

Undefined and Defined Bacterial Preparations for the Competitive Exclusion of *Salmonella* in Poultry - A Review

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ABSTRACT

During the past two decades, there have been many studies on the efficacy of competitive exclusion for the control of *Salmonella* in poultry. Undefined preparations of cultured fecal or cecal microflora generally reduce the prevalence of infected chicks upon challenge with a standard dose of *Salmonella* under laboratory conditions; in contrast, results under field conditions are more variable. The protective capacity of undefined cultures can be affected by several factors including the source of microflora, method for protective culture administration, presence of poultry feed additives, in-laboratory or natural environmental challenge, and hygienic practices on the farm. The formulation of effective defined cultures is most difficult because of insufficient knowledge on the underlying protective mechanism(s) and interactions between gut microflora. Defined cultures are less effective than undefined cultures under laboratory conditions and afford little protection against natural *Salmonella* challenge; their potency decreases upon storage and manipulation of single or mixtures of defined culture isolates.

SCOPE OF THE PROBLEM

Salmonella spp. continue to figure prominently as the principal cause of human foodborne infections in many countries (3,86,95,103). The prevalence of this human pathogen in the natural environment, and in agricultural sectors involved in the rearing of meat animals, poses a major challenge to animal husbandry, and meat processing and manufacturing operations in their efforts to market safe food products (26,103). The propensity for salmonellae to spread from one meat carcass to another during the defeathering or dehairing operations, and to readily contaminate the slaughtering plant environment, tends to undermine the efficacy of contemporary in-plant control measures (65,102). Moreover, the rendering of infected offal into contaminated feed products promotes the recycling of salmonellae into the meat production chain at the farm level (12,101,103). Recent evidence that very few *Salmonella* cells may constitute a human infectious dose is disquieting and emphasizes the importance of reduced *Salmonella* in various sectors of the meat industry (15,26,27). The situation is further exacerbated by the widespread occurrence of multiple antibiotic-resistant *Salmonella* strains and attendant increase in public health risk (18,19,25,40).

In recent years, the importance of poultry as a vehicle

of human salmonellosis has generally paralleled the marked increase in global consumption of poultry meat (93). At the same time, the rate of *Salmonella* contamination in Canadian broiler carcasses at slaughter increased from 23 to 61% between 1974 and 1983 (13). Concomitantly, poultryborne salmonellae were primarily responsible for nearly a twofold increase in the number of foodborne incidents (95). Such a phenomenon recognizes no geographical boundaries and has been noted in other countries (93). Annual cost estimates of human foodborne salmonellosis in Canada are from \$59 to 846 millions (23,94). Similar costs have been reported for West Germany and for England and Wales (86).

The costs for an estimated two million cases of *Salmonella* infections resulting from the consumption of contaminated meat and poultry in the United States has been estimated at \$1.0 billion annually (73). The latter cost was based on medical expenses and lost productivity only. Interestingly, an estimate of \$23 billion was proposed as the annual economic impact of intestinal infectious diseases in the United States (34).

Control measures for the abatement of *Salmonella* within the complex, vertically integrated poultry industry have generally met with limited success. The occurrence of salmonellae in 50% of Canadian hatchery supply flocks, 5% of hatcheries, 55% of broiler farms, 30% of rendered products, 60% of poultry crates, and 60% of chicken and turkey carcasses at slaughter attest to the magnitude of this contemporary problem (24). Establishment and maintenance of *Salmonella*-free breeder flocks and availability of *Salmonella*-free feeds are prerequisites to the effective reduction of *Salmonella* in the poultry industry. Although traditional control measures have been directed at slaughtering, processing, food service and retail operations, control of husbandry practices and the farm environment seemingly need to be strengthened (31). In the last decade, the propensity of the Nurmi concept to provide for the competitive exclusion of salmonellae from the intestinal tract of chicks has been studied extensively at the production level.

This review focuses on recent developments in the application of competitive exclusion for the control of *Salmonella* in poultry using undefined and defined bacterial cultures.

COMPETITIVE EXCLUSION

The intensity of current husbandry practices in the poultry industry potentiates a greater susceptibility of chicks and poults to *Salmonella* infection because of the slow development of indigenous microflora normally found in the intestinal tract of mature birds. Such flora plays a significant role in protecting young birds against intestinal colonization by bacterial species such as *Salmonella* (67). Although chicks develop indigenous microflora early in life through environmental exposure to intestinal bacteria from older birds, replacement of sitting hens with commercial hatcheries and rearing of chickens in cleaned and disinfected broiler houses may have reduced the prevalence of such protective microflora in the poultry environment. The problem is further compounded by the close proximity of penned birds which favors the horizontal spread of *Salmonella* infection.

Nurmi and Rantala (62) first recognized the importance of early establishment of protective microflora in young chicks. Originally, these workers treated chicks with a suspension of crop and intestinal tract materials obtained from adult birds; in subsequent studies, cecal content or feces cultured anaerobically in a liquid medium were used for treatment. These preparations of unknown bacterial composition were administered orally to day-old chicks which were challenged 24 or 48 h later with a standardized dose of *Salmonella*. Results showed that such treated chicks were more resistant to infection than control birds. This prophylactic approach, known as the "Nurmi concept" or "competitive exclusion" (67), opened new horizons for the control of *Salmonella* in poultry.

Laboratory experiments in several countries subsequently confirmed the efficacy of the competitive exclusion (CE) treatment in the protection of newly hatched chicks or turkey poults challenged with up to 10^6 *Salmonella* per chick (55,67). The CE treatment also yielded encouraging results with *Salmonella*-infected mature birds that had been treated with antibiotics prior to the CE treatment (29,48,52). The protective capacity of Nurmi cultures in chicks and poults has been demonstrated against no fewer than 10 *Salmonella* serovars (54). The effectiveness of the CE treatment in the field has been synergistically amplified by sound hygienic practices in farm operations (52). Several authoritative reviews on different aspects of CE treatments have been published (5,54,56,67,79).

Although several mechanisms by which the indigenous intestinal microflora of animal models could inhibit the colonization of invading microorganisms have been proposed (74,78), the exact mechanism(s) involved in the exclusion of *Salmonella* in chicks by the gut microflora of adult birds has yet to be elucidated. Production of volatile fatty acids by obligate anaerobes in the ceca and/or competition between *Salmonella* and resident intestinal microflora for limited binding sites on the cecal mucosa have been suggested (49,67).

Treatment with undefined mixtures

Source. Protective bacterial suspensions or anaerobic cultures are best prepared from cecal or fecal materials

obtained from healthy, *Salmonella*-free adult birds (5,67). The protective potential of the original cecal or fecal material varies from bird to bird where the age of the donor bird plays a determinant role in treatment efficacy (77). Young chicks (2-d-old) are unreliable donors unless pre-treated with cultured intestinal material from adult birds (4,90). Birds from specific pathogen-free (SPF) flocks have also served as donors for protective (37), weakly protective (45), and nonprotective treatments (44). It is noteworthy that the intestinal microflora of SPF birds is generally less protective than that from conventionally reared birds. The specificity of the Nurmi treatment extends beyond the donor avian species as suggested by the ability of CE cultures derived from chickens to protect turkey poults and vice versa (67). The observation that both strict and facultative anaerobes are important components of effective Nurmi cultures underlines the need to prepare such cultures under strict anaerobic conditions (37). Bacteria-free filtrates have proven to be unprotective (82). Serial subculture of Nurmi cultures has been recommended for the dilution to extinction of harmful parasites and viruses potentially present in the source material (67).

Administration. In laboratory experiments, protective treatment has been commonly administered to chicks by gavage into the crop. The inconvenience of this approach for trials involving large numbers of chicks favored the administration of the CE treatment in the first drinking water (99), or by spraying hatching eggs or hatchlings with CE preparations (36). A comparison of these procedures with other methods of administration, such as agar plates or feed slurries seeded with the protective mixture, found the spray method to be the least effective in laboratory scale experiments (91).

Additives. a) Antibiotics. Commercial poultry feeds can contain different antimicrobial, anticoccidial, and/or growth-promoting feed additives. Antibiotics are added to feed or water at low levels (15-25 ppm) to stimulate growth or at high levels (100-200 ppm) for treatment against specific bacterial diseases (67). The use of antibiotics such as tetracycline, penicillin G, streptomycin, and lincomycin/spectinomycin, but not nitrovin or zinc bacitracin, during *in vitro* culturing of intestinal flora from adult fowl, reduced the protective capacity of CE cultures against *Salmonella* colonization in newly hatched chicks (63,70,71,84). However, incorporation of some of these antibiotics in the feed of chicks rarely reduced the efficacy of CE treatments (55). Similarly, medicated feed containing about 200 ppm of either bacitracin, furazolidone, gallimycin, penicillin/streptomycin, chlortetracycline and tylosin, or 10 ppm of nitrovin did not adversely affect the efficacy of CE treatment (67). A recent report indicates that common antibiotic feed additives at growth promoting levels (5-50 ppm) can affect the performance of protective CE cultures. For example, bacitracin or virginiamycin improved CE-dependent protection, whereas flavomycin had no effect, and avoparcin reduced the level of protection (43).

CE cultures have also been used to prevent *Salmonella* reinfection in antibiotic-treated mature birds. Laboratory

experiments involving antibiotic therapy for about 1 week followed by treatment with CE culture on successive days showed marked reduction in the number of infected birds (5,67). Comparable results were obtained under field conditions where ca. 250,000 adult breeder birds with a history of *Salmonella* infection were similarly treated. In these experiments, different avian species received for 12 d the following medicated feeds containing antibiotic levels of 200 ppm, except furazolidine (400 ppm): chickens (chlor-tetracycline and furazolidine), turkeys (neomycin and chlor-tetracycline), and ducks (neomycin) (52). The antibiotics were withdrawn on the day prior to CE treatment, and birds (ca. 19 weeks of age) moved to the laying farm where CE culture was given in the first drinking water. Protection of birds against natural challenge was obtained in all but two of 22 trials. Synergism between antibiotic prophylaxis and a commercial CE preparation (Broilact[®]) has also been reported (48).

b) Anticoccidials. Limited information is available on the combined effects of antimicrobial and anticoccidial feed additives on the performance of CE culture. The combination of monensin (100 ppm) and nitrivin (10 ppm) did not hamper CE-mediated protection of newly hatched chicks (45). However, use of feed containing a high dose of nitrivin (100 ppm) eliminated the determinant gram-negative sporing anaerobes from the ceca and adversely affected the efficacy of CE cultures (11,55). Of five anticoccidial and antimicrobial feed additives tested, combinations of nicarbazin/bacitracin undermined the protective capacity of CE culture (7).

c) Carbohydrates. Although many data on CE-dependent carbohydrate prevention of *Salmonella* colonization in poultry are available, results are generally inconclusive. For example, while arabinose, galactose, or lactose in the feed reduced the prevalence of *Salmonella* in 7- and 14-d-old chicks, lactose-dependent protection was lost in 14- to 21-d-old birds (51). The addition of mannose or lactose in drinking water reduced the incidence of *Salmonella* after 10 d of carbohydrate treatment; in contrast, dextrose, sucrose, and maltose exerted no effect (66). With older birds, the incorporation of lactose or mannose in feed yielded variable results. More specifically, lactose either reduced *Salmonella* in 10- to 30-d-old poults (20), showed no effect in 21-d-old layer birds (21), or increased the prevalence of *Salmonella* in broilers of market age (96). Similar inconsistencies were reported with mannose (47). Instances of apparent synergism between carbohydrates and CE cultures are of equal interest. The addition of lactose to drinking water increased the levels of protection in CE-treated chicks (22,38). Although ineffective when used alone, the addition of fructooligosaccharide to chick drinking water or its incorporation into feed enhanced the protective capacity of CE cultures (6). Clearly, our knowledge on the role of feed additives in the exclusion of salmonellae from the intestinal tract of poultry is limited. Identification of feed additives that undermine the efficacy of competitive exclusion would be a prerequisite to the successful application of this prophylactic regimen to the control of *Salmonella* in poultry.

Safety. Small- and large-scale experiments with undefined cultures have been done for almost 20 years in

different countries and have not precipitated adverse health effects or unfavorable feed conversion ratios in treated birds. As CE treatments usually consist of subcultured intestinal material from donor birds, the likelihood of transmitting nonbacterial pathogens such as viruses and protozoans that cannot multiply in bacteriological culture media is highly unlikely (67). Furthermore, microorganisms of potential concern such as *Mycoplasma* spp., *Listeria monocytogenes*, and *Campylobacter jejuni* cannot flourish under the cultural conditions used in the preparation of protective mixtures (54).

Several measures to assure the safety of undefined cultures have been proposed. CE cultures can be screened directly for the presence of etiological agents commonly associated with human foodborne diseases, for specific avian pathogens, and indirectly by monitoring SPF birds inoculated orally with the protective mixture for development of pathogen-specific antibodies (54,83). The latter biosecurity approach has been applied to CE treatments in large field trials in The Netherlands (59). Another safety measure applied in England restricts the use of treatment material prepared from a company's flock to birds belonging to that company (52).

Commercial preparation. Two commercial preparations are available. Substantial information exists on the preparation produced in Finland under the name Broilact[®] (Orion Corporation Farnos, Turku, Finland). The original preparation which was marketed under the same name, reportedly contained 20-30 different bacterial strains (61). The current product is an anaerobic culture of selected cecal microflora from adult donor birds previously screened for the presence of poultry pathogens. This description derives from an earlier statement that the best protective effect can be obtained using a manipulated anaerobic broth culture free of pathogenic microbes (60). The currently marketed preparation, distributed in a liquid form, is standardized on the basis of what it does not contain rather than on a definitive bacterial composition.

The Broilact[®] and a similar preparation (81) have been used successfully for over 10 years in Sweden and Finland for the protection of nearly 70% of the national broiler production. The efficacy of Broilact[®] against the following serovars was recently demonstrated in laboratory scale experiments: *Salmonella enteritidis* PT4 in The Netherlands (16) and England (17); *Salmonella kedougou* and *Salmonella infantis* in Finland and England (80); and *Salmonella typhimurium* in France (76). The Broilact[®] preparation proved to be ineffective against *Campylobacter* in Finland (1,2).

The efficacy of Broilact[®] against a natural *Salmonella* challenge under field conditions is poorly documented. In Switzerland, failure of the product to protect broilers led to a conclusion that, in the absence of stringent hygienic conditions on farms, Broilact[®] alone could not prevent the infection of poultry with *Salmonella* spp. (28). In another study in England, a combination of Broilact[®] and antibiotics in feed afforded protection in naturally challenged pullets (48).

Another commercial preparation recently developed in England under the name of "Aviguard" (Life-Care Products

Limited, Malvern Link, England) reportedly consists of undefined intestinal bacteria obtained from healthy SPF chickens. This product is available in a lyophilized form. In-house data showed that spraying of day-old broilers with this preparation, under commercial conditions, reduced the levels of *Salmonella* at slaughter. The trials on its efficacy for breeder birds are in progress.

Efficacy under field conditions. One of the main difficulties in field trials is the widespread "natural" *Salmonella* infection of flocks, and difficulty in setting up control flocks that have been subjected to the same level of *Salmonella* challenge as treated birds (55). The efficacy of freshly prepared CE treatments is usually ascertained by a standard laboratory assay prior to large-scale trials using *Salmonella*-free 1-d-old chicks (58,68).

Initial studies in Finland showed that the Nurmi treatment engendered a substantial decrease in *Salmonella* infection of chickens in only one of two field trials (69). In a separate study, the *Salmonella* status of 400 broiler flocks treated with CE culture in the first drinking water was compared to 192 control (untreated) flocks. Only 6.5% of treated flocks were *Salmonella* positive, whereas 21% of control flocks harbored salmonellae (41).

In Sweden (1981-1990), administration of CE treatment to approximately 3.82 million broiler chicks in the first drinking water resulted in only one of 177 treated flocks (results for untreated flocks were not given) becoming infected with *Salmonella* (100). In a more detailed study of 2.86 of these 3.82 million chicks out of which up to 0.15% were sampled, 0.73% of treated and 1.35% of control chicks were found to be infected (99). Such low levels of infection in control birds underline the positive impact of strict government regulations on the hygienic production of broilers (98).

Large-scale field trials in The Netherlands were conducted on approximately 8.0 million broilers contained within 284 flocks (143 treated and 141 untreated). In these experiments, the CE treatment was sprayed onto hatchlings and eggs in the hatchery. The treatment reduced the number of *Salmonella*-positive flocks from 24 to 15% and the number of infected birds within positive flocks from 3.5 to 0.9% (36,59).

In England, the CE treatment was applied to antibiotic-treated, adult breeder birds (approx. 250,000) to prevent *Salmonella* reinfection (52). Upon withdrawal of antibiotics in feed, the protective culture was provided in drinking water on the following day when breeder birds were transferred from a rearing to a laying farm. Synergism between such antibiotic pretreatment and CE prophylaxis was demonstrated in 20 of 22 field trials.

In recent trials conducted by the U.S. Department of Agriculture in Puerto Rico, CE culture was repeatedly administered to chicks; first, by spraying onto chicks in the hatchery, and secondly, in the first drinking water of chicks upon placement in the broiler barns. Results of the first two trials, each based on approximately 22,000 chickens, showed a reduction in *Salmonella* contamination at slaughter from 24 to 5% in cecal samples and from 40 to 12% in rinses of prechill carcasses (14).

In Canada, early results on CE treatment of commercial flocks were equivocal (68). In subsequent trials conducted in research barns, CE culture failed to protect chicks against natural *Salmonella* challenge; however, substantial protection was obtained when some of these treated chicks were challenged in the laboratory (Barnum and Van Dijk, unpublished data). In Germany, the inability of CE culture prepared from SPF birds to effectively protect broiler birds has also been reported (44).

The foregoing results generally suggest that the use of undefined CE cultures in combination with good hygienic control measures on poultry farms does not consistently reduce *Salmonella* infection in commercial poultry flocks.

Cost. The estimation of the costs and benefits of different control measures applied to poultry production and processing in Canada has led to several interesting conclusions (23,24). A projected annual CE treatment cost of \$26.2 millions would engender \$11.6 millions in societal and poultry industry benefits, and provide for a 41% reduction in human salmonellosis. In comparison, irradiation of poultry carcasses at a cost of \$18.5 millions would result in a 90% decrease in human *Salmonella* infections and generate \$19.2 millions in benefits to the society. The CE treatment stood out among other control measures, as the most expensive and one of the least cost effective (23). Canadian cost estimates of \$0.01 per kg of dressed poultry for CE treatment were similar to that reported in The Netherlands where the in-house production and application of such treatment was approximately \$0.01 per chick (23,55).

Treatment with defined cultures

Notwithstanding the excellent safety record of undefined culture treatments in laboratory and field settings, regulatory agencies in most countries remain apprehensive and continue to prohibit the use of these preparations in commercial poultry operations. The unknown bacterial composition of CE cultures apparently raises concerns on the possible transmission of human and/or avian pathogens that may be present in the source materials from donor birds). Moreover, the bacterial composition and the efficacy of undefined preparations could not be standardized. Therefore, concerted efforts in several countries have focused on the identification of key protective elements in undefined cultures with a view towards the development of a product of known bacterial composition. This topic has been the subject of several authoritative reviews (52,55,17,88).

The major problems in developing defined culture treatments include: i) the lack of a sound scientific basis for the selection of potentially protective strains, ii) inadequate selective isolation media for the recovery of minor bacterial components in cecal materials; and iii) the need for reliable diagnostic schemes for the identification of strains as reflected in the general inability to speciate more than 25% of the bacterial components isolated from the ceca of adult birds (53,55).

Although characterization of the microflora in avian ceca has been undertaken (8,10,75,81), a definitive bacterial profile remains elusive. Obligate anaerobes in the chicken ceca are represented by gram-positive anaerobic

cocci (28%), gram-negative, nonsporing rods belonging to *Bacteroidaceae* (20%), gram-positive, nonsporing rods of *Eubacterium* spp. (16%), budding microorganisms (11%), and *Bifidobacterium* spp. (9%) (53).

Suspensions of cecal or fecal materials from adult donor birds or anaerobic cultures of these intestinal materials have generally been used for the isolation of potentially protective microorganisms. A suspension of cecal material which had successfully protected chicks contained approximately (organisms per ml): 10^8 lactobacilli, 10^6 coliforms, 10^4 fecal streptococci, and more than 10^9 anaerobes (9). The lack of conclusive evidence on the protective role of these group(s) of microorganisms, and on the underlying mechanism of protection, has prevented an informed selection of potentially protective isolates.

Monogeneric preparations. Researchers have examined the protective capacity of single bacterial isolates of *Clostridium* spp. (72), *Streptococcus faecalis* (85), *Bifidobacterium* spp. and *Bacteroides hypermegas* (11). Preparations consisting of several strains of a single genus such as *Bacteroides* spp., *Bifidobacterium* spp., and *Escherichia* spp. have also been evaluated (11,91). Although several research groups were unable to demonstrate the ability of mixtures of lactobacilli to protect poultry against *Salmonella* infection (10,92,97), an isolated report did claim success with this group of microorganisms (64). The foregoing reports indicate that CE preparations containing single strains or mixtures of strains from the same genus do not consistently protect chicks against *Salmonella* challenge.

Polygeneric preparations. Probiotics are live microbial feed supplements which beneficially affect the host animal through improvement of its intestinal balance. Moreover, probiotics usually consist of one to eight bacterial strains that commonly belong to *Streptococcus*, *Lactobacillus*, or *Bifidobacterium* spp. (33). Limited data on the probiotic-mediated protection of poultry against *Salmonella* infection suggest that such preparations are ineffective (30). Probiotic strains from the genera *Bacillus*, *Lactobacillus*, or *Enterococcus* spp., administered to day-old chicks via feed, in the first drinking water, or by spraying chicks, failed to protect treated birds against *S. kedougou* (39). Similarly, multistrain preparations of *Lactobacillus* spp. or *Bifidobacterium* spp., or strain mixtures of both genera administered to chicks by gavage or in the drinking water, offered no protection against *Salmonella typhimurium* (92). The foregoing studies indicate that the evaluation of probiotics needs to be intensified using a standard experimental protocol and assay, if their ability to limit *Salmonella* colonization of the poultry intestinal tract is to be ascertained (58). Furthermore, a laboratory-tested probiotic preparation should yield a protection factor value of >4 as a prerequisite for further validation under commercial test conditions (68).

Greater success has been reported with mixtures containing large numbers of bacterial strains from different genera. The first large mixture developed in England contained 48 strains representing the principal groups of microorganisms in the ceca of adult chickens (45). On the basis of their extensive experience with smaller mixtures, the

authors assumed that this complex mixture would not disturb the ecological balance in the chicken gut. Although the efficacy of their large mixture approached that of an undefined cecal suspension upon challenge with 10^3 *S. typhimurium* or *S. kedougou* per chick, it faltered in field trials in the presence of a natural *Salmonella* challenge (57). Incorporation of 17 additional strains of obligate anaerobes to the 48-membered mixture did not enhance the level of protection (46). It is noteworthy that these two chicken-derived mixtures did not afford protection to turkey poults (57).

Studies with defined mixtures formulated in Canada also showed that a large number of different bacterial strains, isolated from intestinal contents of adult chickens, were necessary for protection (91). The most effective mixtures contained either 28 strains from eight genera (35) or 50 strains from 10 genera (89). The efficacy of both mixtures compared favorably with that of undefined fecal culture in protecting chicks against a challenge dose of 10^4 *S. typhimurium* per chick (88). The mixture containing 50 strains was also effective against 10^5 *S. infantis* per chick (87) but failed to protect turkey poults (68). Recent evidence indicated that the potency of both mixtures gradually decreased during cold storage and repeated laboratory manipulation of isolates (35,91). Other mixtures of 10 to 23 strains from different genera were found to be less effective (10,42,91), whereas mixtures of two to five strains were generally ineffective (32).

Although much research has been conducted in this area, a defined culture treatment exhibiting the potency and stability of undefined cultures has yet to be developed. Defined cultures tend towards instability, and they withstand challenge doses of $>10^4$ *Salmonella* per chick poorly (52). At present, the most effective defined mixtures contain a large number of strains from different genera. Clearly, the commercial production of such large mixtures would be most challenging and would require an unflinching coordination of bacterial culture preparation and quality control. Since the protective capacity of defined mixtures diminishes upon storage and laboratory manipulation of component strains and final mixtures, standardization of such preparations would be most difficult. Moreover, our limited knowledge on the underlying mechanism(s) of protection dampens prospects for the successful commercial development of defined treatments in the near future.

CONCLUDING REMARKS

This review on the performance of CE treatments for the control of *Salmonella* in poultry has indicated that: i) Undefined CE cultures reduce the prevalence of infected chicks upon challenge with a standard dose of *Salmonella* under laboratory conditions. ii) The efficacy of undefined cultures in field conditions falters under the repeated challenges of naturally occurring *Salmonella*; however, the approach seemingly provides for a substantial reduction in the contamination of chickens on the farm and of carcasses at slaughter. iii) Synergism between the protective capacity of CE cultures and stringent hygienic measures on the farm reportedly enhances the success of *Salmonella* control pro-

grams. iv) Undefined CE treatments do not adversely affect the health and feed conversion in treated birds, nor do they contain disease agents of public health significance; standardization of such preparations poses a major problem. v) Treatment with defined cultures has proven less effective; the decreased potency of these preparations upon storage and subsequent manipulation is of concern.

The use of undefined CE cultures in large field trials to reduce the levels of *Salmonella* infection in treated birds with no adverse effects on flock productivity and vigor could be considered as an effective control measure. Studies are needed to define more accurately conditions of husbandry and farm hygiene that will optimize the protective potential of CE treatments (52). Research to elucidate the mechanism(s) of protection and to identify determinant factors needs to be strengthened (88). Developmental research in the area of competitive exclusion may prove to be a useful adjunct to contemporary government-industry programs in Canada for the abatement of *Salmonella* in all sectors of the poultry industry (50).

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