Potentials for Colonization Control of *Campylobacter jejuni* in the Chicken

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**ABSTRACT**

*Campylobacter jejuni* is a major cause of enteritis in humans. Chicken is the most important vehicle in transmitting the agent to humans in the United States. The organism colonizes the intestinal tract of the chicken and there enters into a non-pathologic, commensal relation. During slaughter and processing the organism can, and does, adulterate the product. Unsuccessful attempts have been made to "clean" the contaminated carcasses or provide pathogen free flocks and rearing facilities. Therefore, the approach to intervene and diminish *C. jejuni* in the intestinal tract is now being studied. We have been gathering background data on colonization dose, isolate differences regarding colonization, competitive exclusion, expression of outer membrane proteins by the organism, immune response of the chicken to colonization, antibody neutralization of colonization, and how poultry lineage influences susceptibility to colonization. By using this information we hope to diminish colonization of poultry with *C. jejuni*.

With improved capability to selectively culture *Campylobacter jejuni* from feces, it has become clear that the organism is a major cause of enteritis in humans (8). *C. jejuni* is one of the most prominent enteropathogens, and is the agent responsible for widespread human misery, disease, and death. Demin et al. (11) have shown that chicken is the most important vehicle in transmitting the agent to humans within the United States. Public education of the safe preparation of poultry has not been successful in reducing the incidence of poultry associated human enteritis and can never totally eliminate the problem. Recent, adverse publicity pertaining to health hazards associated with poultry has been aired on U.S. national television, has alarmed consumers, and could impact retail poultry purchasing.

*C. jejuni* colonize chickens through a multitude of avenues. Potential avenues are feed, water, wild birds and rodents, fecal droppings from other chickens or farm animals, and various other sources. The original source of *C. jejuni* in a flock of chickens is difficult to determine, but, once in the flock, the gastrointestinal tracts of most members soon become colonized (25). Once colonized, these bacteria usually enter into a non-pathologic, commensal relation in the intestinal tract of the chicken. Consequently, they are of little veterinary concern and the only incentive to prevent colonization is to increase the health quality of the final product. Upon slaughter and carcass processing, fecal matter can contaminate the meat, and the organism adulterates the product.

Although *C. jejuni* is susceptible to a wide range of broiler processing steps (such as antimicrobials, heat, salt, etc.), the disease causing agent is still associated with the poultry product. In addition to "cleaning" the contaminated carcasses, other scientific studies have attempted to eliminate outside sources of contamination, or provide breeder flocks which are devoid of these pathogens. None of these approaches have proven useful on a commercial scale insofar as eliminating or effectively diminishing the problem.

*C. jejuni* are not an essential part of normal flora. Birds can be brought to market without colonization, but this occurs by chance rather than by plan. Therefore, international committees (17,45) have issued strong statements indicating that the problem of poultry contamination by *Salmonella* and *C. jejuni* must be solved during the production of the birds. We suggest it appropriate to investigate a variety of new parameters involved in the commensal relationship between *C. jejuni* and the chicken. Intervention during chicken production would have significance in diminishing campylobacteriosis. Preventing the colonization of poultry with *C. jejuni* could be a most significant means for control.

**LITERATURE REVIEW**

Harris and colleagues provide data indicating that a large percentage of human campylobacteriosis is associated with ingestion of poultry products (15). The report indicated that 22.3% of retail poultry was contaminated with *C. jejuni*, and that the "etiologic fraction" involved with chicken consumption accounted for 48.2% of the *C. jejuni* enteritis cases. The report also indicated that the most frequent serotypes of *C. jejuni* isolated from retail poultry were identical to those found in humans. The CDC (11) showed even stronger correlations implicating chicken as the recur-
rent vehicle in campylobacteriosis. They indicated that chick-
ens accounted for an “etiologic fraction” of 70% of the risks
contributing to the disease. Further work (18) has shown that
Campylobacter serogroups isolated at a poultry processing
plant were the same as some of the predominant serogroups
isolated from humans.

In one study of broilers at slaughter, 79% of the birds
were colonized with Campylobacter (34). Flocks varied in
prevalence of colonization from none to 100%. A prevalence
of less than 100% could be due to the timing of sampling
relative to when the organism was introduced to the flock;
but, it could also be due to differences in resistance to
colonization. Establishment and maintenance of coloniza-
tion involves a complex interaction between the host and
organism. A list of some factors which shape niches of the
gastrointestines include (37):

low pH of the stomach  mucin trapping
peristaltic clearing  competitive exclusion
villos sweep  immune exclusion
toxic bile acids  --secretory antibodies
phagocytes  --cell mediated
oxygen tension  nutrient availability

The precise means by which C. jejuni overcomes these
factors is not known but an important mechanism is postu-
lated to involve adaptations to grow within the mucin layer
(24). The spiral shape of Campylobacter is reminiscent of
normal floral organisms such as Spirochetes which grow in
the mucin layer. The flagella (31) and perhaps the chemotac-
tic response to components of mucin (16) are integral parts
of the ability of the organism to live in mucus. Other
capabilities affecting colonization which have been ascribed
to C. jejuni include survival in phagocytes (20), adherence to
enterocytes (13,27,28), invasiveness (9), and production of
enterotoxins (36).

Dolby and Newell (12) reported that by administrating
intraperitoneal vaccination with C. jejuni of female mice
before mating, the dams conferred immunity to their young,
which were challenged orally 4-6 d after birth with the
homologous strain. They could not demonstrate protection
before mating, the dams conferred immunity to their young,
(37):

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Identifying specific colonization factors for vaccine
production would be highly desirable. As these factors, in all
likelihood, are a relatively small component of the pheno-
typic expression in the organism, amplification of host
exposure to these factors may enhance the immune response
against the germane antigens. Inserting the corresponding
factor genes in an attenuated bacterial expression
vector could provide an increased immunological stimu-
lus resulting in host protection against disease. Baron et al.
(4) reported that they obtained protection against dysentery
cause by Shigella flexneri 2a by using an in vivo-con-
structed recombinant plasmid genes specifying a surface
antigen and transferring the plasmid to a galE Salmonella
typhi Ty21a. Mice immunized with this vaccine strain were
found to be protected against challenge with virulent S.
flexneri 2a.

To accomplish a similar result with C. jejuni, isolation
of, and expression of the genes controlling the colonization
factor(s) expressed by the organism is necessary. Previous
methods of expressing colonized genes from Campylobacter
into E. coli have not been successful. However, recent papers
(23,35,42) report on the development of gene transfe
systems for the genetic analysis of C. jejuni. These papers note
cloning in E. coli and expression of a tetracycline resistance
determinant and ribosomal RNA encoding genes in a trans-
formed C. jejuni. These recent developments provide
evidence for the feasibility of cloning a variety of genes into
expression vectors.

It has been repeatedly demonstrated in many animals
that the immune response to antigens and pathogens are
controlled by the immune response linkage group of genes,
designated “I” in mice and “Dr” in humans (46). These
genes are part of the major histocompatibility complex
(MHC). Immune response genes, designated “G region”,
have also been described in chickens (32). The G region has
been shown to control the antibody response to synthetic
polypeptides (6,14,21), human serum albumin (2), pneu-
monic polysaccharide (3), and Salmonella pullorum (32).
The cell mediated hypersensitivity reaction to tuberculin has
also been associated with the immune response genes in the
chicken (19,22). Resistance to mortality due to infection
with coccidia has also been linked to the MHC (5).

Non-MHC genes have been found in the chicken to
control infection by avian leukosis virus (10). However,
resistance to viruses probably do not encompass mech-
nisms which are effective against enteric colonization such
as by C. jejuni. However, mice have been shown to have a
non-MHC gene which control resistance to Salmonella ty-
phimurium, probably by controlling macrophage functions
(33). Also, the colonization of the gastrointestinal tracts of
pigs by Escherichia coli is controlled by a single gene which
codes for a receptor on the surface of the enterocytes (38,39,40).
Fimbriae on the surface of the E. coli bind to the receptor by
which mechanism the bacteria adheres and then can main-
tain its colonization. Breeding programs for pigs have used
selection for absence of the receptor to develop lines of pigs
which are resistant to colonization by E. coli and therefore
resistant to diarrheal disease caused by the organism (38).
CURRENT RESEARCH DEVELOPMENTS

Congenic pairs of C. jejuni: We are studying differences in phenotypic expressions manifested by congeneric strains of C. jejuni, which do (A74/C), and do not (A74/O) colonize chick ceca when chickens are gavaged with ca. 10^8 cfu (41). The colonizing strain was isolated in our laboratory by gavaging the original isolate at levels of 10^8 cfu per chick and passaging a suspension from the droppings of these birds through the same chicks. After repeating the fecal-oral passage four times the birds were held for an additional 6 d before excising the ceca for isolation of the A74/C. This isolate was then administered by gavavage to a new set of chicks and the acquired colonization trait was noted. These congeneric isolates were serotyped as homologous isolates (41).

Colonization characteristics of C. jejuni: Thirty-five colony forming units (cfu) of a culture of mixed isolates of C. jejuni colonized the ceca of one-half of the newly hatched chicks challenged by oral gavage. Thus the colonization dose-50% (CD-50) here is 35 cfu. A challenge dose of 3,500 cfu/chick colonized the cecae of all 35 chicks challenged. Challenge doses of ca. 10^5 cfu of C. jejuni per chick, resulted in consistent cecal colonization regardless of whether the birds were one, two, or three days post-hatch. Four isolates showed consistently strong cecal colonization abilities, while two isolates colonized the ceca in only 20 of 122 chicks when challenged at levels of 10^5 cfu per chick. One of these poorly colonizing isolates was repeatedly transferred by fecal-oral passage through chicks, and subsequently, this isolate was able to consistently colonize chicks.

Competitive exclusion (CE) is an approach in which native microflora exerts an excluding influence upon human enteropathogenic bacteria (i.e., Salmonella spp.) CE microflora did not qualitatively diminish the colonization rates for C. jejuni. Birds treated with 5 different CE cultures were colonized in 81 of 84 chicks, while control chicks were similarly consistently colonized (35 of 35 chicks).

Outer membrane protein analyses: It is likely that the ability of a gram negative organism to find its niche is reflected in the constituents of the outer membrane. We (29; manuscript in preparation) have compared sarcosyl-insoluble outer membrane proteins (OMP) (26) from C. jejuni A74/O and A74/C. Rabbits were immunized with these proteins and electroimmunoblots (43) were performed. A major antigenic band at ca. 30 kdal was detected in A74/O but was conspicuously absent from A74/C. Sera were cross absorbed with A74/O or A74/C formalin fixed isolates and used in Western blots. The result suggested that epitopes common with the 30 kdal antigen are found in the A74/C. When cross absorption studies were performed using insolubilized OMPS, an antigen of approximately 69 kdal was found that was unique to the colonizing A74/C strain. We believe that the 30 kdal antigen in the A74/O may be a defective form of a necessary colonization factor which is present in a functional form in the A74/C, possibly the 69 kdal factor.

Monoclonal antibodies: Our intentions are to produce monoclonal antibodies to this 30 kdal antigen, and use this reagent as a probe for the functional colonization factor, protein antigen. We have produced monoclonal antibodies against C. jejuni using whole cells to sensitize BALB/c mice (unpublished). We obtained several distinct hybridoma lines, all of which recognized the major 45 kdal OMP expressed by the organism, as determined by Western blot analysis. Currently we are using immunization protocols designed to enhance the probability that a MoAb will be developed that will recognize the 30 kdal OMP of the A74/O which we hypothesize will also recognize a putative colonization factor of A74/C.

Antibody neutralization of colonization: By comparing the opsonized and the non-opsonized C. jejuni for CD-50% values, we are assessing the influence of antibody directed against the organism. Preliminary data indicate that colonization factors can be neutralized by providing appropriate immunoglobulins. The A74/C isolate was opsonized with a 1:40 dilution of sera produced either against the OMPs of the A74/C or the A74/O isolates. Normal rabbit serum and phosphate buffered saline (PBS) were used for controlled comparisons. One-day-old chicks were challenged with treated organisms to compare the CD-50% values. The colonization was reduced at least 100-fold among those chicks given the C. jejuni treated with the anti-A74/C sera as compared with the PBS control chicks. The colonization was reduced 10-fold when this treatment was compared with the C. jejuni treated with normal rabbit serum. The implication of these data is that colonization of chickens by C. jejuni can be diminished with appropriate stimulation of the immune system.

Immunoglobulin response to C. jejuni colonization: Analyses of circulating IgG levels and biliary slgA levels were made through enzyme linked immunosorbent assays (ELISA) (30). Samples were taken from chickens colonized with either human-clinical or chicken-carcass isolates of C. jejuni, and from uncolonized chickens. The ELISA used the homologous isolate of C. jejuni as the capture antigen, and was developed with the specific goat anti-chicken IgG or C. jejuni as the capture antigen, and was developed with the specific goat anti-chicken IgG or C. jejuni as the capture antigen, and was developed with the specific goat anti-chicken IgG or C. jejuni as the capture antigen, and was developed with the specific goat anti-chicken IgG or C. jejuni as the capture antigen, and was developed with the specific goat anti-chicken IgG or C. jejuni as the capture antigen, and was developed with the specific goat anti-chicken IgG or C. jejuni as the capture antigen. Preliminary data indicate that colonization factors can be neutralized by providing appropriate immunoglobulins. The A74/C isolate was opsonized with a 1:40 dilution of sera produced either against the OMPs of the A74/C or the A74/O isolates. Normal rabbit serum and phosphate buffered saline (PBS) were used for controlled comparisons. One-day-old chicks were challenged with treated organisms to compare the CD-50% values. The colonization was reduced at least 100-fold among those chicks given the C. jejuni treated with the anti-A74/C sera as compared with the PBS control chicks. The colonization was reduced 10-fold when this treatment was compared with the C. jejuni treated with normal rabbit serum. The implication of these data is that colonization of chickens by C. jejuni can be diminished with appropriate stimulation of the immune system.

Poultry lineage influencing susceptibility to colonization: Chicks from three commercial lines were tested for colonization susceptibility to three strains of C. jejuni at two inoculation dose levels. Chicks of one commercial line were more resistant to colonization, when challenged with 10^6 to 10^4 organisms, than were chicks of a different commercial line. The third commercial line manifested intermediate colonization potentials. The chicks were challenged at 2 d of age and then assayed for colonization 6 d later. We believe
that the response is too rapid to be influenced by active immune mechanisms but may be a reflection of maternally passed antibodies or may reflect some other genetically transmitted factors.

CONCLUSION

In conclusion, we suggest that one of the next, and most important approaches to be taken in diminishing the presence of C. jejuni in the food chain will be to intervene during the production of the chicken. To accomplish these goals our laboratory is considering some of the more fundamental elements involved in intestinal colonization. Included in our approaches are studying colonization factors expressed by the organism, immune response by the chicken host to the organism, antagonistic microflora in the intestinal tract, and the role of host genetics in resistance and susceptibility to colonization.

REFERENCES


