The enteritis complex in domestic rabbits: A field study

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Abstract
A study of the causative agents of enteritis in domestic rabbits from 44 different accessions is described. In descending order of frequency, the organisms most commonly demonstrated were intestinal and hepatic coccidia (Eimeria species), Escherichia coli, Clostridium spp., Salmonella, Bacillus piliformis, and rotavirus. The species of Eimeria identified included those moderately pathogenic and coccidia of low pathogenicity. Using seven antisera against known enteropathogenic strains of E. coli, only one strain, 015, was identified in three cases. Clostridium perfringens or C. sphaeroforme was demonstrated in the intestinal contents in 11 cases, and lesions compatible with clostridial enteropathy were identified on gross and histopathology. In a serological survey, over 50% of 200 fryer rabbits submitted to Ontario abattoirs and of animals from commercial rabbitries had detectable antibody to rotavirus, indicating the widespread distribution of rotavirus infections in this species. In the cases of enteritis studied, two or more potentially pathogenic organisms were frequently identified, emphasizing that several different organisms may be acting in concert to produce clinical disease.

Résumé
Le complexe de l'entérite du lapin domestique : une étude sur le terrain
Cette étude porte sur l'identification des agents responsables de l'entérite du lapin domestique à partir de 44 dossiers. Par ordre décroissant de fréquence, les organismes isolés ont été une coccidie intestinale et hépatique du groupe Eimeria, E. coli, Clostridium spp., Salmonella, Bacillus piliformis et rotavirus. Du groupe Eimeria, les auteurs ont identifié des coccidies dont le pouvoir pathogène est de faible à modéré. Une seule souche de E. coli (015) a été démontrée chez trois sujets; l'épreuve utilisée comportait sept antisérum contenant des souches entéropathogènes connues de E. coli. Clostridium perfringens ou C. sphaeroforme a été isolé du contenu intestinal dans 11 cas. Les lésions macroscopiques et microscopiques étaient compatibles avec une entéropathie à Clostridium. Lors d'une évaluation sérologique, plus de 50 % des 200 lapins de consommation soumis aux abattoirs en Ontario provenant d'animal d'élevage commercial présentaient des anticorps contre le virus rotavirus. Ceci tend à démontrer que les infections causées par le virus rotavirus sont répandues chez cette espèce. Dans le cas d'entérite à l'étude, les auteurs ont souvent identifié deux organismes pathogènes ou plus, soulignant ainsi le fait que plusieurs agents pourraient agir en concert pour induire la maladie clinique.

(Traduit par Dr Thérèse Lanthier)

Introduction
The commercial rabbit industry is a viable, but frequently overlooked, agricultural enterprise in Canada. In Ontario, there are approximately 400,000 rabbits slaughtered for meat annually. This represents an estimated retail value of $4,000,000 (1). The majority of rabbits are marketed at 8–12 weeks of age as “fryers”, when weighing approximately 2.3–2.5 kg live weight. Adult animals are sold as “roasters”. The enteritis complex is considered to be a major cause of disease, mortality, and economic loss in domestic rabbits, particularly in younger animals (2). The term “enteritis complex” is used to denote the multiple factors that may play a role in the manifestation of this disease. It also indicates the complexity of the disease syndrome and serves to emphasize our inability to identify consistently the primary causative agent in outbreaks of diarrhea in commercial rabbits. Infectious agents known to play a role include intestinal and hepatic coccidia, and infections with bacteria such as Escherichia coli, Clostridium spp., and Bacillus...
*Piliformis*. Dietary and management factors are also recognized to have an effect on the incidence of enteric disease (3,4). The purpose of the study was to investigate the etiologies associated with enteric disease in domestic rabbits in this region using light microscopy, anaerobic and aerobic bacterial cultures, virology, serology, and parasitology.

**Materials and methods**

Producers in Ontario were requested to submit live animals from outbreaks of enteritis in rabbitries. Additional animals were also acquired from research facilities and from veterinarians at regional diagnostic laboratories in the province who referred selected cases for this study. Wherever possible, live animals were obtained for detailed examination. One to four animals were included in each accession. Also included were cases submitted to the necropsy service of the Pathology Department and the Veterinary Laboratory Services (VLS) at the Ontario Veterinary College over a three year period (1988–1991). The Pathology Department and VLS laboratories provide separate services conducted in the same facility. When additional submissions were received from the same premises during the study, comparisons of the nature of problems were made over time. Visits were made to two local abattoirs that process rabbits (Abate Rabbitry, Arthur, Ontario, and Sargent Farms, Milton, Ontario) and blood samples were collected to screen serum samples for antibodies to lapine rotavirus in commercial rabbits.

**Pathology**

Animals submitted live were euthanized by an overdose of sodium pentobarbital (MTC Pharmaceuticals, Cambridge, Ontario). A complete necropsy was performed; specimens submitted for histopathological examination included various regions of small and large intestine, lung, liver, spleen, and kidney. Tissue samples were fixed in neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. In rabbits with hepatic and/or intestinal lesions suggestive of Tyzzer’s disease, paraffin-embedded tissue sections were stained with the Warthin-Starry method in order to demonstrate the typical bacilli in affected tissues.

**Parasitology**

Fecal samples were collected from the colon of rabbits at necropsy. Using fecal flotation, preliminary examination was performed to screen for coccidia. Criteria used to quantitate the infection were as follows: 1–100 oocysts per cover slip on a microscopic slide = +; 100–300 oocysts = + +; >300 oocysts per slide = + + +. Selected positive samples were then incubated in potassium dichromate at room temperature for two to four days to facilitate sporulation and the identification of *Eimeria* species.

**Bacteriology**

Samples of ileal and cecal contents were collected and either stored at −70°C until bacteriology was performed, or submitted directly for bacterial culture. Direct smears were made from the ileal and cecal contents, stained by Gram’s method, and examined for the presence of any predominating bacterial type. Ileal and cecal contents were plated on MacConkey’s agar and incubated for 24 h at 37°C in an ambient oxygen atmosphere. Organisms were identified by conventional methodologies (5). In selected cases, *Escherichia coli* colonies were examined by the slide agglutination test using antiserum against known serotypes of attaching-effacing enteropathogenic *E. coli* (AEEC) previously associated with diarrhea in commercial rabbits in Belgium (6). These included serogroups O109 and O2 (suckling rabbits); O15, O20, O103, O128, and O132 (weanling rabbits). Antisera were purchased from the *E. coli* Reference Center, Pennsylvania State University. Isolates of salmonellae were submitted to the Ministry of Health, Toronto, Ontario for typing. In cases where clostridial infections were suspected, cecal contents were also plated directly on a selective medium for *Clostridium difficile* (CD agar) and incubated under anaerobic conditions in an anaerobic jar with a GasPak (BBL Microbiology Systems, Cockeysville, Maryland, USA) for 48 h at 37°C. Colonial types isolated on CD agar were stained by Gram’s method and suspected *C. difficile* colonies were tested with a *C. difficile* latex slide agglutination kit (Seroab *C. difficile* latex slide agglutination kit, Disposable Products, Adelaide, South Australia). A selective procedure using alcohol shock was used for the isolation of *C. perfringens*, *C. difficile*, and *C. spiroforme* spores from cecal contents (7) prior to plating on a prerduced blood agar plate prepared for *Brucella* spp. and a CD agar plate for anaerobic culture. All colony types were described and numbers noted. When lesions were observed in the respiratory tract at necropsy, samples were collected for aerobic culture.

**Virology**

In randomly selected cases with watery diarrhea deemed compatible with rotaviral infection, cecal contents were collected for detection of rotavirus or rotaviral antigen. One of two methods were used: Either direct electron microscopic examination of fecal contents for the presence of viral particles, or the screening of intestinal contents for viral antigen by an enzyme-linked immunosorbent assay (ELISA) (Testpak Rotavirus, Abbott Laboratories, Abbott Park, Illinois, USA) (8). The procedure entails a colorimetric change in positive fecal samples treated with antirotavirus antibody and alkaline phosphatase conjugate (8). Mouse rotavirus-infected cells fixed to glass slides (supplied by Dr. A.L. Smith, Comparative Medicine, Yale School of Medicine) were the source of viral antigen for testing sera for antibody to lapine rotavirus. For the testing of sera for antibody, samples were diluted 1:10 in phosphate-buffered saline. Fluorescein-labelled antirabbit globulin (Antibodies Inc., Davis, California, USA) and the indirect fluorescent antibody technique were used to test for rotaviral antibody. Specimens for immunofluorescent microscopy were examined using a microscope with an ultraviolet light source, and fluorescence was observed in rotavirus-infected cells in positive samples.
Results
A total of 44 separate accessions from 28 different premises were included in the study. The accessions according to age were as follows: Rabbits aged 10 days - 5 weeks: 6; 6-12 weeks: 32; and over 12 weeks: 6. Multiple accessions from the same premises (P) were as follows: P#1: 7; P#2: 3; P#3: 2; P#23: 3. All of the other accessions studied were single submissions from different premises. Morphological diagnoses based on both macroscopic and microscopic findings were variable, and are included under the specific etiological categories.

Intestinal and hepatic coccidiosis
Coccidial oocysts were demonstrated in approximately 70%, or in 24 of the 34 accessions examined for coccidia (Figure 1). Where speciation was performed, the species identified were as follows: *Eimeria media*: 78%; *E. magna*: 56%; *E. stiedai*: 44%; *E. perforans*: 11%. Mixed infections with *Eimeria* species frequently occurred. Three species were identified in 3/9 samples; two species in 2/9 samples; and one species in 4/9 samples. In over 80% of cases with a relatively high oocyst count, there were lesions compatible with coccidiosis. Lesions in the small and/or large intestine were characterized by edema of the lamina propria, leukocytic infiltration, and mucosal changes that varied from hyperplasia of enterocytes to sloughing and denuding of affected areas of intestine. In animals with hepatic coccidiosis, lesions were characterized by proliferative cholangitis and pericholangitis with perportal fibrosis. In seven of the accessions with coccidiosis, significant numbers of *E. coli* was also recovered on culture from the intestinal tract.

Bacterial infections
*Escherichia coli* was isolated in significant numbers from 15 accessions (Figure 1). Frequently there were concurrent infections. Other organisms associated with coliform infections included coccidia (7 of 15); *Clostridium* spp. (4 of 15); and *Pseudomonas aeruginosa* (3 of 15). Of those isolations of *E. coli* tested with specific antisera, 3 of 10 were identified as serotype O15 in weanling rabbits. None of the isolations tested were positive with the six other sera tested. Therefore, the majority were interpreted to be non-pathogenic strains. In eight accessions with intestinal lesions attributed to be primarily due to *E. coli*, there was blunting and frequently fusion of intestinal villi in affected areas, with proliferation to sloughing of enterocytes and leukocytic infiltration in the lamina propria, heterophils predominating. Variable numbers of bacilli were adherent to the intestinal mucosa in these animals.

The presence of large numbers of clostridia were demonstrated in intestinal contents by direct smear and/or anaerobic bacterial culture in several outbreaks of diarrhea. Isolates were identified as *C. perfringens* or *C. s. diroforme*. Bacteria identified as *C. s. diroforme* had the typical helically-coiled appearance on direct smear of ileal or cecal contents. Cecal lesions consistent with a clostridial infection were characterized by marked edema of the lamina propria, frequently with sloughing of mucosa and focal hemorrhage. Cecal lesions compatible with a *Clostridium*-associated enteropathy were observed in eight accessions, but we were unable to consistently recover organisms of this genus.

*Salmonella* subtype IV was recovered from the small intestine of one animal with enteritis. *Salmonella heidelberg* was isolated from a diarrheic rabbit from another premises. In both cases, there was concomitant intestinal coccidiosis.

Tyzzer's disease was diagnosed in two outbreaks. One epizootic was characterized by moderately high morbidity, profuse diarrhea, and high mortality in affected animals. The other outbreak was in a research facility where the disease was characterized by sporadic deaths associated with profuse diarrhea. On microscopic examination, there was focal hepatic necrosis and a necrotizing transmural typhlitis and colitis, similar to those previously described (9,10). Organisms typical of *B. piliformis* were demonstrated in sections stained by the Warthin-Starry method. One epizootic occurred in a commercial rabbitry. The mortality rate in affected animals was high, and medication with antibiotics in the drinking water had no appreciable effect on the course of the disease. In the other case which occurred in a university setting, other complications included suspected clostridial enteropathy.

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Viral infections
Three of 15 fecal samples tested for rotavirus, using either the ELISA technique or direct electron microscopy, were positive. Rotaviral antibody was present in sera of 9 of 12 and four of four rabbits of various ages from two commercial rabbitries. In the serum samples from fryer rabbits collected at slaughter, antibodies to rotaviral antigen was demonstrated in approximately 60% of the samples tested as follows: Abattoir A: 52 of 100 tested were positive; and from abattoir B: 67 of the 100 samples tested had detectable antibody to rotavirus, using the indirect fluorescent antibody technique. However, no histological changes were detected which were considered to be specific for rotaviral diarrhea.

Mucoid enteropathy
Lesions compatible with mucoid enteropathy were identified in two submissions from one small commercial rabbitry, and in submissions from three other outbreaks of enteric disease. All five submissions had a significant level of intestinal coccidia. The disease was observed in animals that had survived the acute stages of the enteritis complex. Affected animals were usually thin, depressed, and dehydrated. Thick, viscid, transparent to translucent gelatinous material and a few hard fecal pellets were present in the colon.

Discussion
Coccidiosis
Intestinal infections with coccidia of the genus *Eimeria* occur regularly in commercial rabbitries, particularly during the postweaning period. Subclinical infections frequently occur, and infections with two or more species of *Eimeria* are common (2,11). Although rabbits ingest their "night feces" daily (cecotrophy), autoinfections via this route should not be a problem, since incubation at room temperature for at least several hours is required for the oocysts to sporulate and become infectious. Thus regular and thorough sanitation of cage floors is critical in the control of the disease. Of the species identified in our study, *E. magna* and *E. stiedai* which are associated with intestinal and hepatic coccidiosis, respectively, are considered to be the most pathogenic, while *E. media* and *E. perforans* are interpreted to be relatively non-pathogenic (11). There is a marked variation in the pathogenicity among the different strains of intestinal coccidia (11), so the significance of a positive oocyst count depends on the species of *Eimeria* identified on fecal flotation. Following the collection of fecal samples, incubation of the oocysts at room temperature and subsequent speciation is recommended. Oocysts of *E. stiedai*, the cause of hepatic coccidiosis, were identified in six cases, and in four there was concurrent intestinal coccidiosis. Hepatic coccidiosis is a relatively common finding in fryer rabbits. Up to 20% of livers from fryer rabbits may be affected when inspected at slaughter. Depending on the extent of the liver lesions, animals may be in relatively good flesh, emphasizing the variation in the response that is observed postinfection with *E. stiedai*, ranging from death to subclinical disease (Percy DH, unpublished observations). Thorough and regular sanitation of cages is the recommended method for controlling the level of coccidial infestations in commercial rabbitries. At the present time, robenidine is considered by many to be the drug of choice. Robenidine appears to be effective against most strains of intestinal coccidia (13), but not *E. stiedai* (14). Medication of breeding does with robenidine may have a negative impact on the number and weight of animals weaned (15). In clinical outbreaks of intestinal coccidiosis, bacteria such as *E. coli* may play an additive role in the intensity of the disease (12).

Bacterial infections
*Escherichia coli* infections: Based on the studies of researchers in Europe, there have been over 30 strains of AEEC identified in domestic rabbits (6). The bio-types and serogroups of *E. coli* infections of weaned rabbits have been identified (16). There appears to be an age-related virulence pattern (6). *Escherichia coli* may also play a role as an opportunistic infection in cases of enteritis. For example, there is frequently a several-fold increase in intestinal coliform counts in rabbits with clinical evidence of coccidiosis (12). In Western Europe, the distribution of AEEC strains varies with the area studied (16). To our knowledge, there have been no such studies reported in Canada. Of 129 *E. coli* strains from domestic rabbits submitted to The *E. coli* Reference Center, Pennsylvania State University, for serotyping since 1970, serotype O15 was identified from seven cases, serotype O103 from 11 cases, O128 from two cases, O132 from five cases, and O2 from two cases. Serotypes O20 and O109 were not identified (Davis M, The *E. coli* Reference Center, personal communication). These results indicate that at least five of the *E. coli* serotypes associated with enteritis in rabbits in Europe do occur in North America. However, we may have failed to detect additional strains of enteropathogenic *E. coli* by the use of a limited range of antisera. Additional studies will be required in order to determine the AEEC strains of the organism associated with the disease in Canada.

Clostridial infections: "Carbohydrate overload" is a term frequently used in association with clostridial enteropathy in domestic rabbits. The syndrome has frequently been associated with feeding rabbits on a high carbohydrate-low fiber diet. Rabbits ingesting a high energy feed may fail to degrade the majority of carbohydrate (e.g. starch) in the small intestine, resulting in the passage of abnormally high levels of carbohydrate into the large intestine. This substrate may then promote the overgrowth of bacteria such as clostridia, leading to disturbances in the osmolality of intestinal contents, fluid accumulation in the large intestine, diarrhea, production of enterotoxins, and death (4). With one exception, in the outbreaks of enteritis investigated in this study, all rabbits were fed standard commercial rations. Clostridial enteropathy has also been associated with antibiotic treatment, leading to disruption of the normal gut flora (3,17). *Clostridium spiroforme* has been identified as the most likely candidate in many outbreaks in Western Europe, including Britain (18,19), and in Australia (7). It has been suggested that the normal microbial ecology must be disrupted before *C. spiroforme* can colonize in the
intradie (3,19). The organism has a characteristic “corkscrew” appearance in preparations stained by the Gram method, and is considered to be a primary cause of diarrhea with mortality (18). The isolation and positive identification of the organism requires anaerobic culture, preferably with positive identification of the enterotoxin (18). The demonstration of organisms with the typical morphology of *C. spiroforme* was made in three separate submissions. Confirmation of the species was obtained in the one isolate that was submitted to an independent laboratory (Lynch JA, personal communication). However, in spite of the low recovery rate of *C. spiroforme*, there were cases where the history and lesions observed were most compatible with this diagnosis. This serves to emphasize the technical difficulties involved in consistently isolating and demonstrating members of this genus. In one previous study, *C. perfringens* was isolated from only 50% of the cases attributed to this organism, and other cases were confirmed only by demonstrating the type E iota toxin in cecal contents by the mouse inoculation test (4). This procedure was not routinely performed in our study, in part because of animal welfare issues. The presence of potentially pathogenic strains such as *C. perfringens* in fecal samples does not necessarily indicate that the organism is the primary pathogen in the disease process. Throughout this study, there were frequently two or more potentially pathogenic bacterial species recovered from an outbreak of enteritis, emphasizing that it is likely that more than one organism (bacterial, parasitic, or viral) may be contributing to the problem.

*Salmonella* infections: The isolation of *Salmonella* subtype IV and *S. heidelberg* from rabbits from two different premises was an unexpected finding. Both isolates were associated with concurrent intestinal coccidiosis. Isolations of salmonellae from domestic rabbits are relatively rare (20). In both cases in our study, they were interpreted to be copathogens and not the primary cause of the enteritis. There was no prior history of salmonellae infections in these rabbitries, and there was no evidence of systemic disease. The zoonotic potential for infections of this type warrants further study.

Tyzzer’s disease: This disease has been associated with some outbreaks of diarrhea with mortality in domestic rabbits. In the two confirmed cases in this study, acute typhlitis and colitis, and focal hepatic necrosis, which are typical of this disease (9,10). In some outbreaks of Tyzzer’s disease, treatment with tetracycline has met with some degree of success (10). The history, high mortality rate in affected animals, and the focal hepatitis coupled with the necrotizing typhlitis were the characteristic lesions observed. Special stains are required in order to demonstrate the typical intracellular bacilli.

**Viral enteritis**
The mouse rotavirus-infected cells used for the detection of antibody in this study have been used previously. Murine rotavirus has proven to be an appropriate antigen for the detection of antibody to lapine rotavirus (Smith AL, Comparative Medicine, Yale School of Medicine, unpublished data). In another study, human rotavirus was the source of antigen. The percentage of seropositive animals grouped by age in that study varied from 69–100% (21). Viral antigen and antirotaviral antibody have been commonly observed in domestic rabbits, both in clinically-affected and asymptomatic animals. In a previous report, rotavirus was detected in the feces of approximately 25% of rabbits with diarrhea and in 10% of clinically normal rabbits (21). Frequently there is no clinical evidence of disease. These findings serve to emphasize the widespread distribution of rotavirus in commercial rabbitries, including subclinical infections. The significance of this virus as a primary cause of enteritis in commercial rabbitries has not been determined. The single confirmed outbreak of enteritis in young rabbits was in specific pathogen-free stock that shed virus and seroconverted following exposure to the virus (22). It is likely that enteric rotavirus may serve as a copathogen in some outbreaks of enteritis in commercial rabbits, but additional studies are required to determine this. Although transient diarrhea has been produced in young rabbits inoculated with a lapine coronavirus (23), the role of rabbit coronavirus in the enteritis complex has not been determined, and at the present time, coronaval infections are not considered to play a major role in the enteritis complex (2). Coronaval particles have not been observed in diarrheic feces from rabbits prepared for examination by direct electron microscopy at this university (Carman S, personal communication).

**Mucoid enteropathy**
Mucoid enteropathy or “mucoid enteritis” is now considered to be a nonspecific change seen in rabbits surviving an outbreak of enteritis with resultant disruption of the intestinal microbial flora, hyperplasia of goblet cells, gut stasis, and excess mucus production. Since rabbits are hind gut fermenters, any disruption in the intestinal environment may be sufficient to cause significant changes. The disease has been produced experimentally by lowering the pH in the cecum, causing marked changes in the gut flora and lesions similar to those seen in the naturally-occurring disease (24). It is a condition most frequently seen in rabbits which survive for one week or more after the onset of enteritis. It is characterized by cecal impaction and the presence of gelatinous mucus in the colon.

In a comparison of the infectious agents identified in a field study of 21 commercial rabbitries in Europe, the pathogenic agents identified in order of frequency were: Attaching-effacing *E. coli*: 40%; rotavirus: 35.4%; coccidia: 18.5%; and multiple agents in 18.5% of animals examined (2). However, enteric disease due to *C. spiroforme* and Tyzzer’s disease have also been identified by these investigators in subsequent studies (10,18).

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References

11. Varga I. Large-scale management systems and parasite popula-