Evaluation of embryonated eggs as an alternative model for studying pathogenicity and virulence of *Campylobacter* species isolates

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Published online on October 31, 2008.

ABSTRACT
Objectives: To evaluate the pathogenicity potential and relative virulence of *Campylobacter* species isolates using fertile chicken eggs as the model.
Methodology and results: Four bacterial isolates, comprising of 4 *Campylobacter* species from chicken were evaluated using 15-day-old embryonated chicken eggs and yolk sac inoculation technique. The four species, *Campylobacter jejuni* subsp. *jejuni*, *Campylobacter jejuni* subsp. *doylei*, *Campylobacter coli* and *Campylobacter lari* were pathogenic and they killed the embryos, except *C. j.* subsp. *doylei*. Embryos inoculated with *C. j.* subsp. *doylei* hatched after 6 days post-infection with persistently soiled vent. Respective species/subspecies of *Campylobacter* were re-isolated from at least 75% of the dead embryos and hatched chicks. Ranked in order of virulence (based on the post-infection time needed to cause death of embryos), the order was *Campylobacter lari* > *Campylobacter jejuni* subsp. *jejuni* > *C. coli* > *Campylobacter jejuni* subsp. *doylei*.
Conclusions and application of findings: The study demonstrated the potential of using embryonated eggs as a model for studies of pathogenicity and relative virulence of *Campylobacter* isolates in Nigeria or other similar situations where laboratory facilities may not be easily available.

Key words: Embryonated egg, model, pathogenicity, virulence, *Campylobacter* species


INTRODUCTION
*Campylobacter* species are a frequent cause of acute bacterial diarrhea in man. The bacteria are usually contracted through handling or consumption of contaminated poultry meat (Tauxe, 1992; Mead et al., 1999). In Nigeria the important human enteric pathogens contracted from poultry include *C. jejuni* and *C. coli* (Alabi et al., 1986; Ani et al., 1988; Coker & Adeseso, 1994; Coker et al., 2002). The pattern of human campylobacteriosis, particularly *C. jejuni* and *C. coli*, keeps changing due to indiscriminate use of antibiotics and therefore needs constant monitoring.

Despite the potential hazards to human health, the pathogenicity and the relative virulence of the *Campylobacter* isolates are understudied in Nigeria. In part, this deficit results from lack of sufficient number of animal
models for Campylobacter infection in which high resolution virulence measurements are possible, and also due to difficulties encountered in accessing reagents and advanced technologies to carry out proper assays.

There is a need, therefore, to develop and evaluate simple models that are more cost-effective, easily accessible, reasonably sensitive and adaptable to the rural Nigerian conditions. This study evaluated the possibility of using fertile chicken eggs as a model to study the pathogenicity potential and relative virulence of local Campylobacter species isolates.

MATERIALS AND METHODS

Sources of Campylobacter isolates: Bacterial isolates were obtained from the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria. Local isolates of Campylobacter species from layers chicken which were previously identified using the methods of Baron et al. (1994) were used for the study. The isolates were Campylobacter jejuni subsp. jejuni, Campylobacter jejuni subsp. doylei, Campylobacter coli and Campylobacter lari.

Yolk sac inoculation: The technique described by Carter and Cole (1990) was used. Ninety fertile eggs laid by apparently healthy layers aged 8 to 12 months were obtained from six poultry farms in Nsukka town. These fertile eggs were incubated using kerosene-powered incubator at 38 – 40°C for 15 days during which they were candled on the 5th, 10th and 15th day to determine those with growing embryos. Forty-four eggs with growing embryos were selected and used for different bacterial test strains.

The surface of each egg (over the air sac) was disinfected with alcohol and a tiny hole drilled through the shell into the air space using a 22 gauge, 1-inch needle. A 0.5 ml volume of standardized suspension of whole cell culture (10^6 - 10^8 cells/ml) of each test Campylobacter strain was inoculated into the yolk sac through the hole created on the hilt. The hole on the shell was sealed with paraffin wax. Each test was performed in quadruplicate. The control eggs were similarly inoculated with 0.5 ml of normal saline in quadruplicate. The inoculated eggs and four non-inoculated eggs were incubated at 38 – 40°C using kerosene-powered incubator and candled daily for 6 days to determine loss of viability or death of embryo. After 6 days, isolation of Campylobacter from the yolk sac of the dead embryos and hatched chicks were attempted.

RESULTS

The four isolates Campylobacter species isolates tested C. jejuni subsp. jejuni, C. j. subsp. doylei, C. coli and C. lari were pathogenic and all killed the embryos, except C. j. subsp. doylei (table 1). There were varying degrees of virulence amongst the species/subspecies as mortality of embryonated eggs started on the 3rd day and continued up to 6 days post-infection.

Table I: Pathogenicity of selected Campylobacter species in embryonated eggs

<table>
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<tr>
<th>Isolate</th>
<th>1st day*</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
<th>6th day</th>
<th>Total</th>
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CNS = Control inoculated with normal saline; CNI = Control not inoculated; *= Days after inoculation (eggs were inoculated with test bacteria on the 15th day of incubation); ** = Number dead/ Number initially alive after inoculation
All the embryonated eggs inoculated with *Campylobacter jejuni* subsp. *jejuni* and *Campylobacter coli* died on the 4th day post infection (Figures 1A & B). For *Campylobacter lari*, embryo death occurred from the 3rd to the 6th day (Figures 1C, 2A & 2B). Embryos inoculated with *C. j. subsp. doylei*, however, hatched 6 days post infection with chicks that had soiled vents that persisted for 7 days (Figure 2C). All the eggs inoculated with normal saline and those not inoculated at all hatched with unsoiled vents (Figure 3A, 3B) *Campylobacter jejuni* subsp. *jejuni* was re-isolated from 8 of the 12 dead embryos while *Campylobacter jejuni* subsp. *doylei* was re-isolated from 3 out of the 4 hatched chicks. *Campylobacter coli* was also re-isolated from all the 4 dead embryos and 12 out of the 16 dead embryos yielded *Campylobacter lari*. Based on the period between infection time and death of the embryos, the most virulent strain was *Campylobacter lari*, followed by *Campylobacter jejuni* subsp. *jejuni*, *Campylobacter coli* and *Campylobacter jejuni* subsp. *doylei*.

**Figure 1A:** Dead embryos (4th day post-infection) harvested from unhatched eggs infected with *C. jejuni* subsp. *jejuni*; **Figure 1B:** Dead embryos harvested from unhatched eggs infected with *C. coli*. Death occurred on the 4th day post-infection. **Figure 1C:** Dead embryo (3rd day post-infection) harvested from unhatched egg infected with *C. lari*

**Figure 2A:** Dead embryo (5th day post-infection) harvested from unhatched egg infected with *C. lari*; **Figure 2B:** Fully developed chick that died in shell after 6 days post-infection with *C. lari* (21 days of incubation); **Figure 2C:** Chicks hatched from eggs inoculated with *C. jejuni* subsp. *doylei*. Note the soiled vents (arrow head).

**Figures 3A and B:** Chicks hatched from control eggs inoculated with normal saline. The chicks had clean vents.
DISCUSSION
Pathogenicity assay used in this study confirms the report of Carter and Cole (1990) that embryonated eggs can be used to establish pathogenicity and virulence of microorganisms. Our results demonstrated that all the *Campylobacter* species and subspecies tested were pathogenic. Lambert & Maxcy (1984) indicated that isolates of *Campylobacter jejuni* from faecal materials of chicken and turkeys were lethal when introduced via yolk sac into embryonated eggs.

Baoar et al. (1996) used mice as a model to establish the pathogenicity potential and relative virulence of *Campylobacter* species, and obtained similar results to ours. However, the management and handling of mice would require more professional skills and expenses compared to embryonated eggs. The relative virulence of the species and subspecies observed using embryonated eggs has similar pattern to those obtained using tissue cells and serological assay, even though the time of establishment of bacteria post-infection and the relative virulence were better measured using tissue cell and serological assay (Lior, 1984; Alabi et al., 1986; Frost et al., 1998; Eyigor et al., 1999; Fitzgerald et al., 2001; Nadeau et al., 2003). These types of techniques are however not easily available in Nigeria for diagnostic purposes.

The results of this study demonstrated that embryonated eggs could be used to rapidly, accurately and cost-effectively determine the pathogenicity potential and relative virulence of *Campylobacter* species in an area without constant electric power supply and other advanced laboratory facilities. Despite the inability to accurately measure the time of post-infection establishment in the egg model, relative to the use of animal tissue cells and serological assay, it provides an alternative practical means of testing for pathogenicity and relative virulence of *Campylobacter* isolates from clinical conditions.

ACKNOWLEDGEMENTS: Authors are grateful to the Head of Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, for providing *Campylobacter* isolates and laboratory facilities; and acknowledge the contributions of the Laboratory Technologists.

REFERENCES


