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Characterization of Multiple Morphologically Different *Escherichia coli* Colonies From Individual Diagnostic Cases

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Abstract

Isolates of *Escherichia coli* are commonly found in the feces of warm-blooded vertebrates as normal flora. However, in some cases, it can cause severe disease. Calf scours is life-threatening diarrhea caused by infectious organisms including *E. coli*. *E. coli* as the causative agent for scours is generally diagnosed using culture methods. In a preliminary effort to discern whether the colony picked from the plate is the probable *E. coli* strain causing the scours, three to five additional colonies of varying morphology were chosen for genetic and phenotypic comparison within a given calf. **Methods:** Thirty-eight *E. coli* sample plates were obtained from the North Dakota Veterinary Diagnostic Laboratory (NDVDL) calf scours cases. The single colony picked by the NDVDL for PCR analysis was used as comparison against three to five additional colonies isolated from each individual case. All colonies were analyzed for antibiotic resistance, phylogenetic grouping (ECOR), pulsed-field gel electrophoresis (PFGE), and multiple toxins including shiga-toxin (Stx1, Stx2) and heat-stable toxin (Sta) along with adhesion (K99, F41) and Intimin genes. **Results:** The majority of individual scour cases were found to have at least one additional isolate differing in PFGE and antibiotic resistance patterns in addition to ECOR grouping. There was insignificant variation among PCR results for toxins, adhesion, and Intimin genes. However, for those isolates differing genetically, the antibiotic resistance patterns were also different. **Conclusions:** The results indicate that the diagnostic isolate was identical or closely related to some of the additional colonies chosen within a given case. However, among most of the scour cases, genetically diverse *E. coli* were isolated. Future research should include virulence testing of these isolates to ensure that the diagnostic lab isolate is indeed the etiological agent in these calf scour cases.

Materials and Methods

PFGE was performed according to the protocol described by the Center for Disease Control and Prevention[4] using the molecular size standard *Salmonella braenderup* BAA-664 (ATCC, Manassas, VA). Samples were further characterized using the BioNumerics software (Applied Maths, Austin, TX).

Antibiotic Resistance was determined by the Kirby-Bauer method[1] using the BBL Sensi-disc susceptibility test discs (BD Diagnostic Systems, Franklin Lakes, NJ) with the following antibiotics: Ampicillin, Amoxicillin/Clavulonic Acid, Amikacin, Chloramphenicol, Ciprofloxacin, Cefoxitin, Sulfosoxazole, Kanamycin, Naladixic Acid, Streptomycin, Tetracycline, and Cephalothin. Experimental antibiotics were chosen based on availability and cost. The *E. coli* strain 25944 (ATCC) was used as a positive control. Zone measurements were determined by the BIOMIC V[3] (Giles Scientific, Santa Barbara, CA).

PCR analysis for ECOR grouping and virulence factors was completed according to the methods previously described[2,3]. DNA was extracted by inoculating *E. coli* isolates on MacConkey agar plates and incubating overnight at 37°C. A single colony of each isolate was added to 40 µL of TE buffer with 1% 20 mg/mL proteinase K. Samples were incubated at 55°C for 10 min followed by a 10 min incubation at 80°C. DNA was then diluted with 80 µL of sterile water, centrifuged for 5 min, and stored at -20°C for PCR analysis.

Table 1: Primers used in Virulence Factor PCR

Virulence Factor	Forward 5'-3'	Reverse 5'-3'
Stx1	TTCGCTCTGCAATAGGTA	TTCCCCAGTTCAATGTAAGAT
Intimin	ATATCCGTTTTAATGGCTATCT	AATCTTCTGCGTACTGTGTTCA
F41	GCATCAGCGGCAGTATCT	GTCCCTAGCTCAGTATTATCACCT
K99	TATTATCTTAGGTGGTATGG	GGTATCCTTTAGCAGCAGTATTTC
Sta	GCTAATGTTGGCAATTTTTATTTCTGTA	AGGATTACAACAAAGTTCACAGCAGTAA
Stx2	GTGCCTGTTACTGGGTTTTTCTTC	AGGGGTCGATATCTCTGTCC

Results

Number of PFGE Profiles Within Individual Cases

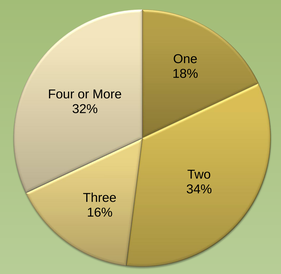
- Of the 38 cases, 35 contained one or more experimental isolate identical to the clinical isolate

Number of Cases with Positive PCR Results

Virulence Factor	Clinical	Experimental
Stx2	0	2
Sta	8	9
K99	8	8
F41	8	8
Intimin	1	7
Stx1	1	6

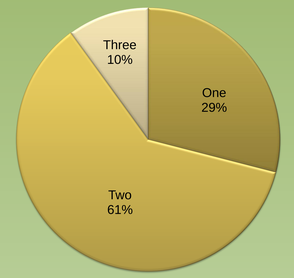
- 53% of cases tested negative for all virulence factors (VFs) among both clinical and experimental isolates
- A total of 9 cases contained experimental isolates testing positive for one or more VFs when the associated clinical isolate tested negative

Number of Antibiotic Resistance Profiles Within Individual Cases Determined by Experimental Antibiotics



- 7 of the 38 cases contained the same antibiotic profile among all isolates
- Of the cases with the same antibiotic profile, 3 cases had isolates with differing PFGE profiles and ECOR groupings. Among these 3, one case differed in VFs

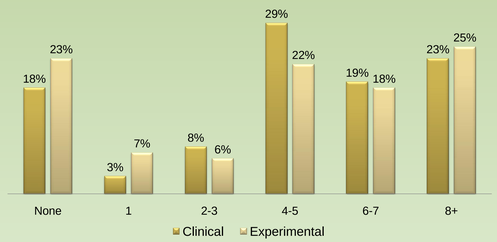
Percentage ECOR Groups Among Cases



- 16 of the 18 cases with isolates testing positive for one or more VFs fell into the ECOR group A or B1, historically considered to be non-pathogenic
- Of the 11 cases containing isolates with identical ECOR grouping, 4 cases contained isolates differing in PFGE, antibiotic resistance, and VF profiles

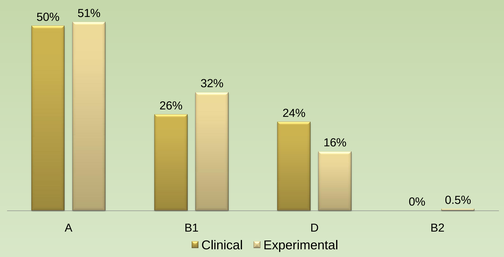
Percent Isolates Resistant to Particular Number Antibiotics

Clinical vs. Experimental



Distribution of ECOR Groups

Clinical vs. Experimental



Discussion and Conclusions

- **According to PFGE results, the majority of cases did contain more than one E. coli isolate. However, 92% of cases contained at least one experimental isolate identical to the clinical isolate.**
- **24% of cases were reported as positive for one or more VF by the NDVDL. However, the current study determined that 47% of cases were positive for one or more VF.**
- **Greater than 65% of all isolates showed resistance to 4 or more experimental antibiotics.**
- **ECOR grouping appears to be unrelated to the pathogenicity of scours *E. coli*.**

E. coli characteristically different from clinical isolates were recovered from many of the cases. One may conclude from this statement that multiple colonies from any given fecal sample should be tested for virulence and antibiotic resistance to ensure the proper etiological agent has been identified. However, questions arise as to whether this may be a realistic plan of action.

- **Is it cost effective to test multiple colonies and more importantly, will the consumer be willing to take on this extra expense?**
- **Do more virulence factors need to be explored as other research suggests additional factors have been shown to cause disease[5]?**
- **What is the limit to the number of isolates to be tested to determine the appropriate antibiotic treatment and again, is this cost worth the extra time and money?**
- **Will there be conflicts with time constraints or technical and material resources to be able to isolate multiple *E. coli*?**

Further research may involve a larger number of scours cases, other virulence factors, and additional antibiotic resistance testing of all isolates by the Veterinary Diagnostic Laboratory method, the Trek Diagnostic Systems Sensititre with the following antibiotics currently used: Ampicillin, Ceftiofur, Chlortetracycline, Clindamycin, Danofloxacin, Florfenicol, Gentamicin, Neomycin, Oxytetracycline, Penicillin, Spectinomycin, Sulphadimethoxime, Tiamulin, Tilmicosin, Trimethoprim/Sulphamethoxazole, Tulathromycin, Tylosin.

A possible scenario to consider is that other factors and alternative methods may need to be explored when determining the best course of action for scours treatment. A single colony may be a good representation, but other factors in terms of virulence and antibiotic resistance could be overlooked.

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