ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 3 (2): 129-134, 2004

Epidemiological Study on the Colonization of Chickens with *Campylobacter* in Broiler Farms in Malaysia : Possible Risk and Management Factors

Saleha A.A.
Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract: Nineteen chicken flocks from ten broiler farms were studied for the colonization of *Campylobacter*. A variety of factors such as farm location and chicken house structure, water source, rearing practice and hygiene management were investigated. Each flock was sampled weekly, from day-old-chicks to slaughter-age chickens and environmental samples which include water, feed, wood shavings, flies and chicken house environment were collected to examine for the presence of *Campylobacter*. In all farms, *Campylobacter* was not detected in one- and seven-day-old chicks. *Campylobacter* was first detected in 38.2% of 14-day-old-chicks and 45.3% of 21-day-old chickens. Samples of feed, wood shavings, flies and chicken house environment were all negative while only 1.5% of untreated water supplies were found positive for campylobacters. Prevalence of campylobacters' colonization was possibly associated with untreated water, presence of other animals and unhygienic management practices; also flying birds could be a source as they were found to harbour campylobacters; in one farm where 'fishing net' was placed over the chicken house to prevent birds from entering, *Campylobacter* was not isolated in the chickens up to slaughter age.

Key words: *Campylobacter*, colonization, broiler chicken, environmental factors

Introduction

Campylobacteriosis is reported as one of the most common bacterial gastroenteritis in humans, often caused by consumption or handling of poultry meat or poultry products. Several studies have shown that the gastrointestinal tracts of poultry, namely chickens are frequently colonized by *Campylobacter* and that the prevalence of *Campylobacter jejuni* in broiler chickens may be as high as 90.0% (Genigeorgis *et al*., 1986; Jones *at al*., 1991). Since these campylobacters can survive routine slaughtering and dressing processing operations, a high proportion of chicken carcasses sold at retail outlets are found to be contaminated and that up to 25.0% of freshly laid eggs may be contaminated (Kazwala, 1993).

The source of *C. jejuni* in broiler chickens at production level is not clear although a number of studies have been carried out (Genigeorgis *et al*., 1986; van de Griessen *et al*., 1992; Humprey *et al*., 1993; Kazwala *et al*., 1993). From these and other studies, several factors were suspected to be the source or vector of campylobacters in chickens; among these factors were the environment of the poultry houses, farm personnel, presence of animals such as dogs, cats and pests as well as insects and farm hygiene and management practices.

This study was undertaken to investigate the source(s) of campylobacters in colonizing the broiler chickens in the farms.

Materials and Methods

The farms: Ten broiler farms were studied, with farms raising about 5000 to 25 000 birds. The broiler houses ranged from one to six per farm, depending on the size of the farm with at least 2500 to 5000 birds per house. The broiler houses were of open type with natural ventilation, with roofs made of either corrugated zinc sheets or 'nipah' palm leaves (as thatch roofs) and the chickens were raised either on deep litter system or raised floor system with slatted floor.

The farms were either located in palm oil estates or on clear land. A number of the farms used tap water while others used pond water which were pumped directly into the water tanks situated at the roof top of the broiler houses.

Isolation of campylobacters from chicks and chickens: The large farm were represented by two to three flocks and the smallholder farms (with 5000 broiler chickens) by one flock. The farms received day-old-chicks from a number of hatcheries. At each farm, cloacal swabs were taken from about 25 to 30 day-old-chicks per flock upon their arrival. The chicks and chickens were sampled every week at each farm till they reached slaughtering age, about 6 - 7 weeks old.

Isolation of campylobacters from feed, water and the environment: At least 50g of feed were taken from each farm - from newly opened bag as well as those in the feed trays. As for the water, samples of at least 100 ml each, were taken from the water tanks and from the drinkers.

Environmental samples consisted of swabs moistened with sterile peptone water which were used to swab the

Table 1: The description of the farms and the chicken houses that were studied

Parameter	Range / Type	No. of farms per type
No. of chickens per farm	5000 - 22000	5000 - 10000 = 5 farms 10000 - 22000 = 5 farms
No. of houses per farm	1 - 6	1 - 3 = 5 farms 4 - 6 = 5 farms
Rearing (growing) days	42 - 45	42 days = 7 farms 45 days = 3 farms
Housing system	Deep litter or raised on slatted floor system	Deep litter = 5 farms Raised on slatted floor = 5 farms
Empty days	14 - 30 days	14 days = 6 farms 21-30 days = 4 farms
Presence of other animals	Yes or no	Yes = 6 farms No = 4 farms
Source of water supply	Surface water (pond), tap water (pipe)	Surface water = 6 farms Pipe = 4 farms
Location	On clear land or in oil palm estate	Clear land = 7 farms Palm oil estate = 3 farms
Roof type	Zinc sheets or thatch roof	Zinc sheets = 4 farms Thatch roof = 6 farms

walls and the floors of the broiler houses; also wood shavings, dust and flies were included. These samples were taken from empty houses only, that is, a week before day-old-chicks were brought in. Wood shavings were used in brooders for day-old-chicks until they were about two weeks old.

All samples were taken aseptically and each placed in separate sterile plastic bags or bottles.

Assessment of farm management and hygiene practices: The management and hygiene practices at the farms were observed as well information gathered through a set of questionnaires forwarded to the farm supervisors and/or workers.

Among the questions covered were husbandry and hygiene practices during the rearing period, cleaning and disinfection practices and time period when the houses were empty; contact with animals, including presence of pests such as rats and flying birds.

Isolation and identification of *Campylobacter*: Cloacal swabs and environmental samples as well as feed and water samples were brought to the laboratory for culture. All samples were examined for the presence of *Campylobacter*. The culture technique and identification procedures for cloacal swabs were as described previously (Saleha, 2002).

The feed, wood shavings and houseflies (*Musca domestica*) caught in the broiler houses, were each placed in separate enrichment broth, made up of *Brucella* broth (BBL) supplemented with cefoperasone [32 mg/l], amphotericin B [10 mg/l], sodium pyruvate [0.25 g/l], sodium metabisulphite [0.25 g/l] and ferrous sulphate [0.25 g/l]. The bottles were then incubated at 37 ^{0}C for 24 h in a micro aerobic atmosphere.

To isolate and estimate the number of *Campylobacter* in water samples, the most probable number (MPN) method, described by Bolton *et al.* (1982) with some modifications by Humphrey and Muscat (1989), was used. One (1) ml portion of each water sample was pipetted into 10 bijou bottles, each containing 5 ml of enriched *Brucella* broth (see above). The bottles were then incubated at 37 ^{0}C for 48 h. After incubation, the broth was streaked onto *Campylobacter* Selective Blood - Free Agar (Oxoid) containing cefoperasone [32 mg/l] and amphotericin B [10 mg/l]. The plates were incubated micro aerobically at 42 ^{0}C for 48 h.

The identification of suspected colonies of *Campylobacter* was done as described previously (Saleha, 2002). The plates streaked with broth containing water samples were read for the presence of *Campylobacter* and the number of campylobacter - negative broth were recorded. The MPN of campylobacters per ml present in each water sample was read from a table derived from the formula of Campbell (1974).

Statistical analysis: Statistical tests of significance was used to assess the significance of differences in isolation rates between groups.

Results

Broiler farms and houses: Ten broiler farms were studied. See Table 1.

Prevalence of campylobacters in day-old chicks to slaughter-age chickens: Each of the 10 farms was sampled weekly (one visit/week/farm) from day-old-chicks till they reached slaughter age. The number of chicks and chickens sampled per visit per farm varied

Table 2: Risk factors investigated for possible campylobacters colonization in broiler chickens at the farms

Possible factors	No. of farms (n=10)	No. of flocks (n=19)	No. of *Campylobacter* - positive flocks (%)
Flock size			
<5000	5	6	5 (83.3)
>5000	5	13	13 (100.0)
Housing type			
deep litter	5	11	11 (100.0)
raised floor	5	8	7 (87.5)
Source of water supply			
pipe/tap	4	5	4 (80.0)
pond/surface	4	14	14 (100.0)
Location			
clear land	7	7	6 (85.5)
oil palm estate	3	12	12 (100.0)
Presence of other animals			
Yes	6	13	13 (100.0)
No	4	6	5 (83.3)
Type of roof			
zinc sheets	4	8	8 (100.0)
thatch	6	11	10 (90.9)

from 25 to 90, depending on the size of the farm. In all farms, campylobacter was not isolated in 1-day-old (455 chicks) and 7-day-old chicks (465 chicks). The detection of the initial presence of campylobacters in the farms was as follows: in 6 farms campylobacters were isolated in 38.2% (191 of 500) 14-day-old chicks; in 3 farms campylobacters were isolated in 45.3% (77 of 170) 21-day-old chickens while in one farm, campylobacter was not isolated in the chicks and chickens, even at slaughter age.

Occurrence of *Campylobacter* in feed, water and environmental samples

Feed: *Campylobacter* was not isolated from 113 samples of feed from newly opened bags as well as in the feed trays.

Water: A total of 206 water samples were taken from water tanks in the chicken houses and drinkers. Three (1.5%) samples were positive for campylobacters - samples from water tanks in three different farms using untreated water from the ponds.

Wood shavings: All 34 samples were negative for *Campylobacter*.

Flies and house environment: *Campylobacter* was not isolated from the 30 flies and from any 114 samples from the chicken houses environment which consisted of two swab samples each of the walls, floors and dust from a total of 19 chicken houses.

Possible risk factors for campylobacters' colonization in the farms: Six factors were analyzed for their association with the colonization of campylobacters in broiler chickens. These factors were flock size, housing type, source of water supply, location of the farm, presence of other animals on the farm and type of roof as shown in Table 2. The results showed that the six factors were not associated with risk of colonization (p value for all six factors > 0.1).

Discussion

Colonization refers to a benign, non-pathological commensal relationship that exists between a host and an organism or parasite. Campylobacters usually enter into this relationship in the gastrointestinal tract of chickens (Stern, 1994). For an enteric organism to establish and maintain colonization, it involves a complex interaction between the host and organism and for *C. jejuni*, the precise mechanism by which it colonizes is not known (Stern, 1994).

The source of *Campylobacter* in broiler chickens at production level is not clear but the incidence of them present generally increases with age (Jones *et al.*, 1991). In this study, the sources of campylobacters in chicks in the farms were also not clear as the factors investigated showed no significant association with the risk of colonization.

It has been reported that vertical transmission of *Campylobacter* infection in poultry is highly improbable (van de Giessen, 1992; Jones *et al.*, 1991). One reason is that *C. jejuni* would not penetrate into the contents of the eggs (Doyle, 1984). Although egg penetration studies revealed that *C. jejuni* could be isolated occasionally from the inner shell and membranes of refrigerated egg (Doyle, 1984), it does not present a threat because *C. jejuni* does not survive for more than

6 h in egg contents (Neill *et al*., 1985).

In the present study, all one - day old and seven - day old broiler chicks were found negative for *Campylobacter* colonization. Thus, this study supported the findings that campylobacters are not transmitted vertically from parents to chicks. This was because these broiler chicks became colonized with *Campylobacter* at about two to three weeks of age. Other studies showed similar findings, however one study reported colonization can occur as early as seven days old (Genigeorgis *et al*., 1986) while others reported at three to five weeks of age (Lindblom *et al*., 1986; Kazwala *et al*., 1990; Jacobs-Reitsma, 1992) and at four to eight weeks of age (Stern *et al*., 2001). A study by Jacobs-Reitsma *et al.* (2001) reported no evidence for vertical transmission of *Campylobacter* in broiler flocks in both breeder and broiler farms.

Once campylobacters have entered a flock, all the chickens in the flock became colonized and stayed colonized up to the time of slaughter (Lindblom *et al*., 1986; Kazwala *et al*., 1990). This finding was shown in this study - there was an increase in the numbers of positive chickens at six or seven weeks of age compared to chicks at two to three weeks old.

In the study by Lindblom *et al.* (1986) on 250 broilers, none was positive for *C.jejuni*, while other studies found 24 - 72% of the flocks were *Campylobacter* - negative (Kapperud *et al*., 1993; Humphrey *et al*., 1993). The present study found only one flock or 6% negative for *Campylobacter* colonization. Among the factors which may contribute to the absence of campylobacters in this particular farm were reasonably good hygiene management of the farm, the owner worked alone (no other worker) and it was the only farm with 'fishing net' placed over the chicken house to prevent pests and birds from entering.

The samples taken from the house environment in a number of epidemiological and risk factors studies include litter, feeds, drinking water, insects, pests and other animals (Kazwala *et al*., 1990; Humphrey *et al*., 1993; Kapperud *et al*., 1993). Generally, feeds and litter are found to be *Campylobacter* - negative. Feeds are dried and pelleted which are likely to be unfavourable for survival of campylobacters (Doyle and Roman, 1982). The work on the survival of *C. jejuni* in broiler feed found that *C. jejuni* underwent rapid death rate in feeds within 24 h when stored at 20 ^{o}C (Humphrey *et al*.,1993). Wood shavings are dry and resinous and their usage on the floor of the broiler house probably has a bactericidal effect on campylobacters as it does for salmonellae (Olesink *et al*., 1971).

Drinking water, in particular untreated water, was found to be associated with *Campylobacter* colonization. In one study, it was found that the predominant source of *C. jejuni* on the farm was the water supply; when intervention programme was instituted, such as chlorination of water and cleaning and disinfection of shed drinking system, the proportion of birds colonized with campylobacters reduced from 81 to 7% which increased to 84% when the intervention programme stopped (Pearson *et al*., 1993). The drinking water samples in three farms were found positive for *Campylobacter* and the sources were untreated surface water.

Insects, particularly house flies, have been shown to play a role in the dissemination of *Campylobacter* infection in chickens (Rosef and Kapperud, 1983; Shane *et al*., 1985). The present study failed to isolate campylobacters from flies; it may be because the number of flies sampled were small. The isolation of *Campylobacter* from darkling beetles and lesser mealworms inside the chicken house by Jacobs - Reitsma (1992) indicated a transmission route from insects to broiler chickens, although it is probably more likely in that study the route was from chickens to insects as the insects were positive after the chickens were found positive. Hazeleger *et al.* (2001) reported that the darkling beetle could play a role in the transmission of *Campylobacter* as the microorganisms for a few days in the beetles.

Another likely source of *C. jejuni* in chicks is by the farm workers, introducing the organisms by their footwear and clothing (Kazwala *et al*., 1990) and by tending other poultry and pigs before entering the broiler house (Kapperud, 1993). The workers had no proper working attire nor do they put on boots. Also, there was no footbath at the entrance of the house. Moreover, hygiene practices were minimal. Hence, the possibility of cross-contamination between houses by workers is high. van de Giessen *et al.* (1992) reported that the use of separate boots for farm workers tending the flocks and washing of hands before dealing with poultry were associated with a reduced risk of acquiring infection with *C. jejuni*, while Humphrey *et al.* (1989) found that dipping boots in disinfectant before farm workers entered broiler house either delayed or prevented colonization with *C. jejuni*. The study by Corry *et al.* (2001) indicated that poorly cleaned and disinfected transport crates are a probable source of infection with campylobacters.

Rodents, in particular rats, and free - flying birds are another likely source which may introduce *C. jejuni* to chicks. They may be reservoirs for campylobacters. There were rats in all the farms studied as reported by the farm workers and owners. The presence of rats on the farm has been shown to be associated with an increased risk (Kapperud *et al*., 1993). A study by Kasrazadeh and Genigeorgis (1987) found 86.7% of rat faeces were positive for *C. jejuni*. Rats, and also mice, may contaminate feed and water which then became the source of *C. jejuni* for the chickens. Once a few chickens were colonized, they further contaminated the feed and drinkers or water troughs and this caused the

colonization to spread. *Campylobacter jejuni* has been isolated from birds, such as crows, pigeons, blue magpies, sparrows and gray starlings (Ito *et al*., 1988; Kapperud and Rosef, 1983). On occasions, during the visits to the farms, two to three birds were seen flying in and out of the broiler houses in all the farms except the farm where no campylobacter was isolated. These birds could be the source and spread campylobacters to the chickens. In two separate studies on flying birds caught around poultry farms and in crows caught around residential areas, it was found that 18% of the flying birds (Saleha *et al*., 2001) and 25.3% of the crows (Chong, 2001) harboured campylobacters.

The management and hygiene practices in the farm do play important roles in the colonization of *C. jejuni* in chickens (Humphrey *et al*., 1993; Kazwala *et al*., 1993). Generally, farms which practice good management and hygiene have lower rate of *Campylobacter* infection (Kazwala *et al*., 1993) and may limit or prevent the horizontal spread of *C. jejuni* (Humphrey *et al*., 1993) within the flock and between flocks.

Acknowledgements

The author would like to thank colleagues for their critical comments, En. Kamarzaman Ahmad for his technical assistance, the broiler chicken farm owners for making this study possible and the University for providing financial aid.

References

Bolton, F.J., D.C. Hinchliffe and L. Robertson, 1982. A most probable number method for estimating small numbers of campylobacters in water. J. Hyg. Camb., 89: 185-190.

Campbell, R.C., 1974. Statistics for Biologists. 2nd .ed. London: Cambridge University Press.

Chong, C.T., 2001. *Campylobacter* in crows: prevalence and antibiotic resistance. Final year project under DVM programme, Universiti Putra Malaysia.

Corry, J.E.L., V.M. Allen, F. Aydin, H.I. Atabay, S.A. Bull, G. Domingue and T.H. Humphrey, 2001. Colonization of broiler chickens with thermophilic campylobacters in three different integrated poultry companies during rearing and slaughter. Abstracts of 11th International Workshop on Campylobacter, Helicobacter and related Organisms in Int. J. Med. Microbiol., 291 (S.31): 41.

Doyle, M.P. and D.J. Roman, 1982. Sensitivity of *Campylobacter jejuni* to drying. J. Food Prot., 45: 507-510.

Doyle, M.P., 1984. Association of *Campylobacter jejuni* with laying hens and eggs. Appl. Environ. Microbiol., 47: 533-536.

Genigeorgis, C., M. Hassuneh and P. Collins, 1986. *Campylobacter jejuni* infection on poultry farms and its effect on poultry meat contamination during slaughtering. J. Food Prot., 49: 895- 903.

Hazeleger, W.C., G.J. Coenen and R.R. Beumer, 2001. Survival of *Campylobacter jejuni* in darkling beetles (Alphitobius diaperinus). Abstracts of 11th International Workshop on Campylobacter, Helicobacter and related Organisms in Int. J. Med. Microbiol., 291 (S.31): 37.

Humphrey, T.J., A. Henley and D.G. Lanning, 1993. The colonization of broiler chickens with *Campylobacter jejuni*: some epidemiological investigations. Epidemiol. Infect., 110: 601-607.

Humphrey, T.J. and I. Muscat, 1989. Incubation temperature and the isolation of *Campylobacter jejuni* from food, milk or water. Letts. Appl. Microbiol., 9: 137-139.

Ito, K., Y. Kubokura, K. Kaneko, Y. Totake and M. Ogawa, 1988. Occurrence of *Campylobacter jejuni* in free-living wild birds from Japan. J. Wildlife Dis., 24: 467-470.

Jacobs-Reitsma, W.F., 1992. Epidemiology and identification of *Campylobacter* bacteria in poultry production. In Proceedings XIX World's Poultry Congress, Amsterdam, I: 371-374.

Jacobs-Reitsma, W.F., C. Becht, T. De Vries, J. van der Plas, B. Duim and J. Wagenaar, 2001. No evidence for vertical transmission of *Campylobacter* in a study on Dutch breeder and Broiler farms. Abstracts of 11th International Workshop on Campylobacter, Helicobacter and related Organisms in Int. J. Med. Microbiol., 291 (S.31): 39.

Jones, F.T., R.C. Axtell, D.V. Rives, S.E. Scheideler, F.R. Tarver Jr., R.L. Wakker and M.J. Wineland, 1991. A survey of *Campylobacter jejuni* contamination in modern broiler production and processing systems. J. Food Prot., 54: 259-262.

Kapperud, G. and O. Rosef, 1983. Avian wildlife reservoir of *Campylobacter fetus* subsp. *jejuni*, *Yersinia* spp. and *Salmonella* spp. in Norway. Appl. Environ. Microbiol., 45: 375-380.

Kapperud, G., E. Skjerve, L. Vik, K. Hauge, A. Lysaker, I. Aalmen, S.M. Ostroff and M. Potter, 1993. Epidemiological investigation of risk factors for *Campylobacter* colonization in Norwegian flocks. Epidemiol. Infect., 111: 245-255.

Kasrazadeh, M. and C. Genigeorgis, 1987. Origin and prevalence of *Campylobacter jejuni* in ducks and duck meat at the farm and processing plant level. J. Food Prot., 50: 321-326.

Kazwala, R.R., J.D. Collins, R.A. Hannan and H.O'M. Crinion, 1990. Factors responsible for the introduction and the spread of *Campylobacter jejuni* in commercial poultry production. Vet. Rec., 121: 305-306.

Kazwala, R.R., S.F.H. Jiwa and A.E. Nkya, 1993. The role of management systems in the epidemiology of thermophilic campylobacters among poultry in Eastern zone of Tanzania. Epidemiol. Infect., 110: 273-278.

Lindblom, G.B., E. Sjogren and B. Kaijser, 1986. Natural *Campylobacter* colonization in chickens raised under different environmental conditions. J. Hyg. Camb., 96: 385-391.

Neill, S.D., J.N. Campbell and J.J. O'Brien, 1985. Egg penetration by *Campylobacter jejuni*. Avian Pathol., 14: 313-320.

Olesink, O.M., G.H. Snoeyenbos and C.F. Smyser, 1971. Inhibitory effect of used litter on *Salmonella typhimurium* transmission in the chicken. Avian Dis., 15: 118-124.

Pearson, A.D., M. Greenwood, T.D. Healing, D. Rollins, M. Shahamat, J. Donaldson and R.R. Colwell, 1993. Colonization of broiler chickens by waterborne *Campylobacter jejuni*. Appl. Environ. Microbiol., 59: 987-996.

Rosef, O. and G. Kapperud, 1983. House flies (*Musca domestica*) as possible vectors of *Campylobacter fetus* subsp. *jejuni*. Appl. Environ Microbiol., 45: 381-383.

Saleha, A.A., 2002. Isolation and characterization of *Campylobacter jejuni* from broiler chickens in Malaysia. Int. J. Poult. Sci., 1: 94-97.

Saleha, A.A., J.R.G. Christopher, I. Aini and K. Ganapathy, 2001. Occurrence of *Campylobacter* in free-flying wild birds. Proceedings of 13th VAM Congress, Kuala Lumpur, 70-71.

Shane, S.M., M.S. Montrose and K.S. Harrington, 1985. Transmission of *Campylobacter jejuni* by the housefly (*Musca domestica*). Avian Dis., 29: 384-390.

Stern, N.J., 1994. Mucosal competitive exclusion to diminish colonization of chickens by *Campylobacter jejuni*. Poult. Sci., 73: 402-407.

Stern, N.J., P. Fedorka-Cray, J.S. Bailey, N.A. Cox, S.E. Craven, K.L. Hiett, M.T. Musgrove, S. Ladely, D. Cosby and G.C. Mead, 2001. Distribution of *Campylobacter* spp. In selected U.S. poultry production and processing operations. J. Food Prot., 64: 1705-1710.

van de Griessen, A., S.I. Mazurier, W. Jacobs-Reitsma, W. Jansen, P. Berkers, W. Ritmeester and K. Wernars, 1992. Study on the epidemiology and control of *Campylobacter jejuni* in poultry broiler flocks. Appl. Environ. Microbiol., 58: 1913-1917.