Journal of Applied Biosciences (2008), Vol. 11: 607 - 611.
ISSN 1997 – 5902: www.biosciences.elewa.org

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

C. A. Akwuobu[1], S. I. Oboegbulem[2], R. A. Ofukwu[3] and K. F. Chah[4]
[1]Department of Veterinary Pathology and Microbiology University of Agriculture, Makurdi, Nigeria
[2]Department of Veterinary Public Health and Preventive Medicine University of Nigeria, Nsukka, Nigeria
[3]Department of Veterinary Public Health and Preventive Medicine University of Agriculture, Makurdi, Nigeria
[4]Department of Veterinary Pathology and Microbiology University of Nigeria, Nsukka, Nigeria

Corresponding author: Phone: +234. 803 778 7706; E-mail: chineduakwuobu@yahoo.com
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. d*oylei.*
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

Citation: Akwuobu CA, Oboegbulem SI, Ofukwu RA. and Chah KF, 2008. Evaluation of embryonated eggs as an alternative model for studying pathogenicity and virulence of *Campylobacter* species isolates. *Journal of Applied Biosciences* 11: 607 - 611.

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 & Adefeso, 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

	Death of embryo after inoculation						
Isolate	1st day*	2nd day	3rd day	4th day	5th day	6th day	Total
C.jejuni subsp. *jejuni*	0/4**	0/4	0/4	2/4	2/4	0/4	4/4
C.jejuni subsp. *jejuni*	0/4	0/4	0/4	4/4	0/4	0/4	4/4
C.jejuni subsp. *jejuni*	0/4	0/4	0/4	4/4	0/4	0/4	4/4
C.jejuni subsp. *doylei*	0/4	0/4	0/4	0/4	0/4	0/4	0/4
C. lari	0/4	0/4	2/4	2/4	0/4	0/4	4/4
C. lari	0/4	0/4	0/4	0/4	2/4	2/4	4/4
C. lari	0/4	0/4	0/4	4/4	0/4	0/4	4/4
C. lari	0/4	0/4	0/4	4/4	0/4	0/4	4/4
C. coli	0/4	0/4	0/4	4/4	0/4	0/4	4/4
CNS	0/4	0/4	0/4	0/4	0/4	0/4	0/4
CNI	0/4	0/4	0/4	0/4	0/4	0/4	0/4

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*.

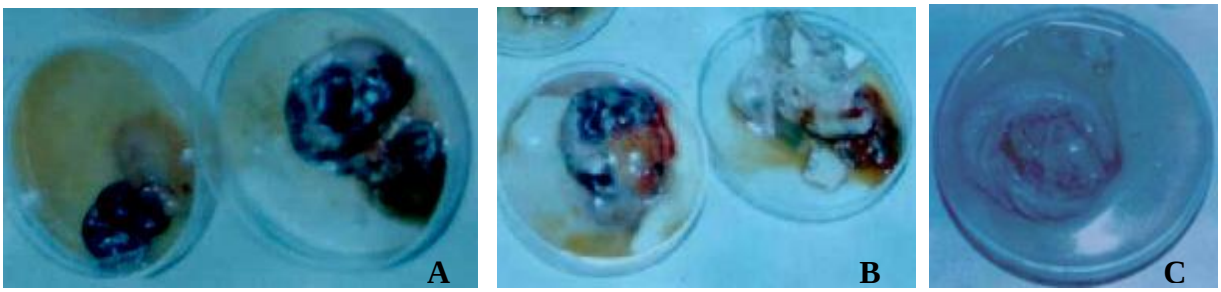


Figure 1A: Dead embryos (4th day post-infection) harvested from unhatched eggs infected with *C. jejuni* subsp. *jejuni;* Figure 1B: Dead embryos harvested from unhatched eggs infected with *C. coli*. Death occurred on the 4th day post-infection. Figure 1C: Dead embryo (3rd day post-infection) harvested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

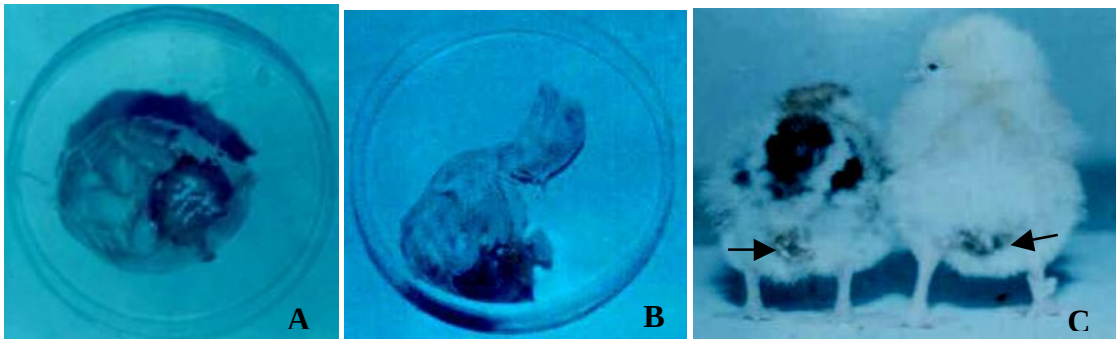
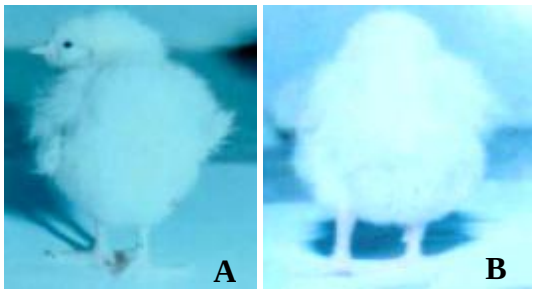


Figure 2A: Dead embryo (5th day post-infection) harvested from unhatched egg infected with *C.lari;* Figure 2B: Fully developed chick that died in shell after 6 days post-infection with *C.lari* (21 days of incubation); Figure 2C: Chicks hatched from eggs inoculated with *C. jejuni* subsp. *doylei*.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

Alabi SA, Coker AO, Dosumlt-Ogunbi O, Odugbemi T, 1986. Biotype and serogroup distribution of *Campylobacter* isolates from children in Nigeria. Journal of Clinical Microbiology x: 856 - 858.

Ani EA, Takahashi T, Shonekan R A, 1988. *Campylobacter jejuni* antibodies in Nigerian Children. Journal of Clinical Microbiology x: 605-606.

Baoar S, Bourgeois AL, Applebee LA, Mourad AS, Kleinosky MT, Mohran Z, Murphy JR, 1996. Murine Intranasal Challenge Model for the Study of *Campylobacter* Pathogenesis and Immunity. Infection and Immunity x: 4933 – 4939.

Baron EJ, Peterson LR, Finegold SM, 1994. *Campylobacter*. In: Bailey and Scott's Diagnostic Microbiology. 9th Edition.

Carter GR. and Cole Jr JR, 1990. *Campylobacter*. In: Diagnostic Procedures in Veterinary Bacteriology and Mycology. 5th Edition. Academic Press, Inc. San Diego, California. 61 - 75 pp.

Coker AO. and Adefeso AO, 1994. The changing patterns of *Campylobacter jejunilcoli* in Lagos, Nigeria after ten years. East African Medical Journal 71: 437 – 440.

Coker AO, Isokpehi RD, Thomas BN, Amisu KO, 2002. Human campylobacteriosis in developing countries. Emerging Infectious Diseases 8 (3).

Eyigor A, Dawson KA, Langlois BE, Pickett CL, 1999. Detection of Cytolethal Distending Toxin Activity and *cdt* Genes in *Campylobacter* species Isolated from chicken carcasses. Applied and Environmental Microbiology 65: 1501 - 1505.

Fitzgerald C, Stanley K, Andrew S, Jones K, 2001. Use of pulsed-field gel electrophoresis and flagellin gene typing in identifying clonal groups of *Campylobacter jejuni* and *Campylobacter coli* in farm and environments. Applied Environmental Microbiology 64: 1429 – 1436.

Frost JA, Oza AN, Thwaites RT, Rowe B, 1998. Serotyping scheme for *Campylobacter jejuni* and *C. Coli* based on direct agglutination of heat-stable antigens. Journal of Clinical Microbiology 36: 335 - 339.

Lambert JD. and Maxcy RB, 1984. Effect of gamma radiation of *C. jejuni.* Journal of Food Science 49: 665 - 667.

Lior H, 1984. A new, extended biotyping scheme for *C. jejuni, C. coli,* and *C. laridis*. Journal of Clinical Microbiology 20: 636 - 640.

Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV, 1999. Food-related illness and death in the United States. Emerging Infectious Diseases 5: 607 - 625.

Nadeau E, Messier S, Quessy S, 2003. Comparison of *Campylobacter* isolates from poultry and human: association between *in vitro* virulence properties, biotypes and pulsed-field gel electrophoresis cluster. Applied Environmental Microbiology 69: 6316 – 6320.

Tauxe RV, 1992. Epidemiology of *Campylobacter* infections in the United States and other industrialized nations. Pp. 9 – 19. In: I. Nachamkin, M.J. Blaser, and L.S. Tompkins (eds.), *C. jejuni* current status and future trends. American Society for Microbiology. Washington, D.C.