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EXPERIMENTAL INFECTION OF DEEP LITTER WITH CAMPYLOBACTER JEJUNI AND TEH USE OF AVAILABLE DISINFECTANTS

BY

ZAKIA A. M. AHMED and JAKKEN EL-JAKEE
Faculty of Veterinary Medicine , Cairo University

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SUMMARY

This work was done to show how litter managment plays an important role in the survival of C. J. of Poultry origin, and the proper use of chemical disinfectants to control the artifical contamination with C. J. in the presence of natural litter components.

The study revealed that, the higher moisture content in litter (20 to 50%) increased the recovery of C. J. at 37°C incubation temperature, and the maximum recovery period in artificially contaminated litter with C.J. was 21 days with $2X10^1$ c.f. u. number under 20 30% M. C. Dryness might deteriorate the survival of C.J. a point which consider for proper litter management and decontamination.

At 42°C incubation temperature, the c.f.u. number decreased from $70X10^1$ to $28X10^2$ between 4th to 15th days of survuval.

2.5% solution of 10% formalin advised to be used gave 100% destruction within 15 minutes.

1: 200,000 and 1: 100,000 solutions of 5% sodium hypochlorite (Clorox) was potent and efficient for decontamination of litter with C. J.

It is imperative to apply proper litter management and to use chemical disinfectants to control, Camplyobacter-Jejuni contamination in litter supposed to be used.

INTRODUCTION

Campylobacteriosis has emerged as a significant zoonotic condition affecting a wide range of food and companion animals. In addition to exotic and free living avian and mamalian species. It is a prevalant as an intestinal comensal in floor housed turkeys broiler breeders and layers-type breeders chickens, (Blaser 1983). Intestinal Campylobacteriosis in human contributed to the consumption of chicken meat. The high carriage rate of Campylobacters in the intestinal tract of broilers and turkeys due to contamination during processing which reflected on high levels of C.J. on poultry meat (Simmone-Shane 1991). Campylobacter Jejuni has recently proved to be one of the most common bacterial agents of enterocolities in human, these bacteria found in the faces of chickens contaminate litter and water (Montrose et al (1985).

This experiment was carried out to study the role of litter management on the survival of Campylobacter-jejuni and the use of proper disinfectants to control it in artificially infected litter having simulous field components (organic matter, microflora and extraneous materials.

MATERIALS

CAMPYLOBACTER STRAIN.

C.jejnui isolated from diarrhetic chickens, the strain was typed in Dept., of Microbiology, Fac. Vet. Med, Cairo. University.

Bacterial culture media:

- Camp. BAP Medium Brucella agar base containing 5% sheep RBCs and supplemented with antibiotics (Kaplan et al., 1980).

- Thioglycollate broth.

Litter:

Deep litter from broiler farm (Straw, bird droppings and other extraneous materials) located in Fac. Vet. Med., Cairo Research centre of Animal and Poultry Management) was freshly collected and used (2 kgm).

Disinfectants used:

- 2.5% solution of 10% formalin.
- 1.25% solution of 10% formalin.
- 1.200,000 solution of 5% sodium hybochlorite (clorox).
- 1:100,000 solution of 5% sodium hyboclorite (clorox).

METHOD

Preperation of bacterial inoculum:

C. jejuni inoculum was adjusted using a thiolglycollate broth dilution technique (Elmer et al 1979). The used inoculum was approximately $3x10^6$ Colony forming units (CFU) according to Montrose, et al 1985).

Preperation of litter:

Four litter samples, 50 gm each were inoculated with 10ml thioglycollate broth/gm litter, the determined c. f. u. number was $3x10^6$ *(Montrose, et al 1985)*. Particular litter samples were taken at 4 days-post inoculation, 1st 2nd and 3rd wk week where 2 grams of each sample were transfered to a culture tube, and about twice the volume of sterile bactopeptone medium was added, the mixture was thoroughly agitated and filtered. From the filterate the standard plate method was applied to determine the vaibility of organism by enumerating the c f.u. on Camp. BAP media agar plate and incubated at 43°C / 48h under microaerophilic condition (Simmon et al *1984)*.

Trial I.: Effect of humidity and temperature on viability of C. jejuni:

In this trail the litter and c. j. strain were exposed to different degree of humidity (20-50%) and temperatures (37°C) and 40% M. C. at 42°C incubation.

1- The litter samples were thoroughly mixed dried and autoclaved at 121°C for one hour. Each 50 gms of litter contained different misture contents began with 20, 30, 40 and 50% (used sterile distilled water, were contaminated with standard C. jejuni ($3x10^6$ c. f. u./gm litter).

All infected litter were incubated at 37°C temperature and were cultured every day for the recovery of C. jejuni.

2- 50 gms of autoclaved litter with 40% moisture content (M. C%) was inoculated with C. jejuni ($3x10^6$ c. f. u./gm litter incubated at 42°C. The count of C. jejuni was checked daily.

Trail II: Effect of some disinfectants on the viability of C. jejuni:

This trail was planned to study the effect of formalin and sodium hybochlorite on viability of C. jejuni five litter samples (each 50 gms) were subjected to the following:-

Four flasks contained 50 gms of non autoclaved litter and proved to be free C. J. was infected with $3x10^6$ c. f. u. gm.

A- 10ml of 2.5% solution of 10% formaline was added to the artifically infected litter in the 1st flask.

B- 10ml of 5% solution of 50% formaline was used in the 2nd flask.

C- 10ml of 1:200,000 solution of 5% sodium hybochlorite wad added in the 3rd flask.

D- 10ml of 1:100000 solution of 5% sod-hybochlorite solution in the 4th flask.

E- The 5th flask with sterile litter was left as control.

D- After 5, 10, 30 and minutes contact time a particular sample 2 gm from each mixture were treated as trial I (Simmon et al, 1984).

c. f. u. numbers were 30, 32 and $28x10^2$ at 20, 30 and 40% M. C. while at 50% M. C. the organism disappeared completely. on the 15th day the c. f. u. number were 9,11 and 11x104 at 20, 30 and 50% M. C. On 21. day, the c. f. u. number decreased nuder all conditions. It was 2x101 at 20, 30 and 40%, M. C. While it disappeared completely at 50% M. C.

Table (1): Survival of Campylobacter0Jojuni in artificialy contaminated litter.

Syrvival Days	M. C%	C.F. U./gm litter				Temperature incubation C°
		10^1	10^2	10^3	10^4	
4		35	70	43	--	
7		50	30	--	--	
15	20	30	25	17	10	37
21		2	0	--	--	
4		100	60	45	--	
7		35	32	--	--	
15	30	17	10	--	--	37
21		2	0	--	--	
4		70	50	60	--	
7		50	28	--	--	
15	40	25	27	12	11	37
21		2	1	--	--	
4		100	80	75	--	
7		60	0	0	--	
15	50	60	0	0	--	37
21		31	29	18	11	
4		50	30	0	--	
7		55	30	--	--	
15	40	60	28	--	--	42
21		50	0	--	--	

RESULTS AND DISCUSSION

Effects of humidity (litter moisture content M. C.%) and temperature of incubation on the viability of campylobacter jejuni:

Results shown in table (1-a) revealed that, at 4-ds-post-artifical litter inoculation with C. J. increased M. C%, increased c. F. u. number, at 20, 30,40 and 50%, the number was 43, 45, 60 and $70x10^3$ respectively. At 7-ds-post-inoculation, the The average c. f. u. was in all M. C.% (95-100x10^1) then decreased to 43 x 10^3 at 20% M. C. The highest number at the same time was 75 x 10^3 c. f. u. at 50% M. C. which indicated, the increase of M. C% give more recovery of C. J. from artifically contaminated litter at temperature incubation 37°C).

These data confirmed that C. J. most likely survives in wet conditions but dryness deteriorate

it (Simon-Shane 1991).

Table (2): Effect of chemical disinfectants on the artifiolaly contaminated litter with C. J.

Disinfectants concentrations	Time contact/minute score of destruction in%				
	"5	"10	"15	"30	"60
1. 2.5% of 10% formal-in	40	80	100	--	--
2. 1.25% of 10% formal-in	42	60	87	91	--
3. 1.200,000 of 5% clorox	50	82	100	--	--
4. 1: 100,000 of 5% clorox	48	70	96	100	--

Results in table (2-b) showed that at 4-ds-post artificial contamination with 3 x 10^6 c. f. u. C. J. /gm litter, the survived c. f. u. at 42°C began with (50-60 x 10^1) at 4-21 days-post inoculation) and decreased to (28-30 x 10^2) at 4-15 ds-post inoculation) then disappeared at 21 days. The hygienic practices of the attendants, presence of flies and the reuse of litter are related to the potential source of infection (Franco, 1989).

<u>Effect of chemical disinfectants on artifical infected litter with campylobacter jejuni:</u>

From data shown in (table 2). It is noticed that, at 2.5% solution of 10% formaldehyde could destroy C. J. 100% after 15 minutes, while 1.25% solution could destroy it after 60 minutes contact. Using clorox (5% active Na-hybochlorite) 1:200,000 of 5% stock solution could damage C. J. 100% after 15 minutes, but, 1:100000 conc- destroyed the organism after 30 minutes contact. From these results proper concentration of 10% formaldehyde (2.5%) advised to be used for disinfection of artifically contaminated litter with Campylobacter jejuni.

Clorox at 1:200000 killed the organism 10[illegible] after 15 minutes but at low concentra[illegible] (1:100000) 30 minutes were needed and bo[illegible] concentrations of clorox were more potent tha[illegible] fromaldehyde this suggesion asured b[illegible] (Genigeoris, 1986).

Therefore, litter management needs mo[illegible] attention, periodic microbiological examinatio[illegible] selection of available and efficient chemic[illegible] disinfectants should be considered.

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