically pil\(^+\) state to a genotypically pil\(^+\)/phenotypically pil\(^-\) state. In addition, irreversible mutations from a pil\(^+\) to a pil\(^-\) state may influence the quantity of piliated cells observed in an environmental system.\(^6,8\) The predominant phase within a particular microenvironment is therefore dependent upon genetic influences and ecological factors. The predominance of a pil\(^-\) phase of strain 1 within the cecal and colonic contents was interesting since a pil\(^+\) phase predominates when this strain is cultured for 72 hours in nutrient broth.\(^a\)

Association of bacteria with the mucus gel provides an alternate mechanism for adherence to a mucosal surface. Infant mice, orally challenged with *Vibrio cholerae* had high numbers of organisms associated with mucus adherent to ileal mucosal sections when examined by scanning electron microscopy.\(^22\) Few organisms were found directly associated

\(^{a}\) Dominick, M. A., NADC, USDA, Ames, IA, unpublished data.
with the epithelial surface. In vitro adhesion assays using *V. cholerae*
and rabbit intestinal slices demonstrated vibrios associated
predominantly with the mucus gel rather than with the mucosal epithelium
or serosa. In vitro adherence of *E. coli* to urinary mucus associated
with isolated uroepithelial cells has been demonstrated with a strain
isolated from a human urinary tract infection. Addition of D-mannose
markedly inhibited attachment of bacteria to the mucus associated with
the isolated uroepithelial cells.

Type 1 pili may promote bacterial trapping within intestinal mucus
of the turkey or adherence to specific receptors in mucus. This is
supported by our observation of partitioning, although incomplete, of
the pil + and pil - phases of strain 1 and 3 into bacteria associated with
the mucus blanket and intestinal contents, respectively. Since the
mucus gel readily adheres to epithelial surfaces, association with
mucus where mucus flow rate is slow may provide *E. coli* the opportunity
to compete for a position within the indigenous microflora of the avian
intestine. This, in turn, would establish an effective carrier state
for piliated *E. coli* and thereby favor continuous environmental
contamination with potentially virulent serotypes.
REFERENCES


PATHOLOGY OF CLOACAL BURSAE IN GNTOBIOTIC TURKEYS
ORALLY INOCULATED WITH VIRULENT ESCHERICHIA COLI

M. A. Dominick and N. F. Cheville

Manuscript prepared for submission
to Veterinary Pathology

From the National Animal Disease Center, Agricultural Research
Service, U.S. Department of Agriculture, P. O. Box 70, Ames, IA 50010.

No product endorsements are implied herein.
SUMMARY

Cloacal bursae from 3-week-old turkeys were examined by light and electron microscopy and bacteriologic techniques at 1, 2, 5, 8, 12 and 16 days after oral inoculation of highly virulent (strain 1) and weakly virulent (strain 3) *Escherichia coli*. In strain 1 inoculated turkeys, granulocytic inflammation developed early in bursae and diminished by post-inoculation day (PID) 8. Bacteria were found in few follicular medullae on PID 5 and 8; bacteremia was detected on PID 1, 2 and 5. In strain 3 inoculated turkeys, pyogranulomatous bursitis was detected at PID 5 and became progressively more severe with time. Follicular alterations included abscessation, lymphocyte necrosis and depletion, reticulo-epithelial hyperplasia and perifollicular fibroplasia. Follicular pads had degenerate and necrotic epithelial cells, intercellular edema, cystic spaces containing acidic mucosubstances and laminar deposits of calcium salts. Bacteria were found within necrotic centers of follicular abscesses, in phagosomes of macrophages and multinucleate giant cells and within extracellular spaces of follicular pads and follicular medullae from PID 5 to 16; bacteremia was not detected. We conclude that *E. coli* may pass through the bursal follicular-associated epithelium, replicate within follicular medullae and produce extensive follicular necrosis.
INTRODUCTION

Colibacillosis occurs commonly in immature turkeys and chickens as acute septicemia or fibrinopurulent airsacculitis. Chronic granulomatous inflammation of the lungs, liver, intestine and mesentery occur less frequently, usually in birds surviving acute disease. Although respiratory infection with E. coli is often secondary to a predisposing mycoplasmal or viral infection, uncomplicated colibacillosis occurs under conditions of overcrowding and poor sanitation.

Contact of intestinal bacteria with the cloacal bursa occurs as organisms are shed in the feces. Peristaltic contractions of the bursal tunica muscularis and contraction of striated muscles during respiration cause an exchange of bursal and cloacal contents. Contact of pathogenic E. coli with the cloacal bursa would readily occur since pathogenic strains of E. coli are part of the intestinal coliform population and persist in high titer in the cecum and colon of gnotobiotic turkeys and chickens.

The cloacal bursa functions as a site for antigen independent development and maturation of B-lymphocytes; it also functions as a peripheral lymphoid organ. Application of colloidal carbon to the anal lips results its rapid transport into the bursal lumen. Phagocytosis of colloidal carbon by the follicular-associated epithelium results in uptake and redistribution of the tracer into the follicular medulla. Uptake of bacteria by the bursal follicular-associated epithelium has not been described.
The objectives of this study were to describe the histologic and ultrastructural lesions of the cloacal bursa after oral inoculation of gnotobiotic turkeys with *E. coli*, and to examine the follicular-associated epithelium and bursal follicles for evidence of bacterial uptake and transport into the follicular medulla.
Bacteria  

E. coli serotypes 078:K80:H9 (strain 1) and 02:K1:H6 (strain 3) that were previously isolated at the National Animal Disease Center from the liver of turkeys with colisepticemia were used in this investigation. Bacteria were incubated in trypticase soy broth (TSB) at 37°C for 24 hours and the suspension was centrifuged at 1000 xg for 20 minutes at 4°C. The supernatant was discarded and the bacterial pellet was resuspended in phosphate buffered saline solution (PBSS, pH 7.2) to a standard suspension of $10^8$ colony-forming units (CFU)/ml of PBSS.

Gnotobiotic turkeys  

Turkey eggs were obtained commercially from a mycoplasma-free flock, hatched and poult s raised in germfree isolators as previously described.

Experimental design  

Eighty gnotobiotic turkeys were separated into two treatment groups (36 birds each) and one uninoculated control group (8 birds). Turkeys in each treatment group were inoculated orally at 3 weeks of age with 1 ml of a standard suspension of either strain 1 or 3. Six turkeys from each treatment group were killed and necropsied on post-inoculation day (PID) 1, 2, 5, 8, 12 and 16. Two uninoculated control turkeys were necropsied on PID 1, 5, 8 and 16. Bursae were collected for bacteriologic examination from 3 of 6 turkeys from experimental groups at each necropsy. Bursae from uninoculated controls and the remaining 3 of 6 turkeys from each experimental group at each necropsy were collected for histologic and ultrastructural examination. Blood samples collected from the wing vein of each bird were cultured for bacteria.
**Bacterial titration** Bursae were weighed and then triturated and serially diluted (10-fold) in cold (4°C) TSB. Numbers of *E. coli*/gm of bursa were determined by pipetting onto blood agar 0.5 ml of serially diluted samples and counting bacterial colonies after 24-hours incubation at 37°C. Blood samples were diluted 1:10 in TSB, incubated at 37°C for 48 hours and then examined for turbidity. The blood-broth mixture was then subcultured on blood agar for 24 hours at 37°C. Bacterial colonies present on agar were identified as the inoculated strain by a slide agglutination reaction with strain-specific antiserum.

**Histologic examination** Bursae were removed intact, opened longitudinally along their dorsal midline with an incision extending from the bursal duct to the craniodorsal pole, and fixed in 10% neutral buffered formalin for 24 hours. Each bursa was sectioned into three segments of approximately equal size. Tissue blocks from each segment were processed by routine technique, sectioned at 5 μm and stained with hematoxylin and eosin and Gomori methanamine silver/hematoxylin. Additional sections were stained by Gram, modified Dieterle, periodic acid Schiff/Alcian blue, pH 2.5 and Masson's trichrome techniques.

**Electron microscopic examination** Samples of bursa were collected from 2 turkeys at each specified PID, fixed for 2.5 hours in cold (4°C) 2.5% glutaraldehyde, washed in cacodylate buffer, post-fixed in 2% osmium tetroxide, dehydrated in graded alcohols and propylene oxide and flat embedded in epoxy resin. A minimum of 6 blocks was examined from each bursa.
Morphometric procedures  Three randomly selected plicae from each of 3 segments of bursa per turkey were examined by light microscopy at a magnification of 111 x. Transectional areas of 5 follicles per field and their corresponding medullary transectional areas were measured by tracing the microscopic image projected onto a computerized digitizing tablet. Cortical transectional areas and cortex/medulla transectional area ratios were calculated indirectly.

Relative volumes of normal follicles, degenerate or atrophic follicles, interstitium and epithelium per bursal plicae were determined by point count stereology. Randomly selected bursal plicae were examined at 100 x using a 10 x objective lens and a 10 x eyepiece lens. Point counts were made using a 121-point coherent grid formed from a 1 cm x 1 cm reticle mounted in the eyepiece. The transectional points \( P_A \) for each parameter measured, the total points \( P_T \) and points falling in extrabursal space \( P_{EB} \) were used to calculate the volume density \( V_V \) of each parameter relative to the bursal plicae volume as follows:

\[
\text{Relative } V_V = \frac{P_A}{P_T - P_{EB}}
\]
RESULTS

Gross lesions Turkeys given strain 3 had <1 mm foci of necrosis irregularly distributed on the surface of bursal plicae at PID 5 that increased in number progressively to PID 16. Gross lesions were not seen in bursae of turkeys given strain 1.

Histologic and ultrastructural lesions At PID 1 and 2, bursal alterations were similar in turkeys inoculated with strain 1 and 3. Submucosa subjacent to interfollicular epithelium had moderate to high numbers of eosinophils and heterophils in a multifocal distribution (Fig. 1). Overlying epithelium was heavily infiltrated by similar cell types and was focally eroded. Follicles adjacent to regions of submucosa heavily infiltrated by granulocytes had necrotic lymphocytes within their cortices and medullae. Microabscesses in a few follicular medullae were seen in 3 turkeys inoculated with strain 3.

Ultrastructurally, intercellular spaces of the follicular-associated epithelium were prominent in turkeys inoculated with either strain. Lateral cell membranes of the follicular-associated epithelium had extensive filopodia and cell membrane interdigitations. Macrophages within intercellular spaces had multiple residual bodies containing amorphous electron dense material, lipid globules and myelin figures.

From PID 5 to 16, bursal inflammation differed among strain 1 and 3 infected turkeys. In strain 1 infected turkeys, the dominant lesion on PID 5 was local, severe, granulocytic inflammation within the interfollicular epithelium and superficial submucosa. Numbers of
Fig. 1. Superficial submucosa of bursal plicae infiltrated by large numbers of granulocytes 2 days after oral inoculation of gnotobiotic turkeys with strain 1. Hematoxylin and eosin; x 280. Inset - Higher magnification showing granulated cytoplasm of heterophils and eosinophils in submucosa. Hematoxylin and eosin; x 518
granulocytes, macrophages, plasma cells and lymphocytes within the superficial submucosa progressively diminished from PID 8 to 16. Cystic spaces containing pale fibrillar material were seen infrequently within follicular pads. Intrafollicular microabscesses were seen in less than 1.0% of follicles. After PID 5, bacteria were demonstrated within a few follicular medullae but not within follicular pads by both Gram and modified Dieterle staining techniques.

Ultrastructurally, the follicular-associated epithelium of bursae from strain 1 infected turkeys had expanded intercellular spaces which contained many membrane-bounded vesicles. Vesicles were most abundant along plasma membranes of macrophages in intercellular spaces and along basolateral plasma membranes of the follicular-associated epithelium. Follicular-associated epithelial cells had prominent vacuoles and vesicles in the apical cytoplasm and numerous vesicles associated with multiple Golgi complexes. Bursal follicles had necrotic lymphocytes within follicular medullae and macrophage phagosomes.

Bursal inflammation in strain 3 infected turkeys increased in severity with time. Microabscessation, cyst formation and squamous metaplasia of follicular pads were seen with increasing frequency through PID 16. Ultrastructurally, the most frequent alteration of the follicular-associated epithelium was intercellular edema. Vesicles 40 to 80 nm in diameter were seen within intercellular spaces and in cytoplasmic vacuoles lined by long slender microvilli. Coated pits and coated vesicles were abundant along macrophage and epithelial plasma membrane surfaces (Fig. 2). Follicular-associated epithelium of some
Fig. 2. Follicular-associated epithelium of bursa from turkey necropsied 16 days after oral inoculation with strain 3.
Expanded intercellular space and cytoplasmic vacuoles (*) containing numerous membrane-bounded vesicles (arrow, insert)
Necrotic follicular-associated epithelial cell (NE)
Bar = 2 μm. Inset - membrane-bounded vesicles in intercellular space (►). Coated pit along plasma membrane surface (arrow).
× 62,400
follicular pads formed cystic spaces lined by squamous or cuboidal cells with irregularly distributed, short, blunt microvilli. Cyst lumina contained edema fluid, heterophils, macrophages and laminar bodies of calcium salts deposited on PAS-positive, Alcian blue (pH 2.5) positive mucosubstances (Fig. 3).

By PID 5, bacterial colonies were seen in cystic spaces (Figs. 4, 5), microabscesses and necrotic foci in follicular pads. On PID 16, multinucleate giant cells with bacteria in their cytoplasm were at the base of some follicular pads.

Follicular lesions varied markedly in turkeys infected with strain 3 and included lymphoid depletion, lymphocyte necrosis and phagocytosis (Figs. 6a, 6b), reticuloepithelial hyperplasia of follicular medullae, and follicular microabscessation (Fig. 7). Some microabscesses in follicular medullae extended into the overlying follicular-associated epithelium. Follicular medullae with lymphoid depletion were composed of reticuloepithelial cells, multinucleate giant cells, macrophages, heterophils, fibroblasts and plasmacytes with distended rough endoplasmic reticulum.

Intrafollicular abscesses and pyogranulomas were rare on PID 8 but were widely distributed in bursal plicae by PID 16 (Figs. 8, 9). Pyogranulomas had necrotic centers circumscribed by a zone of heterophils, macrophages and multinucleate giant cells. A zone of necrotic lymphocytes within a thin remnant of follicular cortex surrounded some pyogranulomas. Edematous interstitium had fibroblasts arranged concentrically around the pyogranuloma and was infiltrated diffusely
Fig. 3. Follicular-associated epithelium of bursa from turkey necropsied 16 days after oral inoculation with strain 3. Macrophages (M) in edematous intercellular space (⋆) and laminar deposit of calcium salts (arrow) in cystic space within follicular pad. Bar = 5 μm.
Fig. 4. Follicular pad of bursa from turkey necropsied 5 days after oral inoculation with strain 3. Colony of *E. coli* in follicular pad directly below surface epithelium. Gram stain; x 518.

Fig. 5. Follicular pad of bursa from turkey necropsied 5 days after oral inoculation with strain 3. *E. coli* within intercellular space of follicular-associated epithelium. Gram stain; x 900.
Fig. 6a. Follicular medulla in bursa from control poult necropsied at PID 5. Necrotic lymphocytes are absent. Bar = 5 μm

Fig. 6b. Follicular medulla in bursa of turkey inoculated with strain 3 and necropsied at PID 5. Necrotic cells (lymphocytes) within phagosomes of macrophages (arrows). Bar = 5 μm
Fig. 7. Follicular alterations in bursae of turkeys inoculated with strain 3. Intramedullary microabscess at PID 5 (a) x 75. Reticulo-epithelial hyperplasia and lymphocyte necrosis (arrow) at PID 5 (b) x 100. Abscessation of follicle and follicular pad at PID 8. Note the thin rim of remaining follicular cortex (c) x 75. Multinucleate giant cells in follicle with atrophic follicular pad at PID 16 (d) x 100. Hematoxylin and eosin stain.
Fig. 8. Cloacal bursa from turkey inoculated with strain 3 and necropsied at PID 16. Bursal plica with intrafollicular abscesses, pyogranuloma and marked thickening of the interfollicular stroma. Hematoxylin and eosin stain; x 32

Fig. 9. Cloacal bursa of turkey inoculated with strain 3 and necropsied at PID 16. Pyogranuloma with necrotic center surrounded by a thin zone of macrophages and multinucleate giant cells. Marked perifollicular fibroplasia and perivascular cuffing of macrophages and lymphocytes (arrow). Hematoxylin and eosin stain; x 325
by macrophages, plasma cells, lymphocytes and heterophils. Short, irregular strands of immature collagen were demonstrated between adjacent cords of fibroblasts in tissue sections stained by Masson's trichrome technique. Arterioles and venules in the interstitium had prominent perivascular aggregations of lymphocytes, macrophages and heterophils (Fig. 9).

On PID 5, bacteria were in foci of lymphocyte necrosis and reticuloepithelial hyperplasia (Figs. 10, 11) and in microabscesses of follicular medullae. From PID 8 to 16, bacterial colonies were also in necrotic centers of intrafollicular pyogranulomas and abscesses. Ultrastructurally, multinucleate giant cells and macrophages in follicular medullae had several intact bacteria in single phagosomes and numerous residual bodies and cytoplasmic lipid globules (Figs. 12, 13, 14). Bacteria were also found in electron dense cellular debris surrounded by macrophages and reticuloepithelial cells in follicular medullae (Fig. 15).

Bursal morphometry Follicular cortical, follicular medullary and total follicular mean transectional areas increased significantly (P < .01) with time in inoculated and control turkeys (Table 1). The overall mean follicular area was significantly less (P < .05) in strain 3 inoculated turkeys than the overall mean follicular areas of strain 1 inoculated and control turkeys. Cortical areas were similar among the three groups. Medullary areas of control turkeys were significantly greater (P < .05) than medullary areas of strain 1 and 3 inoculated turkeys. The significant difference between follicular areas of
Fig. 10. Follicular medulla in bursa from turkey inoculated orally with strain 3 and necropsied at PID 5. *Escherichia coli* (arrow) within segment of follicular medulla with marked lymphoid depletion and reticuloepithelial hyperplasia. Gram stain; x 520

Fig. 11. Follicular medulla of bursa from turkey inoculated orally with strain 3 and necropsied at PID 5. *Escherichia coli* within intramedullary microabscess. Gram stain; x 520
Fig. 12. Follicular medulla of bursa from turkey inoculated orally with strain 3 and necropsied on PID 12. Multinucleate giant cells (MG) with bacteria in phagosomes (arrows); macrophages (M) with multiple residual bodies and reticuloepithelial cells (RE). Bar = 5 μm
Fig. 13. Follicular medulla of bursa from turkey inoculated orally with strain 3 and necropsied at PID 16. Macrophages (M) with lipid globules (L), residual bodies (R) and bacteria in phagosomes (arrow). Bar = 1.5 μm
Fig. 14. Follicular medulla of bursa from turkey inoculated orally with strain 3 and necropsied at PID 16. Several *E. coli* within a single phagolysosome of macrophage within follicular medulla.

Bar = .5 μm
Fig. 15. Follicular medulla of bursa from turkey inoculated orally with strain 3 and necropsied at PID 16. *Escherichia coli* in cellular debris surrounded by macrophages (M) and reticulo-epithelial cells in follicular medulla. Bar = 5 µm. Inset — *Escherichia coli* with intact cell walls are separated from the cellular debris by an electron lucent space. Bar = 0.25 µm
Table 1. Mean transectional areas and area ratios of bursal follicles, follicular cortices and follicular medullae following oral inoculation of gnotobiotic turkeys with virulent E. coli.

| PID | Follicular Area | | | Cortical Area | | |
|-----|----------------|----------------|----------------|----------------|----------------|
|     | Strain 1 | Strain 3 | Control | Strain 1 | Strain 3 | Control |
| 1   |    .149  |    .127  |    .126  |    .064  |    .066  |    .046  |
|     |    (.001) |    (.009) |    (.017) |    (.001) |    (.008) |    (.006) |
| 2   |    .139  |    .115  |     -    |    .064  |    .052  |     -    |
|     |    (.006) |    (.006) |     -    |    (.004) |    (.005) |     -    |
| 5   |    .125  |    .138  |    .168  |    .054  |    .064  |    .071  |
|     |    (.010) |    (.006) |    (.010) |    (.007) |    (.003) |    (.009) |
| 8   |    .159  |    .132  |    .169  |    .069  |    .059  |    .074  |
|     |    (.013) |    (.006) |    (.009) |    (.007) |    (.004) |    (.006) |
| 12  |    .179  |    .145  |     -    |    .080  |    .069  |     -    |
|     |    (.019) |    (.005) |     -    |    (.011) |    (.002) |     -    |
| 16  |    .168  |    .155  |    .192  |    .077  |    .075  |    .090  |
|     |    (.012) |    (.001) |    (.015) |    (.007) |    (.001) |    (.014) |

*Observations reported in mm² as the mean ± SEM.*
<table>
<thead>
<tr>
<th>Strain</th>
<th>Medullary Area</th>
<th>Cortex/Medulla Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>.085</td>
<td>.068</td>
<td>.080</td>
</tr>
<tr>
<td>(.001)</td>
<td>(.008)</td>
<td>(.011)</td>
</tr>
<tr>
<td>.075</td>
<td>.063</td>
<td>-</td>
</tr>
<tr>
<td>(.004)</td>
<td>(.002)</td>
<td></td>
</tr>
<tr>
<td>.071</td>
<td>.074</td>
<td>.097</td>
</tr>
<tr>
<td>(.003)</td>
<td>(.004)</td>
<td>(.011)</td>
</tr>
<tr>
<td>.090</td>
<td>.073</td>
<td>.095</td>
</tr>
<tr>
<td>(.006)</td>
<td>(.002)</td>
<td>(.003)</td>
</tr>
<tr>
<td>.099</td>
<td>.076</td>
<td>-</td>
</tr>
<tr>
<td>(.009)</td>
<td>(.007)</td>
<td></td>
</tr>
<tr>
<td>.091</td>
<td>.080</td>
<td>.103</td>
</tr>
<tr>
<td>(.005)</td>
<td>(.001)</td>
<td>(.003)</td>
</tr>
</tbody>
</table>
strain 3 inoculated and control turkeys resulted from reduced medullary areas of strain 3 inoculated turkeys. Ratios of cortical to medullary areas were greatest in strain 3 inoculated turkeys and were significantly different \((P < .05)\) from strain 1 and control turkeys.

Relative volumes of follicles within bursal plicae decreased in turkeys inoculated with \textit{E. coli} \((P < .01)\) (Fig. 16). Overall mean relative volumes of bursal follicles for control, strain 1 inoculated and strain 3 inoculated turkeys were 0.79, 0.75 and 0.70, respectively. In addition, the relative volume of bursal follicles in strain 3 inoculated turkeys declined significantly \((P < .05)\) with time to a mean value of 0.66 on PID 16.

Reduction in follicular volume following inoculation with \textit{E. coli} was associated with an increase in interstitial volume (Fig. 16). Overall mean interstitial volume of strain 3 inoculated turkeys was twice the interstitial volume of control turkeys. The overall mean interstitial volume of strain 1 inoculated turkeys was intermediate between values for control and strain 1 inoculated turkeys. Relative volumes of bursal epithelium were unaltered by infection with \textit{E. coli} (Fig. 16). Atrophic, degenerate or abscessed follicles were not seen in control turkeys or in the majority of strain 1 inoculated turkeys. Strain 3 inoculated turkeys had \(< 1\%\) atrophic or degenerate follicles on PID 2 and 5. Degenerate follicles accounted for 5.0\% of the follicular volume on PID 8 and 12 and 9\% of the follicular volume on PID 16.

\textbf{Bacterial titration} \quad Both strains of \textit{E. coli} readily colonized the bursa (Fig. 17). On PID 1 and 2, mean titers of strain 1 exceeded
Fig. 16. Volume densities (mean ± SEM) of bursal follicles, interstitium and epithelium relative to bursal plical volume. Lines represent linear regressions of measured parameters for strain 1 inoculated (—), strain 3 inoculated (——) and control (——) turkeys.
Fig. 17. *Escherichia coli* titers expressed as the mean and range per gram of bursa following oral inoculation with strain 1 (dotted line) or strain 3 (solid line).
titers of strain 3 by 1 log unit/gram of bursa. Bursal titers of
*E. coli* were similar among inoculated birds from PID 5 to 12. On
PID 16, average titers of strain 3 were 100-fold higher per gram of
bursa than titers of strain 1. Bacteremia was detected in 3 turkeys
inoculated orally with strain 1 and necropsied on PID 1, 2 and 5 but
was not detected in turkeys inoculated with strain 3.
DISCUSSION

We have demonstrated that E. coli can pass through the follicular-associated epithelium and replicate within follicular medullae of the turkey bursa of Fabricius. Since medullae of bursal follicles are poorly vascularized and bacteremia was not detected in strain 3 inoculated turkeys, E. coli within follicular pads and medullae is strongly suggestive of bacterial penetration of the follicular-associated epithelium.

Sequential phagocytosis, transport and exocytosis of intact bacteria by follicular-associated epithelium (M cells) has been described in rabbit Peyer's patches. Intact bacteria have not been seen within follicular-associated epithelium or follicular medullae of cloacal bursae of chickens following intracloacal administration of live E. coli, Staphylococcus albus and suspensions of live or dead Brucella abortus. The bursal follicular-associated epithelium has been shown to phagocytose 1 μm diameter latex spheres and transport them into the intercellular space.

The origin and nature of the vesicles in cytoplasmic vacuoles and intercellular spaces of the follicular-associated epithelium of chronically infected turkeys was not determined. We speculate that these structures resulted from excessive membrane turnover associated with a marked increase in endocytosis and exocytosis by the follicular-associated epithelium following oral inoculation of E. coli.

Our studies indicate that chronic pyogranulomatous bursitis can be a feature of avian colibacillosis in gnotobiotic turkeys. In a previous
study, *E. coli* did not associate with follicular pads or incite cytopathic alterations of the bursa after intracloacal inoculation of conventional turkeys with *E. coli* serotype 078:H9. Lymphocyte necrosis and depletion, reticuloepithelial hyperplasia and perifollicular fibroplasia are features of follicular degeneration during the normal involution of the bursa. Persistence of *E. coli* within follicular medullae apparently hastens this degenerative process.

Severe cytopathologic alterations within follicular pads and bursal follicles were likely associated with resistance of strain 3 to host defense mechanisms. Strain 3 readily produces K1 capsular antigen which protects *E. coli* from the bacteriocidal activity of complement. Using mouse peritoneal macrophages in the presence of normal serum, K1 antigen was also found to protect *E. coli* from phagocytosis. K1 strains have other properties that promote bacterial invasion into tissue. The presence of Col V plasmid in K1 strains may interfere with phagocytosis of *E. coli* and complement deposition following activation by the alternate pathway. Col V plasmids also enhance the pathogenicity of K1 strains by coding for production of aerobactins that increase bacterial uptake of iron. In this study, the presence of multiple intact bacteria within single phagosomes of macrophages and multinucleate giant cells suggests strain 3 may resist phagocyte killing mechanisms.

Uptake of *E. coli* by the bursal follicular-associated epithelium and their presentation to the follicular medullae may enhance systemic immunologic responsiveness to *E. coli* in the turkey and chicken.
Intracloacal inoculation of killed *Brucella abortus* results in high serum antibody titers to *brucella.* Retardation of both bursal development and immune reactivity occurs with ligation of the bursal duct at the 19th day of incubation. A marked increase in plaque-forming cells in the spleen occurs after intracloacal antigen administration in chicks. Since 15% of the intestinal coliform population of a healthy chicken is composed of pathogenic strains of *E. coli,* contact of pathogenic *E. coli* with the bursal lymphoid tissue would readily occur and may represent a mechanism to enhance humoral resistance to systemic infection with *E. coli.*
REFERENCES


SUMMARY AND DISCUSSION

Both highly and weakly virulent *E. coli* colonize and persist throughout the intestine of gnotobiotic turkeys. Persistently high numbers of *E. coli* in the cecum and colon of gnotobiotic turkeys is indirect evidence for a strong bacterial-mucosal association. The role of capsular, cell wall and type 1 pilus antigens in promoting *E. coli* colonization of the intestinal mucosa is incompletely understood; however, persistence of *E. coli* in washed intestinal segments likely involves mucosal receptor-bacterial ligand interactions.

Translocation of *E. coli* from intestine to liver likely occurs early in *E. coli* infection in gnotobiotic turkeys. In germ-free and conventional chicks, it has been reported to occur primarily in the first 4 days of life. However, invasion of *E. coli* through the intestine may not represent an important mechanism in the pathogenesis of colisepticemia in conventional turkeys where the native intestinal microflora may also inhibit bacterial translocation. In gnotobiotic mice, translocation of *E. coli* from the gastrointestinal tract to mesenteric lymph nodes is inhibited by the normal cecal flora.

Type 1 pili do not play a significant role in adherence of pathogenic *E. coli* to intestinal epithelium of turkeys. Piliated organisms were not found adherent to intestinal epithelium from segments of intestine heavily colonized. A direct relationship between piliation and virulence was not clearly demonstrated in our *in vivo* model since piliated forms of both highly and weakly virulent strains were in the mucus blanket of the cecum and colon. High serum agglutinating antibody
titers to type 1 pili in turkeys inoculated with strain 3 (02) provides additional evidence for in vivo expression of type 1 pili by a weakly virulent strain.

Phase variation between a piliated and non-piliated form of E. coli occurs within cecal and colonic microenvironments of the turkey. Factors promoting pilus phase variation in the turkey cecum and colon are undefined but likely involve genetic and environmental influences on bacterial phenotype.\(^{26,27}\) The predominance of a non-piliated phase of strain 1 in cecal and colon contents was interesting since the piliated form predominates when this strain is cultured for 72 hours in nutrient broth (Dominick, NADC, USDA, Ames, IA, unpublished data).

Type 1 pili may promote bacterial trapping in intestinal mucus of the turkey or adherence to specific receptors in mucus. This is supported by our observation of partitioning, although incomplete, of piliated and non-piliated forms of E. coli into bacteria associated with the mucus blanket and intestinal contents, respectively. Since intestinal mucus readily adheres to intestinal epithelium, association of E. coli with the mucus blanket would provide an alternate mechanism for adherence to a mucosal surface.\(^{107,108}\)

Contact of E. coli with the cloacal bursa occurs as organisms are shed in the feces. We have demonstrated that E. coli can pass through the follicular-associated epithelium and replicate within follicular medullae of the cloacal bursa of the turkey. Intact bacteria have not been seen in follicular pads or follicular medullae of chicken bursae after intracloacal administration of E. coli,\(^{97}\) Staphylococcus albus,\(^{97}\)
or *Brucella abortus*. However, sequential phagocytosis, transport and exocytosis of intact bacteria by follicular-associated epithelium (M cells) has been described in rabbit Peyer's patches.

Our studies indicate that pyogranulomatous inflammation of the cloacal bursa is a feature of colibacillosis in the turkey. In a previous study, morphologic alterations of the bursa were not seen after intracloacal inoculation of conventional turkeys with *E. coli* serotype 078:H9. The severe cytopathologic alterations of follicular pads and bursal follicles were likely associated with bacterial resistance to host defense mechanisms.

From these studies, we conclude that colonization and persistence of *E. coli* in the intestinal tract of gnotobiotic turkeys is not correlated with virulence. Secondly, translocation of *E. coli* from intestine to liver in the turkey is likely inhibited by the indigenous microflora of the intestine. Thirdly, type 1 pili do not mediate in vivo adherence of pathogenic *E. coli* to intestinal epithelium of turkeys but may promote bacterial trapping in intestinal mucus or adherence to specific receptors in mucus. Lastly, *E. coli* can penetrate the follicular-associated epithelium of the cloacal bursa and cause extensive follicular necrosis.
REFERENCES


8. Soerjadi, A. S.; Stehman, S. M.; Snoeyenbos, G. H.; Weinack, O. M.; Smyser, C. F. Some measurements of protection against para-
typhoid Salmonella and Escherichia coli by competitive exclusion

and generation time of Escherichia coli that are virulent or

Identification and isolation of somatic pili from pathogenic

11. Harry, E. G. The survival of Escherichia coli in the dust of

environmental contamination with septicaemia strains of
Escherichia coli and their incidence in chickens. Vet. Rec. 77:
241-245; 1965.

13. Carlson, H. C; Whenham, G. R. Coliform bacteria in chicken broiler
house dust and their possible relationship to colisepticemia.

14. Harry, E. G. A study of 119 outbreaks of colisepticemia in

Calnek, B. W.; Helmboldt, C. F.; Reid, W. N.; Yoder, H. W. ed.
Diseases of poultry. Ames, Iowa: Iowa State University Press;
1978.


58. Bar-Shavit, Z., Ofek, I.; Goldman, R.; Mirelman, D.; Sharon, N.
Mannose residues of phagocytes as receptors for the attachment of

59. Perry, A.; Ofek, I.; Silverblatt, F. J. Enhancement of
mannose-mediated stimulation of human granulocytes by type 1
fimbriae aggregates with antibodies on Escherchia coli surfaces.

60. Smith, H. Microbial surfaces in relation to pathogenicity.


62. Glynn, A. A.; Howard, C. J. The sensitivity to complement of
strains of Escherichia coli related to their K antigens.
Immunology 18: 331-346; 1970.

63. Howard, C. J.; Glynn, A. A. The virulence for mice of strains of
Escherichia coli related to the effects of K antigens on their
resistance to phagocytosis and killing by complement. Immunology

64. McCabe, W. R.; Carling, P. C.; Bruins, S.; Greely, A. The relation
131: 6-10; 1975.

65. Stendahl, O.; Normann, B. Surface characteristics of Escherichia
coli strains in relation to development of bacteremia. Acta


80. Banks, W. J. ed. Histology and comparative organology: A
text-atlas. Baltimore, MD: Williams and Wilkins Co.; 1974;
191-192.
81. Imondi, A. R.; Bird, F. H. The turnover of intestinal epithelium
82. Chodnick, K. S. A cytological study of the alimentary tract of the
domestic fowl (Gallus domesticus). Q. Jl. Microsc. Sci. 88:
419-443; 1947.
83. Toner, P. G. Fine structure of the argyrophil and argentaffin
cells in the gastro-intestinal tract of the fowl. Z. Zellforsch.
63: 830-839; 1964.
84. Penttila, A. Enterochromaffin cells in the chicken duodenum during
prenatal development. Z. Zellforsch. 91: 380-390; 1968.
85. Weinack, O. M.; Snoeyenbos, G. H.; Smyser, C. F. Reciprocal
competitive exclusion of salmonellae and Escherichia coli by
native intestinal microflora of the chicken and turkey. Avian
86. Fuller, R.; Jayne-Williams, D. J. Resistance of the fowl (Gallus
domesticus) to invasion by its intestinal flora. II. Clearance
1970.
87. Berg, R. D.; Owens, W. E. Inhibition of translocation of viable
Escherichia coli from the gastrointestinal tract of mice by

89. Olah, I.; Glick, B. The number and site of the follicular epithelium (FE) and follicles in the bursa of Fabricius. Poultry Sci. 57: 1445-1450; 1978.


103. Firth, G. A. The normal lymphatic system of the domestic fowl. 

104. Payne, L. N. The lymphoid system. Bell, D. J.; Freeman, B. M. 

105. Toner, P. G. The fine structure of the globule leukocyte in the 

106. Fuller, R.; Jayne-Williams, D. J. Resistance of the fowl (Gallus 
    domesticus to invasion by its intestinal flora. II. Clearance 
    1970.

107. Etzler, M. E. Lectins as probes in studies of intestinal 
    1979.

108. Forstner, J.; Taichman, N.; Kalnins, V.; Forstner, G. Intestinal 
    goblet cell mucus: Isolation and identification by 

109. Sorvari, T.; Sorvari, R.; Ruotsalainen, P.; Toivanen, A.; Toivanen, 
    P. Uptake of environmental antigens by the bursa of Fabricius. 

110. Sorvari, R.; Sorvari, T. E. Bursa Fabricii as a peripheral 

ACKNOWLEDGMENTS

I wish to first express my sincere thanks to my wife, Sally, for the love, support and encouragement she provided throughout my graduate training. Special thanks go to my son, Joseph, for the timely and pleasant diversions he provided me during the more difficult moments in the preparation of this dissertation.

I extend my gratitude to my co-major professors, Drs. Norman F. Cheville and John P. Kluge for the guidance and support they provided during my graduate training in pathology. I would also like to thank Dr. Cheville for the influence he has had on my personal development and professional direction. The support and suggestions of the other members of my graduate committee, Drs. Lawrence H. Arp, William P. Switzer and Charles O. Thoen are deeply appreciated.

I am forever grateful for the invaluable technical expertise provided by Allen Jensen in bacteriologic techniques, Karen Schlueter and Phil Ekema in electron microscopy, John Flickinger, Paul Schlotfeldt and Cindy Martens in histology and special stains, Wayne Romp, Tom Glasson and Gene Hedberg in photography and illustrations and Janice Eifling and Carol Moeller in library services. My thanks also to Janice Olson for the excellent job done in typing this dissertation.

My sincere gratitude is extended to Dr. Peter J. Matthews, Animal Supply Officer, and his coworkers E. Deane Dennis, Roger Spaete, Ervin Zook, Martha Church, Cindy Blessing and Syd Hartman for their efforts in providing me with gnotobiotic turkeys. Without their assistance this research could not have been undertaken.
Lastly, I am sincerely grateful to Dr. Phillip O'Berry for providing me with the equipment and facilities necessary to complete this research.