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Susceptibility of Chickens to *Campylobacter fetus* subsp. *jejuni*

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ABSTRACT

Poultry has been implicated in the transmission of *Campylobacter* organisms to humans. To determine the susceptibility of chickens to *Campylobacter* organisms, *C. fetus* subsp. *jejuni* isolated from human patients was inoculated intraperitoneally, intravenously or orally into chickens. *C. fetus* subsp. *jejuni* infections developed in chickens by all three routes of administration. Antibiotics routinely and continuously used as poultry feed additives to control protozoan parasites and enhance feed efficiency were tested for bactericidal action to *C. fetus* subsp. *jejuni*. Salinomycin and monensin, two widely used antibiotics, had no activity against *C. fetus* subsp. *jejuni*.

INTRODUCTION

Campylobacter fetus subsp. *jejuni* has been recently recognized as a causative agent of human gastroenteritis. The development of selective culture techniques designed to isolate *Campylobacter* species from stool specimens has demonstrated

that *Campylobacter* is distributed worldwide and is commonly associated with diarrheal patients as is *Salmonella* or *Shigella* (Karmali and Fleming, 1979).

These discoveries have prompted much speculation and experimentation on the mode of transmission of *Campylobacter fetus* subsp. *jejuni*. Poultry has been implicated in the transmission of *Campylobacter* organisms (Butzler, et al., 1977; Grant, et al., 1980; Leuchtefeld, et al., 1980; Park, et al., 1981; Smibert, 1969; and Smith and Muldoon, 1974). Experimental *C. fetus* subsp. *jejuni* inoculations in poultry have produced varied results. Ruiz-Palacios *et al.* (1981) used 3-day-old chickens inoculated orally with human isolates of *C. fetus* subsp. *jejuni* to demonstrate the invasive power of the bacterium and the pathology of the intestinal mucosa resulting in diarrhea. Manninen et al. (1982), however, reported no deaths or diarrhea in 3-day-old chickens inoculated orally with isolates of *C. fetus* subsp. *jejuni*. The use of mammalian hosts for intestinal colonization of *C. fetus* subsp. *jejuni*, such as mice, rats and rabbits, has had mixed success (Field et al., 1981 and Manninen, et al., 1982). Experimental infections of *C. fetus* subsp. *jejuni* in animals by various methods of inoculation has not been documented.

Virtually all commercial broiler operations routinely use antibiotics in the feed to prevent various protozoan diseases. These antiprotozoan antibiotics such as salinomycin and monensin are designed for continuous use in the feed for virtually the entire life of birds grown specifically for meat production. In addition to their antiprotozoan properties, salinomycin and monensin are bactericidal against certain procaryotes (Miyazaki, et al., 1974; Agtarap and Chamberlin, 1968). If these antibiotics are bactericidal against *Campylobacter* organisms in the intestinal tract of chickens, then the routine administration of these antibiotics could reduce the potentiality of broiler chickens as a source of *Campylobacter* infections in humans.

The purpose of this study was to test the susceptibility of chickens to *C. fetus* subsp. *jejuni* by inoculating the bacteria intraperitoneally, intravenously and orally in chickens and to determine the efficacy of two widely used anti-protozoan antibiotics, salinomycin and monensin, to *C. fetus* subsp. *jejuni*.

MATERIALS AND METHODS

Bacterial strains:

Three strains of *C. fetus* subsp. *jejuni* were isolated from humans suffering from gastroenteritis (Shirrow, 1977). The isolates were combined and grown aerobically in brain-heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) at 41°C. Twenty-four hour cultures were stored in the broth at −20°C. Frozen organisms were then thawed and used by the second subculture.

Pure cultures of *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli* (Carolina Biological Supply Co., Burlington, N.C.) were used with *C. fetus* subsp. *jejuni* to test the efficacy of antibiotics used routinely in the poultry industry. Twenty-four hour cultures of these bacterial species were stored at 8°C on nutrient agar slants, over-laid with sterile mineral oil until needed.

Experiment I: Chicken Susceptibility Tests.

(i) *Chickens*. Day-old leghorn cockerels obtained from a commercial hatchery were maintained in clean, cardboard, disposable pens and given unmedicated chick starter feed and water *ad libitum*. The room temperature was maintained at 35°C with continuous lighting.

(ii) *Preparation of Inoculum*. Twenty-four hour broth cultures of *C. fetus* subsp. *jejuni* were centrifuged (3000 × g for 10 min.), the supernatent drawn off, and the bacterial cells washed once with sterile saline. The washed bacterial cells were resuspended with sterile saline to obtain the inoculum. A portion of the inoculum was serially diluted in sterile water, inoculated on duplicated 5% sheep blood agar plates (Columbia agar base, BBL Microbiology Systems) and incubated at 42°C in an atmosphere consisting of 5% oxygen, 10% carbon dioxide and 85% nitrogen for 48 h to determine colony-forming units (CFU) per ml. The inoculum concentration was approximately 10^7 CFU/ml. Chickens injected intraperitoneally were given 0.2 ml of inoculum. Chickens injected intravenously were given 0.1 ml of inoculum in a wing vein and a 7.5 cm animal feeding needle (Popper and Sons, Inc., New Hyde Park, N.Y.) was used to give 0.5 ml of inoculum orally.

(iii) *Experimental Design*. Seven-day old chicks were selected at random and placed in four groups with six birds/group. Each group was housed in identical cages and given the same feed and water. Group 1 was inoculated intravenously with inoculum. Group 2 was inoculated intraperitoneally and Group 3 was inoculated orally. Group 4 was designated the control group and was not inoculated with *C. fetus* subsp. *jejuni*. Special procedures were maintained to prevent cross contamination. At seven days post-inoculation, the birds were killed by cervical dislocation, the breast smeared with disinfectant and the peritoneal cavity was aseptically opened to expose the viscera. Sterile swabs were taken on the surface of the heart, lungs, intestine and peritoneal lining. Blood and bile samples were also taken. The samples were incubated aerobically in brain-heart infusion broth at 41°C for 48 h. Parallel samples were incubated in 5% sheep blood agar plates at 41°C in 5% oxygen-10% carbon dioxide-85% nitrogen for 48 h. Colonies which morphologically resembled *C. fetus* subsp. *jejuni* were sub-cultured for identification (Leuchtefeld, et al., 1980).

Experiment II: Calculation of minimal inhibitory concentration (MIC) of salinomycin and monensin.

(i) *Antibiotics*. Salinomycin (Pfizer, Inc., Terre Haute, Ind.) and monensin (Elanco Products Co., Indianapolis, Ind.) were obtained from the manufacturers in powdered form. The antibiotics were dissolved in 70% ethyl alcohol and filtered through a sterile 0.45 um membrane filter (Gelman Instrument Co., Ann Arbor, Mich.). One gram of active drug was dissolved in 10 ml of alcohol. One-tenth milliliter of alcohol-drug suspension was mixed with 9.9 ml of brain-heart infusion media to obtain 1000 μg of active drug per 1 ml of media. Serial double dilutions were conducted to obtain a range of drug concentrations and determine the MIC of the drug for each microorganism tested. Alcohol dilutions (without drug) were conducted concurrently to determine if the alcohol alone affected the growth of the bacteria in the media.

(ii) *Standardization of Inoculum*. The turbidity of 24 h broth cultures used for inoculum was adjusted by visually comparing the cultures to that of a 0.5 McFarland turbidity standard before inoculating the serial dilutions with an inoculation loop (National Committee for Clinical Laboratory Standards, 1980). The inoculated serial dilutions of *C. fetus* subsp. *jejuni* were incubated aerobically at 41°C. The other bacterial species (*B. cereus*, *S. aureus* and *E. coli*) were incubated at 37°C.

RESULTS

Chicken Susceptibility Tests.

Chickens were susceptible to *C. fetus* subsp. *jejuni* by all the inoculation methods (Table 1). One chicken inoculated intravenously died on Day 3 postinoculation and three others had active *C. fetus* subsp. *jejuni* infections when they were examined on Day 7 postinoculation. During the postinoculation period, the chickens inoculated intravenously became increasingly listless, pale, inactive and on necropsy all the chickens had enlarged hearts.

Chickens inoculated intraperitoneally had the highest mortality rate with 50% of the birds dying during the postinoculation period. The three remaining chickens has swollen intestines, extensive adhesions and a large focus of infection at the site of inoculations.

Chickens given *C. fetus* subsp. *jejuni* orally had no mortality during the postinoculation period but five of the six chickens had viable *C. fetus* subsp. *jejuni* organisms on the surface of the liver and heart. The intestines, livers and hearts were swollen in all these chickens.

The viscera of all the control birds appeared sterile. None of the birds demonstrated viable *C. fetus* subsp. *jejuni* or any other bacterial species upon autopsy.

Salinomycin and monensin had no bactericidal effect on *C. fetus* subsp. *jejuni* and *E. coli* in the range of concentration tested (Table 2). However, both antibiotics were effective against the Gram positive organisms; *S. aureus* and *B. cereus*. Parallel tests with alcohol controls (without drug) did not inhibit growth of any of the bacterial species at the volumes used in determining minimal inhibitory concentrations of salinomycin and monensin.

DISCUSSION

In every poultry operation, there is spontaneous mortality or the death of birds with no apparent cause. Usually the number of birds involved is relatively small and no pattern to the deaths is apparent so the birds are not examined closely. Most of these deaths occur in the first three weeks of life. When these spontaneous deaths are examined, the majority of birds have swollen intestines and other indications of enteritis. Veterinarians are familiar with the hepatic hemorrhagic lesions associated with *Campylobacter fetus* infections in poultry. However, some of the *Campylobacter* infected birds examined in this study had swollen intestines indicative of enteritis with normal-looking livers. This observation coupled with the fact that *Campylobacter* organisms are fastidious and require special cultural and isolation techniques for identification suggests that some spontaneous mortality in poultry operations may be due to *Campylobacter* infection.

Salinomycin and monensin are examples of polyether antibiotics used by the poultry industry to control coccidial organisms (Reid, 1978). In addition to their anticoccidial action, the polyether antibiotics are extensively used because of minimal tissue deposition, slow development of drug resistant strains of protozoan parasites and low toxicity to the host. Both salinomycin and monensin are added continuously in the feed ration from the day the chicks hatch until five days before the birds are marketed. Although salinomycin is not yet sold in the United States, it is used in many foreign countries at a concentration of 70 parts per million (ppm). Monensin is approved by the Food and Drug Administration as a poultry feed additive at concentrations as high as 121 ppm. It is estimated that 80-85% of

the broilers produced in the United States are fed monensin continuously as a feed additive and the antibiotic is sold worldwide.

Because salinomycin and monensin are antibiotics (produced by *Streptomyces albus* and *S. cinnamonensis* respectively), it is not surprising that they have bactericidal action. This study confirms other reports that polyether antibiotics are effective against many Gram positive microorganisms such as *Staphylococcus aureus* and *Bacillus cereus* (Miyazaki, et al., 1974). However, both antibiotics demonstrated no activity against *Escherichia coli* or *C. fetus* subsp. *jejuni* (Miyazaki, et al., 1974), which are Gram negative bacteria.

The data suggest that chickens are highly susceptible to *C. fetus* subsp. *jejuni* infections and hepatic lesions are not always apparent in active infections. The polyether antibiotics, salinomycin and monensin, used as feed additives, have no bactericidal effect on *C. fetus* subsp. *jejuni*. Therefore, poultry and poultry products have the potential for *C. fetus* subsp. *jejuni* contamination and a source for human campylobacteriosis.

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Table 1. The incidence of *C. fetus* subsp. *jejuni* infections in 7 day old chickens inoculated by various methods.

Group	Method of Administration	Postinoculation Mortality[a]	Positive Visceral[b,c] Culture	% of Survivors Positive for Campylobacter
1	Intravenous	1/6	3/5	60%
2	Intraperitoneal	3/6	3/3	100%
3	Oral	0/6	5/6	83%
4	Control (Uninoculated)	0/6	0/6	0%

[a]Numerator indicates number of birds died postinoculation, denominator indicates the number of birds in the group.
[b]Cultures include visceral swabs, blood and bile cultures.
[c]Numerator indicates number of survivors infected, the denominator indicates the number of survivors in the group.

Table 2. Minimal inhibitory concentration (MIC) of salinomycin and monensin with *C. fetus* subsp. *jejuni* and three other bacterial species cultured in Brain-Heart Infusion broth and incubated aerobically.

	MIC (μg/ml)[b,c]	
Microorganism[a]	*Salinomycin*	*Monensin*
C. fetus subsp. *jejuni*	> 1000.0	> 1000.0
Escherichia coli	> 1000.0	> 1000.0
Staphylococcus aureus	< 7.81	< 7.81
Bacillus cereus	< 0.49	< 0.98

[a]*C. fetus* subsp. *jejuni* was incubated at 41°C while the other bacterial species were incubated at 37°C.
[b]Dilution range was between 1000 and 0.489 μg of drug/ml of culture media.
[c]The MIC's were averages of 3 replicates.