Bacterial enteritis in ostrich (*Struthio Camelus*) chicks in the Western Cape Province, South Africa

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ABSTRACT Ostrich (*Struthio camelus*) chicks less than 3 mo age are observed to experience a high mortality rate that is often associated with enteritis. This study was undertaken to investigate the infectious bacteria implicated in ostrich chick enteritis. Postmortems were performed on 122 ostrich chicks aged from 1 d to 3 mo and intestinal samples were subjected to bacterial culture. Bacterial isolates were typed by PCR and serotyping. *Escherichia coli* (*E. coli*; 49%) was the most frequently isolated from the samples followed by *Clostridium perfringens* (*C. perfringens*; 20%), *Enterococcus* spp. (16%), and *Salmonella* spp. (7%). Of the *E. coli*, 39% were categorized as enteropathogenic *E. coli*, 4% enterotoxigenic *E. coli*, and no enterohaemorrhagic *E. coli* were found. The majority (93%) of *C. perfringens* was Type A and only 7% was Type E. *C. perfringens* Types B through D were not present. The *netB* gene that encodes NetB toxin was identified from 16% of the *C. perfringens* isolated. All the *C. perfringens* Type E harbored the *netB* gene and just 10% of the *C. perfringens* Type A had this gene. Three *Salmonella* serotypes were identified: *Salmonella* Muenchen (*S.* Muenchen; 80%), *S.* Hayindongo (13%), and *S.* Othmarschen (7%). The indication is that the cause of enteritis in ostrich chicks is bacterial-involving: enteropathogenic *E. coli* and enterotoxigenic *E. coli*; *C. perfringens* Types A and E (with the possible influence of *netB* gene); and *S.* Muenchen, *S.* Hayindongo, and *S.* Othmarschen.

Key words: ostrich chick, bacterial enteritis, *Clostridium perfringens*, *Salmonella*, *Escherichia coli*

INTRODUCTION

A mortality rate of 30 to 40% in the first 3 mo life of the ostrich chick (*Struthio camelus*) has come to be accepted as "normal." In certain instances of disease outbreak, mortalities can reach 80 to 100%, and the morbidity of those individuals that remain results in stunted growth (Verwoerd et al., 1998). Enteritis is considered to be a major cause of this mortality which constitutes a major drawback to ostrich farming (Samson, 1997; Huchzermeyer, 2002).

Bacterial infections have been identified as the primary cause of gastrointestinal disease and poor management practices are a recognized contributing factor to infection (Samson, 1997). The bacterial pathogens most frequently involved in infectious enteritis of ostriches are: *Escherichia coli* (*E. coli*), *Campylobacter jejuni*, *Pseudomonas aeruginosa*, *Salmonella* spp., and *Clostridium* spp. (Huchzermeyer, 1998; Herraez et al., 2005; Doneley, 2006). This study aimed to identify the bacterial causes of enteritis in ostrich chicks.

Clostridium perfringens (*C. perfringens*) can cause enteritis in ostrich chicks (Huchzermeyer, 1998). The bacterium is a normal inhabitant of the gut and predisposing stress factors such as change of diet are thought to lead to its proliferation leading to the disease condition (Huchzermeyer, 1998; Gholamiandekhordi et al., 2006). *C. perfringens* is differentiated into Types A through E according to the possession of 4 major toxins α, β, ϵ, and ι (Songer, 1996). The release of these toxins is believed to play a part in the pathogenesis of *C. perfringens* but it is not clear which toxins are involved (Songer, 1996; Crespo et al., 2007). The role of other toxins produced by *C. perfringens*, such as enterotoxin and beta2 toxin, in causing enteritis is considered to be insignificant (Crespo et al., 2007; Keyburn et al., 2010; Smyth and Martin, 2010). The NetB toxin has been implicated as the major contributing factor to enteritis in chickens although other complimentary factors may still exist (Keyburn et al., 2010; Smyth and Martin, 2010).

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E. coli is a prevalent member of the normal intestinal microflora of humans, animals, and birds, generally from birth (Levine, 1987; Oswald et al., 2000; DebRoy and Maddox, 2001). Contaminated environmental sources (vegetation, soil and water) contribute to exposure, soon after birth (Quinn et al., 2011). Some *E. coli* strains are pathogenic and have been associated with specific diseases in humans and animals: gastroenteritis, urogenital disease, septicemia, and pleural infections (Oswald et al., 2000). *E. coli* is commonly isolated from sick ostrich chicks but not much investigation has been done into this intestinal pathogen (Verwoerd et al., 1998; Nardi et al., 2005). Six categories of *E. coli* are recognized to cause intestinal or diarrheagenic disease: enterotoxigenic *E. coli*, enteroinvasive *E. coli*, enteropathogenic *E. coli*, enterohaemorrhagic *E. coli*, enteroaggregative *E. coli*, and diffusely adherent *E. coli* (Levine, 1987; Nataro and Kaper, 1998). In a study by Nardi et al. (2005) only enterotoxigenic *E. coli* was isolated from chicks with clinical signs of enteritis.

Different *Salmonella* serotypes cause enteritis in ostriches especially chicks. The clinical sign of diarrhea is often observed and in some instances sudden death may occur. Other cases may display nonspecific signs of anorexia and depression (Welsh et al., 1997a). No host-specific salmonella serotypes are associated with disease in ostriches unlike in chickens where *Salmonella* Pullorum (*S.* Pullorum) and *S.* Gallinarum are recognized to cause disease (Verwoerd et al., 1998). *Salmonella* serotypes that have been identified as causing enteritis in ostrich chicks include *S.* Typhimurium and *S.* Ituri amongst others (More, 1996; Huchzermeyer, 1998; Welsh et al., 1997a; Welsh et al., 1997b).

MATERIALS AND METHODS

Sampling Area

Samples were obtained from farms which were experiencing problems related to enteritis. The farms were located in the Klein Karoo region of the Western Cape Province of South Africa and were mainly found within a 50 km radius from the town of Oudtshoorn. The sampling area is shown in Figure 1.

The farms were designated Letters A to R according to the order in which they were visited. A total of 18 farms were included in the study. Birds showing no signs of enteritis were sourced from Farm Q and served as normal controls.

Ethical Approval

Ethical approval for the study was received from the Animal Ethics Committee of the Faculty of Veterinary Science, University of Pretoria (Project Number V050-11). Written consent from farmers, of the respective farms where sample collection took place, was obtained after the purpose of the study had been explained to them.

Sampling Procedure

A total of 122 ostrich chicks were sampled during 2011 and 2012. Sampling occurred in March/April and September/October of each yr to coincide with the chick season, when ostrich chicks were available on the farms. The age group of birds sampled was between 1 d and 12 wk age. These were chicks which had died from an enteritis-related problem and moribund chicks showing the principal clinical sign of diarrhea with or without depression, anorexia, and lethargy. The normal controls (n = 6) obtained from Farm Q, were aged between 4 d and 6 wk and displayed no signs of enteritis. These birds were euthanized by cervical dislocation and severance of the spinal cord.

Postmortem examination was performed on dead birds within 8 hours of death in order to minimize the effects of autolytic changes and overgrowth of opportunistic microbial organisms in the carcass. They were stored at ambient temperature in the shade for the period before examination. Intestinal tissues (small intestine, colon, and caecum) were collected. Intestines showing gross lesions indicative of enteritis (inflamed, hemorrhagic, distended, fluid-filled, and pseudomembranous) were sampled. Samples denoted as "intestine" were not characterized as a particular section of intestine at sampling. Samples were sent to the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria for analysis.

Bacterial Culture

The samples were inoculated onto blood and MacConkey agar and incubated aerobically at 37°C for 18 to 24 h. For preliminary identification, the Gram stain, catalase, and oxidase tests were performed on selected colonies. The indole, xylose, urease, and citrate tests were performed to confirm the identification of suspect *E. coli* colonies (Quinn et al., 1994). The API10S (bioMérieux, France, Ref. 10100), miniaturized biochemical sugars test strips were used to identify other *Enterobacteriaceae*.

Salmonella was isolated from the samples by a selective enrichment culture method. Samples were inoculated into buffered peptone water and incubated aerobically at 37°C for 18 to 24 h. From buffered peptone water, samples were inoculated into Rappaport–Vassiliadis broth and incubated aerobically at 41.5°C for 18 to 24 h. Thereafter, samples were cultured on xylose lysine desoxycholate agar and incubated aerobically at 37°C for 18 to 24 h. Colonies, suspected to be *Salmonella* spp., were transferred from xylose lysine desoxycholate agar and grown on blood agar in an aerobic environment at 37°C for 18 to 24 h. The Gram stain, catalase, oxidase, and indole tests were performed to further characterize the isolates. The API10S (bioMérieux), miniaturized biochemical sugars test strips were used to confirm the isolates as *Salmonella*.

Figure 1. Map of South Africa and the Western Cape Province. Sampling area delineated by the circle.

To isolate *Clostridia*, samples were inoculated onto blood agar and incubated anaerobically at 37°C overnight. Suspect colonies were confirmed by Gram stain, catalase, and gelatinase tests and by the characteristic growth on lactose egg yolk milk agar (Willis and Hobbs, 1959; Quinn et al., 1994).

C. perfringens Toxin Typing

DNA was extracted from a pure culture of *C. perfringens*. One to 2 colonies were placed in 100 μL distilled water, boiled at 97°C for 10 min, and centrifuged at 10,000 g for 10 min. The supernatant was used as template for PCR (Keyburn et al., 2008). The concentration of extracted DNA was measured in a spectrophotometer (PowerWave XS2, BioTek, Winooski, VT, Product Number 7301000) and ranged from 53.6 to 443.1 ng/μL.

A multiplex PCR modified from the method described by Yoo et al. (1997) was used. The PCR mixture contained 25 μL Dream Taq PCR Master mix (Thermoscientific), 20 μM each primer, and 3 μL DNA template made up to a total volume of 50 μL with nuclease-free distilled water. The PCR primers are shown in Table 1.

A Veriti 96-well thermal cycler (Applied Biosystems) was used to perform the PCR. The program was as follows: 5 min at 94°C, 30 cycles of 1 min at 55°C, 1 min at 72°C, and 1 min at 94°C (Yoo et al., 1997). The amplified product (10 μL) was then analyzed by electrophoresis using a 2% agarose gel.

Detection of netB Gene

DNA which was extracted for toxin typing of *C. perfringens* was also used as DNA template for the detection of *netB* gene. The PCR mixture was composed of 12.5 μL Dream Taq Green

Table 1. Primers for *Clostridium perfringens* toxin typing (Yoo et al., 1997).

Toxin	Primer	Nucleotide sequence	Amplicon size (bp)
α-toxin	CPA-forward	5′-GTTGATAGCGCAGGACATGTTAAG-3′	402
	CPA-reverse	5′-CATGTAGTCATCTGTTCCAGCATC-3′	
β-toxin	CPB-forward	5′-ACTATACAGACAGATCATTCAACC-3′	236
	CPB-reverse	5′-TTAGGAGCAGTTAGAACTACAGAC-3′	
ε-toxin	CPE-Forward	5′-ACTGCAACTACTACTCATACTGTG-3′	541
	CPE-Reverse	5′-CTGGTGCCTTAATAGAAAGACTCC-3′	
ι-toxin	CPI-Forward	5′-GCGATGAAAAGCCTACACCACTAC-3′	317
	CPI-Reverse	5′-GGTATATCCTCCACGCATATAGTC-3′	

PCR Master Mix (2x) (Thermoscientific), 0.8 μM primers, and 5 μL template DNA. Distilled PCR grade water was used to make up the volume of the reaction mixture to 25 μL. The forward primer was AKP 78 5′-GCTGGTGCTGGAATAAATGC-3′, and the reverse primer was AKP 79 5′-TCGCCATTGAGTAGTTTCCC-3′ (Keyburn et al., 2008), synthesized by Inqaba Biotech, South Africa. A confirmed positive *netB* gene sample was used as the positive control and PCR-grade water as the negative control.

The thermocycler program was performed as previously described (Keyburn et al., 2008). A 1.5% agarose gel was used to analyze the PCR product (5 μL) by electrophoresis at 100 V for 45 min. The size of positive amplicons was determined by the use of a 100 bp molecular marker (Hyperladder IV, Bioline, Western Cape, South Africa, Product Number Bio-33029). A digital image of the gel was taken using a photo documentation system (Molecular Imager ChemiDoc XRS+ System, Bio-Rad, South Africa) for capture and storage of the results.

The positive control for the *netB* gene was developed from the samples obtained in this study. The amplification product of 3 positive samples and the primer AKP 78 were sequenced (Inqaba Biotech, South Africa). The sequencing data was analyzed on the National Center for Biotechnology Information (**NCBI**) website (http://www.ncbi.nlm.nih.gov/) with the Basic Local Alignment Search Tool to determine the degree of similarity between the sequences obtained in this study and those published in Genbank, Accession Numbers FJ189481 through FJ189503. Thereafter, one of these established *netB* gene positive samples was used as the positive control.

E. coli Virulence Factor PCR

Characterization of *E. coli* by detection of virulence factor genes by PCR was carried out by the Bacteriology Section at the Agricultural Research Council, Onderstepoort Veterinary Institute South Africa. Multiplex PCR was performed using the specific primers for genes: LT, STa, STb, EAST1, Stx1, Stx2, Stx2e, *paa*, and AIDA-1 (Mohlatlole et al., 2013). This multiplex PCR determined the presence of enteropathogenic *E. coli* by possession of the *paa* gene; enterotoxigenic *E. coli* by possession of LT, STa, STb genes; and enterohaemorrhagic *E. coli* by possession of Stx1, Stx2, Stx2e genes.

Table 2. Different bacterial species isolated from samples obtained in this study.

Bacteria	Number isolated	Percentage of total samples (n = 151)
E. coli	107	49.1%
C. perfringens	44	20.2%
Salmonella spp.	15	6.9%
Enterococcus spp.	35	16.1%
Klebsiella spp.	4	1.8%
Enterobacter spp.	3	1.4%
Citrobacter spp.	3	1.4%
Staphylococcus spp.	1	0.5%
Streptococcus spp.	1	0.5%
Bacillus spp.	1	0.5%
No bacteria isolated	4	1.8%

Salmonella Serotyping

The slide agglutination test was used for serotyping of *Salmonella* isolates at the Bacteriology Laboratory of the Agricultural Research Council, Onderstepoort Veterinary Institute. The isolates were identified according to the antigenic formula provided by the White–Kauffmann–Le minor scheme (Grimont and Weill, 2007).

RESULTS

Bacteria Isolated From Samples

A total of 151 samples were collected from 122 ostrich chicks and tested for bacteria. Bacterial species isolated from the samples are indicated in Table 2. The most frequently isolated bacterium was *E. coli* (49.1%), followed by *C. perfringens* (20.2%), *Enterococcus* spp. (16.1%), *Salmonella* spp. (6.9%), *Klebsiella* spp. (1.8%), *Enterobacter* and *Citrobacter* spp. (1.4% each), and *Staphylococcus*, *Streptococcus*, and *Bacillus* spp. (0.5% each). There were no bacteria isolated from 1.8% of the samples.

Clinical Signs, Postmortem Findings

The principal presenting clinical sign of the 122 ostrich chicks examined was death. Postmortem revealed varying degrees of intestinal inflammation (indicative

of enteritis) which affected different sections: small intestine, colon, and caecum. Four major symptoms seen prior to death were: diarrhea (n = 47), depression/weakness (n = 27), failure to thrive (n = 5), and respiratory signs (n = 3; Table 3).

Normal Controls

The normal control chicks (n = 6) were in good health with no clinical signs and there were no abnormal findings at postmortem. The bacteria identified from the samples: small intestine, colon, and caecum from each chick (n = 18), in order of decreasing frequency, were *E. coli*, 13 (72.2%); *Enterococcus* spp., 11 (61.1%), *Bacillus* spp., 4 (22.2%) and *C. perfringens*, 3 (16.7%). There was only one sample, a small intestine, from which no bacteria were isolated. No *Salmonella* were isolated from the normal control samples.

Only 3 (23.1%) of the 13 *E. coli* isolates harbored any of the virulence factors tested. The combinations of these virulence factors were EAST1 alone from 2 *E. coli* isolates and EAST1 with *paa* from one *E. coli* isolate. The prevalence of the *E. coli* virulence factors from the normal controls (23.1%) was much less than that from sick birds (79.2%). All the *C. perfringens* isolates from the normal controls were Type A and they did not carry the *netB* gene.

C. perfringens Types and Occurrence of netB Gene

C. perfringens was isolated from the small intestine, colon, and caecum of the ostrich chicks from 12 of the 17 farms. The majority (93.2%) of the isolates were *C. perfringens* Type A which was present on the 12 farms. *C. perfringens* Type E (6.8%) was only isolated from samples collected on Farm B (Table 2).

The *netB* PCR results showed 15.9% of the *C. perfringens* isolates were positive for *netB* gene. All the *C. perfringens* Type E and 9.8% of *C. perfringens* Type A isolates possessed the *netB* gene. The *netB* gene positive isolates were found on Farms B and F.

Sequencing of the netB Toxin Gene

The Basic Local Alignment Search Tool results indicated that the sequence for the *netB* toxin gene had 94% similarity to the sequences, Accession Numbers FJ189481 through FJ189503, available on the NCBI website (http://www.ncbi.nlm.nih.gov/). The sequencing results of the 3 PCR products were identical to each other.

E. coli and Virulence Factor Determination

E. coli were isolated in samples collected from 14 of the 17 farms. *E. coli* was isolated exclusively in 53.3% of the positive samples and isolated with other bacteria in the remaining positive samples.

Table 3. Clinical signs and numbers of *E. coli*, *C. perfringens*, and *Salmonella* spp. isolated from the farms.

		Clinical signs					Bacteria isolated							
								C. perfringens						
Farm	Number of samples	Diarrhea	Sudden death	Depression/ weakness	Failure to thrive	Respiratory signs	*E. coli*	Type A	Type B	Type C	Type D	Type E	*netB*	*Salmonella* spp.
A	28	18	4	3	0	0	23	10	0	0	0	0	0	13
B	23	0	6	11	3	0	23	1	0	0	0	3	4	1
C	31	9	10	0	2	0	25	7	0	0	0	0	0	0
D	7	0	4	0	0	0	6	1	0	0	0	0	0	0
E	5	0	5	0	0	0	5	5	0	0	0	0	0	0
F	4	0	4	0	0	0	4	4	0	0	0	0	3	0
G	6	4	0	0	0	0	3	5	0	0	0	0	0	0
H	3	3	0	0	0	0	3	3	0	0	0	0	0	0
I	3	0	0	3	0	0	3	1	0	0	0	0	0	0
J	2	0	2	0	0	0	1	0	0	0	0	0	0	0
K	3	0	0	0	0	3	1	0	0	0	0	0	0	1
L	8	2	0	6	0	0	2	0	0	0	0	0	0	0
M	1	0	1	0	0	0	0	0	0	0	0	0	0	0
N	19	9	1	0	0	0	4	0	0	0	0	0	0	0
O	2	0	2	0	0	0	0	1	0	0	0	0	0	0
P	4	0	0	4	0	0	4	0	0	0	0	0	0	0
Q	18	0	0	0	0	0	13	3	0	0	0	0	0	0
R	2	2	0	0	0	0	0	2	0	0	0	0	0	0

The characterization of the virulence factors from 106 of the 107 *E. coli* isolates, in this study, revealed that EAST1 was the most prevalent gene (59.4%) as it was identified from 63 *E. coli* isolates. This was followed by: *paa*, 41 (38.7%); AIDA-1, 5 (4.7%); STa, 2 (1.9%); and STb, 2 (1.9%). The virulence factors LT, Stx1, Stx2, and Stx2e were not detected. Twenty-two (20.8%) *E. coli* isolates were negative for the virulence factors tested.

The combination of these genes in the *E. coli* isolates was: EAST1 alone (34.9%); *paa* alone (16.0%); EAST1 and *paa* (19.8%); EAST1 and AIDA-1 (0.9%); EAST1, *paa*, and AIDA-1 (2.8%); AIDA-1 alone (0.9%); STa alone (0.9%); STb alone (1.9%); and STa and EAST1 (0.9%).

The *E. coli* isolates were categorized based on their possession of certain virulence factor genes as indicated in Table 4. Enteropathogenic *E. coli* made up 38.7%, enterotoxigenic *E. coli* 3.8%, and no enterohaemorrhagic *E. coli* was found.

Salmonella Serotypes

Salmonella was isolated from samples collected on 3 farms in this study. The following *Salmonella* serotypes were isolated; *Salmonella* Muenchen (*S.* Muenchen; 80%) *S.* Hayindongo (13.3%), and *S.* Othmarschen (6.7%). *S.* Muenchen and *S.* Hayindongo were isolated from Farm A on 2 separate occasions and only one type was isolated from all samples on each occasion. Farms B and K had one isolate of *S.* Muenchen and *S.* Othmarschen, respectively. *Salmonella* was mostly isolated with other bacteria, mainly *E. coli* and *C. perfringens*. *S. Muenchen* was exclusively isolated from Farm A.

DISCUSSION

E. coli was the most frequently isolated bacterium and found on most of the farms in the study. This finding correlates with the observation that *E. coli* is the predominant enteric bacteria isolated from ostrich chicks suffering from enteritis (Verwoerd et al., 1998). *E. coli* is known to form part of the normal intestinal flora (Levine, 1987) and multiplex PCR for virulence factor encoding genes was performed in order to determine whether any pathogenic strains were isolated.

Approximately 40% of isolates were determined to be enteropathogenic *E. coli* based on the presence of the *paa* gene. Possession of the *eae* gene has been accepted to identify enteropathogenic *E. coli* but considering the finding that the *paa* gene is associated with the presence of the *eae* gene, the *paa* gene was used in this study (Batisson et al., 2003; Toma et al., 2003). It is however acknowledged that ideally this should be confirmed by testing the isolates in this study for the *eae* gene to independently confirm this statement. Approximately 4% of isolates were found to be enterotoxigenic *E. coli* by possession of ST genes only. Enterohaemorrhagic *E. coli* were not found as no Stx genes were present in the isolates. Isolates from which no virulence factor genes were identified (20%) can be considered to be nonpathogenic *E. coli*.

Studies that have investigated pathogenic *E. coli* in ostrich chicks with diarrhea are very limited. One study identified enterotoxigenic *E. coli* possessing the LT gene only, from 4/24 (16.6%), 3-month-old chicks with diarrhea and no enterohaemorrhagic *E. coli* were found by PCR for the presence of the Stx gene (Nardi et al., 2005). Tests aimed at the other categories of diarrheic *E. coli* were not pursued. The low prevalence of enterotoxigenic *E. coli* (4%) and the finding of no enterohaemorrhagic *E. coli* in this study is comparable to what was found by Nardi and co-workers (2005). The enterotoxigenic *E. coli* in the study of Nardi et al. (2005), however, differ in that they possessed the LT gene only whereas those in this study possessed the ST gene only; this signifies differing strains of enterotoxigenic *E. coli* (Kaper et al., 2004).

The majority of *C. perfringens* were Type A and a few were Type E. The few published studies that mentioned necrotic enteritis of ostriches did not identify the toxinotype except for one study which mentioned the isolation of *C. perfringens* Types A and D (Samson, 1997; Huchzermeyer, 1998; Huchzermeyer, 2002; Kwon et al., 2004). *C. perfringens* Type A is considered the principal cause of necrotic enteritis in poultry (Keyburn et al., 2008). Similarly, it appears that *C. perfringens* Type A is more likely to be involved with enteritis in ostrich chicks.

The *netB* gene was identified in 16% of all the *C. perfringens* isolates. Only 10% of the Type A isolates possessed the *netB* gene, whereas all the Type E isolates possessed the *netB* gene. This reports for the first time the presence of *netB* gene on *C. perfringens* Type E isolates, and the isolation of Type E with the presence of the *netB* toxin gene in samples collected from ostrich chicks.

Sequencing results of the *netB* gene fragment amplified in this study indicated a 94% similarity to the *netB* gene sequences (Accession Numbers FJ189481 to FJ189503) on the NCBI website (http://www.ncbi.nlm.nih.gov/). The nature of the product of this *netB* gene and its pathogenicity may have to be determined as it may differ from the NetB toxin described by Keyburn et al. (2008).

Of the 3 different serotypes of *Salmonella* identified in this study, 2 of them, *S.* Hayindongo and *S.* Othmarschen, have not been identified in previous publications of *Salmonella* serotypes isolated from ostriches (More, 1996; Welsh et al., 1997b; Huchzermeyer, 1998; Verwoerd et al., 1998). In cases where *Salmonella* was isolated, a single serotype was identified. That serotype was associated with disease on a single farm at a particular time of disease occurrence (More, 1996). On Farm A, *S.* Hayindongo was isolated in March 2011 and *S.* Muenchen was isolated in the following chick season in September 2011. This was also previously

Table 4. Virulence factor genes identified from *E. coli* isolates.

E. coli category	Determinant	Total number isolates (%)
Enteropathogenic *E. coli*	*paa* positive; LT, ST, and Stx negative	41 (38.7%)
Enterotoxigenic *E. coli*	LT and/or ST positive	4 (3.8%)
Enterohaemorrhagic *E. coli*	Stx positive	0 (0.0%)

reported when *S.* Typhimurium was isolated from all the cases on a single farm from a study of 11 different farms (More, 1996).

The main observation from this study is that the cause of enteritis in ostrich chicks is bacterial-involving: enteropathogenic *E. coli*, enterotoxigenic *E. coli, C. perfringens* Type A and E (with the uncertain influence of *netB* gene), *S.* Muenchen, *S.* Hayindongo, and *S.* Othmarschen. Further studies should focus on confirming the role of these bacteria as etiological agents of enteritis of ostrich chicks in experimental disease models.

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