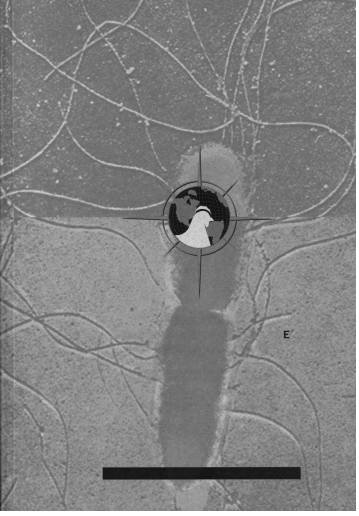
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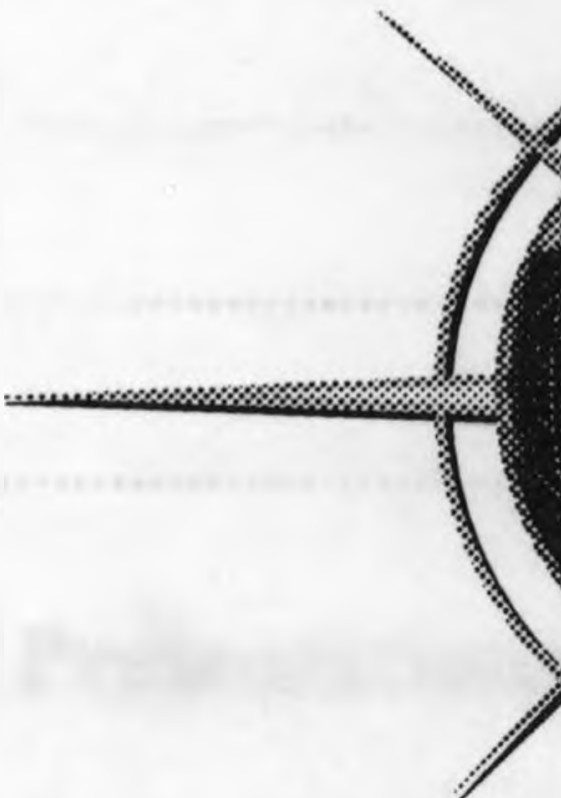

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May 28, 1998

To All 1998 AAAP Symposium participants,

Welcome to Baltimore and welcome to what we think will be a very informative and valuable symposium for you. The symposium format has been expanded to two days this year because of the very important nature and breadth of the topic. The program committee has spent two years planning and organizing the program to provide you the most current information from the most informed experts in the field. Our goal is to help the poultry industry by providing a forum to exchange information and enhance understanding of some current food safety issues. The participation in this symposium by the poultry industry, academia, and governmental agencies indicates that we are taking an important step toward our goal.

We hope you find your participation rewarding.

ely,

John R. Glisson
President, A

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Symposium Schedule

Saturday, July 25

8:00 a.m. Welcome and Introduction (Richard Gast)

Session I: Defining the Problem

Organizers: Robert Eckroade and Richard McCapes

8:05 Obstacles to Control
- Richard McCapes

8:30 Important and Relevant Attributes of the Salmonella Organism
- Tom Humphrey

8:55 Contamination of Raw Foods of Avian Origin
- Ann Marie McNamara

9:15 Meat-Type Poultry Industry Initiatives
- G. Thomas Holder

9:35 Egg Industry Initiatives to Control Salmonella
- Kenton Kreager

9:55 Break

Session II: Sources and Transmission

Organizers: Yan Ghazikhanian and Peter Holt

10:25 Control of Salmonella Contamination of Poultry Feed
- George McIlroy

10:50 Incidence and Impact of Salmonellae in Broiler Hatcheries
- Nelson Cox

11:15 Big Fleas Have Little Fleas on Their Back to Bite Them: Environmental Problems in Poultry Production
- Clifford Wray

11:40 Announcements

11:45 **Lunch**

1:00 p.m. Biosecurity and Disinfection for Salmonella Control
- Gregorio Rosales

1:25 Predisposing Factors
- Peter Holt

Session III: Detection and Testing

Organizers: J. Stan Bailey and W. Douglas Waltman

1:50 Isolation of Salmonella from Poultry Environments
- W. Douglas Waltman

2:15 Break

2:45 Serology and Salmonella
- Richard Gast

3:10 Role of Microbiological Testing in HACCP
- J. Stan Bailey

3:35 **Discussion**
- Charles Beard, Moderator

4:25 Announcements

5:00-7:00 **Poster Session with Cocktails and Hors d'oeuvres**

Organizers: Jean Guard-Petter and Nicholas Primm

Sunday, July 26

8:30 a.m. Welcome and Introduction (Charles Hofacre)

Session IV: Control of Salmonella During Live Animal Production

Organizers: Kenton Kreager and K. V. Nagaraja

8:35 An Overview of Salmonella Control
- Charles Hofacre

9:00 Use of Antibiotics to Control Salmonella in Poultry Production
- John Glisson

9:25 Prospects for "Competitive Exclusion" Treatment in Controlling Salmonellas and other Foodborne Pathogens in Poultry
- Geoffrey Mead

9:50 Break

10:20 Vaccination against Salmonella Infection: Killed and Subunit Vaccines
- K. V. Nagaraja

10:45 The Use of Live, Attenuated Vaccines against Salmonella in Poultry
- Paul Barrow

Session V: Control of Salmonella During Processing

Organizers: Elizabeth Krushinskie and Amy Waldroup

11:10 Intervention Strategies in the Processing Plant
- Helen Brown

11:35 Announcements

11:40 **Lunch**

1:00 p.m. Preharvest Crop Contamination and Salmonella Recovery from Broiler Carcasses at Processing
- Billy Hargis

1:25	The Effect of Poultry Processing Steps on Populations of Bacteria on Fresh Broiler Chicken Carcasses - Scott Russell
1:50	Chemical Control of Microorganisms on Raw Poultry - Amy Waldroup
2:15	**Break**
2:45	The Effect of Management Practices (Collection, Processing, Storage, and Distribution) on the Growth of Salmonella enteritidis in Naturally Contaminated Shell Eggs - Andrew Rhorer
3:10	Irradiation Pasteurization: Efficacy, Marketability, Consumer Acceptance - Christine Bruhn
3:35-4:45	**Discussion and General Summary** - Charles Beard, Moderator

About the Speakers

J. Stan Bailey is a Research Microbiologist with USDA-ARS at the Russell Research Center in Athens, Georgia. He has authored or coauthored over 340 scientific publications in the area of food microbiology, concentrating on improved microbiological quality of poultry, *Salmonella* methodology, and rapids methods of identification. He is currently responsible for research directed toward controlling and reducing contamination of poultry meat products by *Salmonella.* He was appointed to be a Fellow of the American Academy of Microbiology in 1994. He has served as Chair of the Food Microbiology Division of the American Society of Microbiology in 1992 and as Secretary for the AOAC Microbial Methods Committee from 1992 to 1996.

Paul A. Barrow was awarded the PhD degree in 1978 from the University of Reading. After post-doctoral work at the London School of Hygiene and Tropical Medicine and a short term at Unilever Research, he joined Houghton Poultry Research Station (which eventually became part of the Institute for Animal Health) in 1981. He is head of the *Salmonella* group and is currently studying mechanisms whereby *Salmonella* colonize the alimentary tract of poultry. He has an additional interest in serological monitoring, vaccination, and antibiotic resistance. He is the author or coauthor of more than 60 papers in refereed journals.

Helen G. Brown received the PhD degree in Food Science from the University of Arkansas in 1984. During 13 years at the University of Arkansas, she conducted research, taught Food Science courses, and coordinated student activities. She has been a Research Specialist at Tyson Foods, Inc. in Springdale, Arkansas since 1991. She began her career with that company as an oil specialist and has expanded her responsibilities to work on any area related to the quality of Tyson's products. This involves interaction with University researchers, live production, processing, and suppliers. For the past several years, she has worked on evaluating new technologies in the processing plant with respect to improving the quality of the end product.

Christine M. Bruhn received the PhD degree in Consumer Behavior from the University of California, Davis. As a Consumer Food Marketing Specialist, she studies consumer attitudes toward food safety and quality and conducts education programs which inform consumers about new products and technologies. She chairs, FANSA, the Food and Nutrition Science Alliance, composed of the American Society for Nutritional Sciences, the American Dietetic Association, the American Society for Clinical Nutrition, and the Institute of Food Technologists. She is past chair of the Institute of Food Technologists Food Science Communicators and the Nutrition Division, and served as a Scientific Lecturer from 1992 to 1997.

Nelson A. Cox received the PhD degree in Bacteriology from Louisiana State University in 1971. Since joining the staff at the Russell Research Center in Athens, Georgia that same year, he has authored or co-authored more than 450 scientific publications in the area of food microbiology, concentrating on *Campylobacter*, *Salmonella*, *Yersinia*, *Listeria*, and cultural methods of identification. He is a Fellow of the American Academy of Microbiology and an Adjunct Professor of Poultry Science at the University of Georgia. He has been issued 5 patents which have all been licensed by commercial companies.

Richard K. Gast received the PhD degree in Poultry Science from The Ohio State University in 1987. He has served since then as a Research Microbiologist at the USDA-ARS Southeast Poultry Research Laboratory in Athens, Georgia. He is also an adjunct faculty member in the Departments of Poultry Science and Avian Medicine at the University of Georgia. His research interests in recent years have focused on the very diverse issues involved in the prevention, detection, and control of *S. enteritidis* infections in chickens. He was the author of the chapter on paratyphoid *Salmonella* infections in the most recent (10^{th}) edition of *Diseases of Poultry*.

John R. Glisson is a professor in the Department of Avian Medicine at the University of Georgia. He holds DVM and PhD from the University of Georgia. His research program has focused on bacterial diseases of domestic poultry. Currently, he is the Clinical Services Chief for the Poultry Diagnostic and Research Center and is the President of the American Association of Avian Pathologists.

Billy Hargis earned the DVM and PhD degrees at the University of Minnesota and is a diplomate of the American College of Poultry Veterinarians. He has been a faculty member of the Departments of Veterinary Pathobiology and Poultry Science at Texas A&M University since 1987, where he is currently a Professor. He maintains active undergraduate teaching and graduate education/research programs with much of his research directed toward controlling salmonellosis in poultry and reducing poultry-related food-borne illness through innovative antemortem control programs.

Charles L. Hofacre is Manager of Professional Service for Bayer Animal Health's Poultry Business Unit in Watkinsville, Georgia. Before joining Bayer, he has served as director of veterinary medicine for an international turkey breeder and as vice-president of poultry health and quality assurance and member of the board of directors for a leading international broiler breeder. From 1992 to 1996, he served on the General Conference Committee of the National Poultry Improvement Plan, the poultry health advisory committee to the Secretary of Agriculture. He is the author or coauthor of numerous scientific poultry publications. He holds the DVM degree from The Ohio State University and the PhD degree in Medical Microbiology from the University of Georgia.

G. Thomas Holder received the DVM degree from the University of Georgia in 1964 and has been an integrated poultry veterinarian for 24 years. He currently serves as Director of Avian Health for Allens Hatchery, Inc. in Seaford, Delaware.

Peter S. Holt has been a Research Immunologist with the USDA-ARS Southeast Poultry Research Laboratory in Athens, Georgia since 1987. He is a recipient of the PhD degree in Immunology from the University of Missouri School of Medicine. His current research focuses on the development of mucosal immunity in chickens against intestinal and respiratory pathogens with the ultimate goal of creating efficacious vaccines for inducing strong mucosal immune responses. He is on the editorial board of the journal *Poultry Science* and is an adjunct faculty member of the Department of Avian Medicine at the University of Georgia.

Tom J. Humphrey, PhD is currently Head of the PHLS Food Microbiology Research Unit in Exeter, UK. Before joining the PHLS in 1981, he was a Lecturer in Food and Agricultural Microbiology at the University of Plymouth, where he now holds a Visiting Chair in Food Microbiology. He has also been appointed a Visiting Professor in the School of Biological Sciences of the University of Exeter. The current research activities of his unit are focused on investigating the responses of *Salmonella* and *Campylobacter* to environments common in food and animal production.

Kenton Kreager has 18 years of experience in layer service work with two breeders and one feed company. For the last 14 years he has been employed by Hy-Line International in Dallas Center, Iowa, where he is in charge of that company's worldwide customer service and company diagnostic laboratory. He received the DVM degree from Iowa State University in 1979 and is a Diplomate of the American College of Poultry Veterinarians.

Richard H. McCapes, DVM is President-elect of the U. S. Animal Health Association. He was in the turkey breeding business prior to joining the faculty at the School of Veterinary Medicine, University of California, Davis and chaired the Department of Epidemiology and Preventive Medicine before retirement. He is a past Chair of both the Veterinary Medicine Advisory Committee to the FDA and the Secretary's Advisory Committee on Foreign Animal and Poultry Diseases to the USDA and is a past President of the American Association of Avian Pathologists.

George McIlroy qualified as Veterinarian from Trinity College, University of Dublin in 1975. He has served as a Veterinary Officer in the Field Service of the Department of Agriculture for Northern Ireland, a Senior Veterinary Research Officer responsible for Epidemiology with the Veterinary Science Division at Stormont Laboratories, and Associate Director and Head of Technical and Veterinary Services for Ross Breeders. He was responsible for setting up the voluntary poultry *Salmonella* monitoring program in Northern Ireland ahead of the introduction of compulsory legislation. In 1996, he joined John Thompson and Sons Ltd, the major compound mill in Ireland, to create a major new biosecure manufacturing facility to produce and supply *Salmonella*-free feed for poultry.

Ann Marie McNamara, has been the Director of the Microbiology Division, Scientific Support, of the USDA Food Safety and Inspection Service since 1992 and is responsible for planning, directing, and coordinating the scientific activities of three branches and for identifying new areas of research and new microbiological monitoring and surveillance programs. Awarded the ScD degree in Microbiology by the University of Pittsburgh, she has also previously served as a Senior Staff Microbiologist at the Clinical Microbiology Service of the National Institutes of Health Clinical Center.

Geoffrey C. Mead received the PhD degree from the University of London. In 1967, he joined the Agricultural Research Council's Food Research Institute at Norwich and worked for 18 years on microbiological aspects of poultry production, processing, and storage. A reorganization of food research within the council led to his group's transfer to continue their work at the Meat Research Institute in Bristol, where he eventually became Head of the Hygiene Section and Acting Head of the Microbiology Division. In 1992, he became Vestey Professor of Food Safety and Veterinary Public Health at the Royal Veterinary College, University of London. He is currently European President of the World's Poultry Science Association and holds the Temperton Fellowship for Poultry Research.

K. V. Nagaraja is Professor of Veterinary Medicine at the University of Minnesota. He received the DVM degree from Mysore Veterinary College in 1966 and the PhD degree fro the University of Minnesota in 1979. He is a member of the U. S. Animal Health Association Standing Committee on Transmissible Diseases of Poultry and is Vice-Chairperson of that organization's Committee on *Salmonella*. His extensive research activities, focusing principally on poultry salmonellosis, have been reported in 400 publications, including refereed journal articles, abstracts, book chapters, and extension publications.

Andrew R. Rhorer has been Senior Coordinator of the National Poultry Improvement Plan since 1991. He has also recently served as Egg Industry Liaison for the Salmonella enteritidis Task Force and Director of the USDA-APHIS Poultry Health Initiative. Awarded the MS degree in Food Science by Purdue University in 1982, he has also previously served as Executive Vice-President of the Indiana Poultry Association, Executive Director of the Indiana State Egg Board, Executive Director of the Indiana Turkey Market Development Council, and Executive Director of the Tri-State Poultry Federation.

Gregorio Rosales received the DVM degree from the National Autonomous University of Mexico and joined a broiler company as a staff veterinarian in 1979. He served as an associate professor at the College of Veterinary Medicine in Mexico between two terms of study at the University of Georgia, which awarded him the PhD degree in 1988. Since then he has worked in broiler primary breeding. He was been employed by Ross Breeders Incorporated since 1991 and is currently Vice President of Veterinary Services.

Scott M. Russell received his PhD degree in Poultry Science from the University of Georgia. After his undergraduate education, he worked in the poultry industry for GoldKist and Wayne Farms for three years as a Microbiologist, Quality Control Manager, Production Scheduler, and Production Manager. He has been an Assistant Professor of Poultry Science at the University of Georgia for four years, with both research and extension responsibilities. His main areas of interest are developing rapid and automated methods for identifying and enumerating pathogenic and spoilage bacteria from foods of animal origin and identifying methods for eliminating pathogenic and spoilage bacteria from poultry during rearing and processing.

Amy L. Waldroup has been at the University of Arkansas since 1987, and is currently a Professor of Poultry Products Technology in the Center of Excellence for Poultry Science. She teaches courses related to egg and poultry products technology and her research efforts have focused on methods to control pathogens and spoilage organisms on processed poultry. Research generated in her lab has led to the approval of chlorine dioxide, on-site generated chlorine, and ozone for treatment of processed poultry. She holds a PhD degree in Food Science and Technology from Texas A&M University.

W. Douglas Waltman was awarded the PhD degree by the University of Georgia in 1985. After serving as an NIH Postdoctoral Fellow at the University of Alabama at Birmingham, he has served since 1988 as a Microbiologist at the Georgia Poultry Laboratory in Oakwood, Georgia. He created and has annually supervised the National Poultry Improvement Plan's National Workshop on Monitoring and Detection of *Salmonella* in Poultry and Poultry Environments and is the author of numerous scientific journal articles and book chapters on diagnostic methodology for *Salmonella* in poultry.

Clifford Wray graduated from the University of Edinburgh as a Veterinarian in 1963 and received the PhD degree from the University of Liverpool in 1969. He served as an Assistant Veterinary Investigation Officer at the East of Scotland College of Agriculture and has been at the Enteric Bacteriology Section at the Central Veterinary Laboratory (UK) since 1972. He is presently Head of Section for the National and OIE Reference Laboratory for *Salmonella*. He has been awarded Fellowship in the Royal College of Pathologists for his published work and is a consultant to WHO on *Salmonella* vaccines, verocytotoxic *E. coli*, and antimicrobial resistance.

Abstracts of Oral Presentations

Obstacles to Control

R. H. McCapes
President-elect
United States Animal Health Association
Davis, California

This will be a discussion of factors which contribute to salmonella contamination of raw foods of avian origin and obstacles to their control. Emphasis is given to the live bird, slaughter and processing phases of the poultry meat and egg industry production system and 16 salmonella control points within that system. Special attributes of the salmonella organism, host animal and industry structure are discusses, as well as control strategy options in a changing regulatory and consumer environment.

Important And Relevant Attributes Of The Salmonella Organism

Professor Tom Humphrey, PHLS Food Microbiology Research Unit,
Church Lane, Heavitree, Exeter Devon. EX2 5AD, United Kingdom

Contaminated poultry meat and eggs, particularly the latter, continue to be internationally important vehicles of human salmonellosis. The principal salmonella serotype involved is *Salmonella enteritidis*. There are over 50 phage types (PTs) of this bacterium but PT4 is the most widespread and probably the most important. As well as being an important human pathogen PT4 can also cause severe clinical problems in growing broiler flocks.

Chickens can be colonised with and occasionally infected by a wide range of salmonella serotypes but *S.enteritidis* predominates. The organism possesses several attributes which allows it some selective advantage in the poultry environment. Many strains, particularly of PT4, are highly invasive in chickens and can infect from a low dose and by a variety of routes including contaminated aerosols and dust particles. Under certain conditions strains of PT4 will show enhanced tolerance to a range of potentially hostile environments. Isolates with enhanced tolerance are also those which have a predilection for reproductive tissues. It is probably this latter attribute which is the most important in the continued dominance of PT4. Reproductive tissues can be positive in the absence of faecal carriage which presents problems in surveillance and can lead to the contamination of egg contents which pose a direct threat to public health and also allow the vertical transmission of the bacterium. Carriage in infected birds can be long-standing but the bacterium will also easily spread to birds housed in the same unit.

Another important attribute of *S.enteritidis* isolates is their ability to persist for a long time in the farm environment. In the United Kingdom the infection of salmonella-free replacement flocks, a partial consequence of the persistence of PT4, is a major problem for the egg and poultry meat industries.

The lecture will include discussions on the attributes of PT4, *S.typhimurium* definitive type (DT) 104, and like salmonellas and will also involve a consideration of intervention strategies being adopted by the UK poultry industries.

Contamination of Raw Foods of Avian Origin

Ann Marie McNamara, Sc.D.
Director
Microbiology Division
Office of Public Health and Science
Food Safety and Inspection Service
U.S. Department of Agriculture
Washington, D.C. 20250

The Microbiology Division, Food Safety and Inspection Service, United States Department of Agriculture, routinely conducts bacteriological surveys of raw animal products. These surveys are designed to determine the types, prevalence, and levels of pathogenic bacteria and indicator organisms found on raw poultry carcasses and in ground poultry products. This session will compare and contrast the results of these surveys as they apply to raw foods of avian origin.

Meat-type Poultry Industry Initiatives

G. Thomas Holder, DVM, Dipl. ACPV
Director, Avian Health
Allen's Hatchery, Inc.

It is not possible, contrary to what some consumers believe, to produce salmonella free poultry short of irradiation. What realistic level of salmonella can be expected in birds going to the processing plant? Several governmental agencies want regulatory power over live production but efforts should be in the area of research. Should salmonella be measured quantitatively rather than positive or negative?

Interventions by the poultry industry to reduce salmonella and best management practices will be discussed.

Egg Industry Initiatives to Control Salmonella

Kenton Kreager, DVM, ACPV
Hy-Line International
Dallas Center, Iowa

The commercial layer industry in the U.S. demonstrates a high degree of concern and activity toward the control of S. enteritidis (SE) in eggs and egg products. A survey of the nation's largest producers, representing over 100,000,000 layers, indicated the techniques used in SE control depend on their perceived cost-effectiveness. Nearly all respondents participate in one of the defined egg quality assurance programs and a majority of those test flocks at various ages by culture of birds or housing environment. Most would voluntarily divert eggs to pasteurization from known SE-positive flocks. Salmonella vaccines, competitive exclusion products, and organic acid feed additives are generally not used by the industry, although a willingness to evaluate new products was indicated. All chicks purchased come from NPIP "U.S. S. enteritidis Monitored" breeders and hatcheries and about half of the producers conduct additional testing of chicks or chick box pads upon arrival at the farm. Growing houses are more routinely washed and disinfected between flocks than adult layer houses, but known SE-positive layer houses are given more cleaning/disinfection effort. Routine antibiotic administration is not considered a method of salmonella control. Feed ingredients are often selected based on likelihood of salmonella contamination and/or tested prior to use. Rodent and insect control is given high priority and consultants or commercial services are often used to design effective programs which are then usually administered by employees. Egg wash temperatures/pH and egg storage/transport temperatures are closely monitored by producers, who demonstrated a good knowledge of the desired target levels. Employees are often trained in disease control issues and are expected to help conduct the company's biosecurity policies.

Control of Salmonella Contamination of Poultry Feeds

Dr. S. George McIlroy
Group Technical Director
John Thompson & Sons Ltd.
Belfast, Northern Ireland

Human and animal disease caused by Salmonella probably costs billions of dollars each year. In the UK, it is conservatively estimated that human illness associated with Salmonella costs around 25M dollars per annum. Much of this is blamed, rightly or wrongly, on the consumption of poultry products.

Detailed investigation by the Ministry of Agriculture, Fisheries & Foods in the United Kingdom have indicated that feed can be the commonest source of Salmonella for poultry flocks. Furthermore, a recent House of Commons Agricultural Committee Report on Food Surveillance has recommended that government should provide the impetus to achieve Salmonella free feed in the future.

This paper highlights the contamination of poultry feeds and raw materials by Salmonella in the United Kingdom and Europe over recent years. The paper also describes the current methods being applied to control Salmonella contamination and comments on the level of success achieved. The paper highlights the necessity of achieving effective control of Salmonella contamination of poultry feed as a pre-requisite for a total biosecurity programme to control Salmonella during live animal production.

Incidence and Impact of Salmonellae in Broiler Hatcheries

N.A. Cox
USDA, ARS, Russell Research Center
Box 5677, Athens, GA 30604-5677

Breeder flocks and commercial hatcheries represent the earliest and probably the most critical control points for salmonellae entry into the commercial integrated poultry operations. The implications of salmonellae in the hatchery can be far reaching. To begin with, salmonellae can enter the hatchling chick through an assortment of body openings, such as the mouth, nasal, navel, eye or cloaca. Regardless of entry route, very low numbers of salmonellae are required to colonize the chick. A chick that becomes colonized in the hatchery can subsequently spread salmonellae contamination to other chicks in the hatchery and to flock mates during grow-out. When such a flock reaches the processing plant, salmonellae contamination both interior and exterior can be released into the processing plant and contaminate the final product from that and other subsequently processed flocks. Data from several field trials have been used to track *Salmonella* serotypes originating from the hatchery to the final processed carcass. Competitive exclusion treatments have been shown to protect chicks from low level challenge by environmental salmonellae; however, pre-exposure to salmonellae in the hatchery can reduce the effectiveness of such a treatment.

Big Fleas Have Little Fleas on Their Back to Bite Them: Environmental Problems in Poultry Production

C. Wray and R. Davies
Central Veterinary Laboratory
New Haw
Addlestone
Surrey
United Kingdom

The food chain is an important source of *Salmonella* for poultry. The organism is widespread in the environment and many watercourses and ponds may be contaminated with *Salmonella* by the activities of man and other animals. The organism can also survive for long periods of dusts in poultry houses despite cleaning and disinfection. As a consequence, insects may become contaminated and act as vectors.

A number of studies have indicated that the mealworm beetle (*Alphitobius diaperinus*) may become infected and excrete *Salmonella*. Likewise, flies have been shown to be possible vectors and the organism may become established and multiply in them. We have shown that blowfly larvae (*Lucilia serricta*), which may contain up to 10^4 c fus *Salmonella*, are attractive to chickens, and when ingested the cuticle has a protective effect on the gut and chickens are consequently become infected with very small doses of *Salmonella.*

Birds and mammals may also become infected either directly or indirectly from insects. Although the presence of *Salmonella* in rodents may on many occasions indicate environmental contamination, many investigations have attested to the importance of rodents in the persistence of *Salmonella* on poultry farms. Our studies have shown that mice may secrete *Salmonella* intermittently for at least 18 weeks, the number of organisms per pellet ranging from <10 to 10^5. Chickens may readily be infected should they consume these pellets.

At depopulation, rodents move out of buildings and return to re-stocking, contaminate the house and perpetuate the *Salmonella* cycle. Cats may become infected by eating infected rodents and spread *Salmonella* to animal feeds, etc. Infected animals, especially birds, may spread *Salmonella* to other holdings.

Thus, *Salmonella* control policies must include pest control *in its finest sense* if the spread and persistence of *Salmonella* is to be prevented on poultry farms.

BIOSECURITY AND DISINFECTION FOR SALMONELLA CONTROL

A. Gregorio Rosales DVM, Ph.D and Eric L. Jensen DVM, MAM

Today, large integrated broiler operations and the primary breeding companies supplying them are extremely vulnerable to the transmission of pathogens such as *Salmonella spp.* Therefore, the major emphasis in preventing infections with salmonellas and other pathogens is to avoid their introduction to farms and hatcheries. This is accomplished by establishing and implementing effective biosecurity programs. A biosecurity program comprises a series of regulations for the location and design of farms; movement of personnel and equipment; manufacturing and distribution of feed; rodent and pest control; cleaning and disinfection procedures; disease surveillance and risk assessment. These regulations should be simple, cost-effective, and reviewed regularly in accordance with the challenge situation and resources available. Also, the program must be established as a team effort and through a complete understanding and commitment to the specified goals.

The implementation of a comprehensive biosecurity program will protect the value of the capital invested and contribute to the long-term profitability of the operation.

Cleaning and disinfection practices are an important part of the biosecurity program. These procedures are essential between production cycles to reduce or eliminate the presence of salmonellas that may infect subsequent flocks. Careful planning, attention to detail, selection of the suitable disinfectants and application methods are critical to make the effort cost-effective. Bacteriological verification by swabbing and culture is critical to assess the success of these procedures. Company personnel must be aware and trained on the biological, environmental, food and occupational-safety, and regulatory issues that apply to the use of products used for cleaning and disinfection.

Predisposing Factors

Peter Holt
USDA
Southeast Poultry Research Lab
Athens, Georgia

Commercial poultry may be exposed to salmonellae at many points during their lifetimes, the outcome of which will vary depending upon the *Salmonella* species involved. Flock infection with the avian pathogens *S. pullorum* or *S. gallinarum* routinely results in a systemic infection with high rates of morbidity, mortality, and persistent infection. Conversely, infections in poultry by paratyphoid salmonellae are generally milder and more self-limiting. However, certain situations can alter the host/parasite interactions between the birds and the salmonellae which can have a dramatic effect on the severity of the resultant infections. Factors, such as age, concurrent infections, intoxication, and stress can all affect the severity and progression of a paratyphoid *Salmonella* infection. Age is an especially important variable in that newly hatched birds are 100-1000-fold more susceptible to *Salmonella* infections than older birds, the infections are more often lethal, and the organism can persist in tissues for extended periods following infection. Similarly, increased *Salmonella* shedding, infection persistence, and pathology are observed in birds infected with disease agents such as infectious bursal disease virus, reticuloendotheliosis virus, and coccidia or following the ingestions of mycotoxins. Molt induction and transport stress have also been shown to exacerbate *Salmonella* problems in poultry, increasing the susceptibility, shedding, and horizontal transmission of the organisms through the flock. The identification of these and other situations which predispose the birds to paratyphoid *Salmonella* infections is a crucial first step for the producers in reducing the incidence and levels of *Salmonella* in their flocks. This will ultimately enhance the safety and wholesomeness of the poultry products reaching the consuming public.

Isolation of *Salmonella* from Poultry Environments

W. Douglas Waltman
Georgia Poultry Laboratory
Oakwood, Georgia

The ability to monitor for and detect *Salmonella* in poultry is the first step in reducing *Salmonella* on farms and ultimately in the product bought by the consumer. Developing baseline data is important in devising intervention strategies, biosecurity measures, and good management practices for reducing *Salmonella*. Thereafter, monitoring for *Salmonella* is a vital part of assessing these measures to determine their efficacy and to make necessary changes when needed.

The isolation of *Salmonella* begins with the collection of representative samples from the poultry environment. These should be collected from several sites in the house and should be stored and transported to the laboratory appropriately and timely. Once in the laboratory, these samples should be set up as soon as possible.

When attempting to isolate *Salmonella* from environmental samples, three characteristics of these samples should be considered: a) *Salmonella* are usually in low numbers, b) other bacteria are usually in very high numbers, and c) *Salmonella* may be stressed or injured.

A nationwide survey of laboratories in the United States involved in *Salmonella* detection from poultry found a high degree of variability in the media and methodology used to isolate *Salmonella*. A lack of standardization had been shown in earlier studies in Europe to adversely affect the ability of different laboratories to isolate *Salmonella* from the same samples. Standardization of media and methods for isolating *Salmonella* may not be forth coming or necessary, however, there are some media and methods that are clearly superior to others and these should be adopted. The use of selenite as an enrichment media should be discontinued due to its lack of sensitivity, toxicity, and waste disposal problems. The use of plating media containing novobiocin has been shown to eliminate *Proteus* spp. and dramatically decrease false positive results, while increasing sensitivity. Other plating media, such as XLT4, have also proven to be better than some of the older media.

Serology and *Salmonella*

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Since the late 1920's, detecting specific antibodies in the blood of infected poultry has provided the first line of defense against losses caused by *Salmonella pullorum* and *S. gallinarum*. Known to cause vertically transmissible, systemic diseases in poultry, these host-adapted pathogens are controlled principally (as in the U. S. National Poultry Improvement Plan) via serological identification of infected replacement breeding stock before the onset of egg production. Largely because of the effectiveness of widespread serological testing programs, the incidence of pullorum disease and fowl typhoid has remained low in many nations for several decades.

In recent years, much of the focus for *Salmonella* detection in poultry has shifted to the non-host-adapted "paratyphoid" serotypes (such as *S. enteritidis* and *S. typhimurium*), which are primarily of concern as agents of food-borne disease in humans. Although diverse serological methods have been developed and applied to successfully detect paratyphoid infections with both sensitivity and consistency, the use of antibody tests in this context has never secured anything remotely approaching universal approval. Among the objections to the use of serology for detecting infections of poultry with paratyphoid Salmonellae are (1) infections without a significant systemic phase may never elicit a detectable antibody response, (2) antibody detection provides only an historical determination of prior exposure to *Salmonella*, and cannot assess whether a flock currently presents a risk to public health, (3) extensive antigenic cross-reactivity can significantly complicate identifying a particular serotype as the cause of infection, and (4) the diverse environmental sources and reservoirs of paratyphoid Salmonellae may compromise any real hope of controlling infections with these organisms in production flocks by simply identifying infected breeders.

Nevertheless, several attributes of antibody tests are distinctly advantageous in comparison to bacteriological methods such as culturing environmental samples. Serological assays can more easily be automated to accommodate large numbers of samples within limited laboratory space and can deliver results far more rapidly. The presence of specific antibodies in the yolks of eggs laid by infected hens affords a sample that can be collected with minimal labor, without causing stress to the birds or posing a risk to flock biosecurity. Because antibody titers can persist long after the clearance of active infection, serology is generally of limited value for identifying currently infected flocks in order to prevent their eggs from being released to consumers. However, for other purposes, the persistence of antibody positivity can be an asset. For example, antibody tests can be highly useful as screening tools to help identify whether particular flocks should be subjected to expensive and time-consuming bacteriological monitoring. Risk reduction (quality assurance) programs for controlling *S. enteritidis* in egg-laying chickens are often based on a regimen of recommended management practices. By determining whether these programs have succeeded in preventing exposure to *S. enteritidis*, serological testing can provide an efficient index of the cost-effectiveness of risk reduction activities.

The Role of Microbiological Testing in HAACP

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The Food Safety and Inspection Service (FSIS) established published "The Final Rule on Pathogen Reduction and Hazard Analysis and Critical Control Point (HACCP) Systems" in July of 1996. By definition, HACCP is designed to be preventative, to identify critical control points and to implement procedures to control, monitor and verify the success of the system. The FSIS mandated HACCP system has two principal microbiological testing components. First, performance standards for *Salmonella* have been set that all slaughter plants that produce raw, ground meat and poultry will have to meet. Also, each slaughter plant is required to conduct microbial testing for generic *E. coli* to verify that their process control systems are working as intended to prevent fecal contamination.

Sampling plans and microbiological test currently being used by FSIS and the poultry industry will be reviewed and critiqued. Additionally, the relationship between generic *E.coli*, *Salmonella*, and fecal contamination and their relationship to process control will be examined. Finally, the possibility of future changes in sampling plans and microbiological techniques on the improved efficiency of HACCP will be discussed.

An Overview of Salmonella Control

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If we are to be successful in reducing salmonella in poultry, we need to accept one primary concept: **Salmonella is a Management Disease!** In reality, unless we are dealing with *S. enteritidis* phagetype 4 or turkey poults, salmonellosis is usually not a disease. However, veterinarians are the link because they know microbiology, epidemiology, and most importantly, management; therefore, salmonella is considered a disease. There are many ways to approach the control of salmonella. What I will try to do is give an outline of the key areas to review or focus on in developing a program. However, for a salmonella control program to be successful it will have to be customized to fit an individual organization's daily bird management program. In other words, a successful salmonella control program is one that becomes an integral part of the daily routine of every employee in the organization.

Use of Antibiotics to Control Salmonella in Poultry Production

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Salmonella species in poultry may be highly pathogenic to birds, such as *Salmonella pullorum*, or mildly to moderately pathogenic to birds, such as *Salmonella enteriditis*, or totally non-pathogenic to birds. Transmission can occur from breeders to progeny by transovarian contamination of hatching eggs or by contamination of the hatching environment because of deficient hatching egg hygiene. Antibiotics have been used by various programs to reduce the transmission from infected breeders to progeny. Much of the use of antibiotics to control *Salmonella sp.* in poultry has occurred in developing nations. For instance, in most developed countries, *Salmonella pullorum* and *Salmonella gallinarum* have been eliminated from commercial poultry by serological monitoring of flocks and elimination of infected birds. In some less developed nations, the eradication or control of these organisms has been attempted utilizing antibiotics.

More commonly, antibiotics have been used to suppress or eliminate the shed of the paratyphoid salmonellae from infected breeders to their progeny. The shed rate from infected breeders can be impacted dramatically by antibiotic use in the breeders. Also, antibiotics are injected into day-old poultry or *in-ovo* before hatch. Although this practice is not necessarily intended for salmonella control, it can also suppress the salmonella infection in young birds.

Antibiotic usage for salmonella control has been limited by the availability of effective antibiotics for use in poultry. The poultry industry in most countries has minimized the use of those classes of compounds used in human medicine, such as fluoroquinolones, to preserve their effectiveness.

PROSPECTS FOR "COMPETITIVE EXCLUSION" TREATMENT IN CONTROLLING SALMONELLAS AND OTHER FOODBORNE PATHOGENS IN POULTRY

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In newly hatched chicks, the rapid establishment of an adult-type intestinal microflora, via the oral route, produces almost immediate resistance to colonisation by any food poisoning salmonellas that gain access to the rearing environment. Exploitation of the "competitive exclusion" (CE) effect is now an accepted part of the overall strategy by which poultry-associated salmonellas are being controlled in some countries. However, the degree of protection provided by CE treatment is strongly influenced by the nature and source of the treatment material. From the many studies carried out on CE, it is clear that the most effective and consistent protection is obtained with the use of complex, undefined microfloras from certain adult donor birds. Defined mixtures of treatment bacteria have been less protective because the nature of the CE effect *in vivo* is not fully understood and the essential properties of the protective organisms are unknown. Therefore, any attempt to select protective strains can only be made on an empirical basis. Although several undefined treatment preparations are now available commercially, some regulatory authorities have been reluctant to sanction their use due to fears that unsuspected avian and human pathogens could be transmitted to recipient birds. In practice, such products are screened for a wide range of potential pathogens and, in parts of Scandinavia, there is a long history of problem-free use.

Outside Scandinavia, CE treatment is used mainly for breeder chicks that could be compulsorily slaughtered if they become infected with the invasive serotypes, *S. enteritidis* and *S. typhimurium*. Unfortunately, the protection sometimes breaks down as the birds come into lay and stress increases their susceptibility to salmonella infection. Generally, cost constraints within the Industry preclude any treatment of broilers. This situation could change if poultry flocks were to become infected with the verotoxigenic *Escherichia coli* 0157, an organism causing serious human illness and presently associated mainly with ruminants. At least one of the current CE products aimed at salmonella control will also protect against the *E. coli*. There is the further possibility of obtaining protection against *Campylobacter jejuni/coli*, which is widespread in commercial poultry flocks and a major cause of human enteritis. Available CE products are relatively ineffective in this respect and evidence suggests that different kinds of bacteria are involved in the protective process. Those studied by the author were obtained from free-range, adult hens and they conferred a high degree of resistance in treated chicks to a subsequent challenge from *C. jejuni*. The organisms appear to be obligate anaerobes that grow poorly in conventional culture media and further research is needed to determine their identity and protective mechanism.

Vaccination against *Salmonella* infection
Killed and Subunit Vaccines

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Salmonellosis in poultry is caused by either the host adapted serotypes such as *Salmonella gallinarum* or by *Salmonella pullorum* or by non-host adapted types which are very often pathogenic to humans. Prophylactic vaccination is a possible method of preventing vertical transmission of *Salmonella*. For the large scale use of prophylactic vaccines, they must be both safe and effective. The effectiveness of these vaccines may vary with the method of its preparation. Because killed vaccines are not as immunogenic as live vaccines, adjuvants of many types from alum through oil emulsions to polynucleotides have been used to increase their antigenicity. An ideal vaccine should mimic the immunological stimulation associated with natural infection, evoke minimal side effects, be readily available, cheap stable and easily administered. There are a number of experimental studies reported on the use of bacterins and attenuated live cultures as vaccines in the prevention of avian salmonellosis. Components extracted from *Salmonella* such as ribosomes or supernatant factors or polysaccharide-protein conjugates have also been investigated as potential candidates for new vaccines. Partial resistance to both systemic and oral *S. enteritidis* challenge was seen in mice vaccinated with heat killed *Salmonella pullorum* vaccine containing Freund's complete adjuvant. Experimental inactivated *S. enteritidis* Phage type 4 vaccine has been examined in chickens. A single subcutaneous vaccination at 3 weeks of age or two vaccinations at three and six weeks provided good protection against challenge with 10^9 colony forming units (CFU) or 10^8 CFU of virulent *S. enteritidis* given intramuscularly or intravenously. Oil-emulsion bacterins have been evaluated for reducing fecal shedding of *S. enteritidis*. In one experiment, an experimental vaccine prepared from acetone-killed *S. enteritidis* oil emulsion and a commercially available vaccine were compared for protection of laying hens against intestinal colonization of *S. enteritidis* following its oral administration. In this study, each vaccine was administered twice at 4 weeks interval. Two weeks after the second vaccination, birds were challenged orally with 10^8 CFU. Both vaccines significantly reduced the incidence of intestinal colonization and mean number of *S. enteritidis* shed in the feces at 1 week post challenge. However, more than half of the vaccinated hens still shed *S. enteritidis*. In one other investigation, acetone killed oil emulsion vaccine prepared from a phage type 13 of *S. enteritidis* was given to hens of 23 weeks and 45 weeks of age. A second injection was given 6 weeks after the first and three weeks after the second vaccination, all hens were challenged with an oral challenge of 10^9 cells of *S. enteritidis* phage type 14b. In both groups of birds *S. enteritidis* was isolated from fewer internal organs and pools of egg contents from vaccinated hens than unvaccinated controls. Subcutaneous inoculation at three or at three and six weeks old with a formalin inactivated oil

adjuvated *S. enteritidis* Phage type 4 vaccine containing 10^{11} CFU/ml protected chickens against a massive experimental challenge with 10^{9} CFU by either intramuscular or intravenous administration at 5 or 8 weeks old.

Numerous experiments in mice with *S. typhimurium* and *S. enteritidis* infections have shown that inactivated parenteral vaccines failed to elicit significant cell mediated immunity. Production of poor inconsistent protective immunity with killed vaccines may be due to the rapid destruction and elimination of the organism from the host system and also the destruction of the relevant antigens during vaccine preparation. Inactivated vaccines, despite eliciting acceptable levels of humoral immunity are poor inducers of T cell mediated immunity which is required to enhance the local mucosal immune response for preventing colonization of *Salmonella* in the gut and curtail the subsequent systemic spread of the organism. The protective efficacy of inactivated vaccines is further limited by their low immunogenicity especially in unprimed hosts and their inability to induce cytolytic T-cells. Serum antibody titers are of uncertain value as indicators of the overall extent of protection against *Salmonella*.

Interest in the development of an effective vaccine has led researchers to explore the possibility of using outer membrane proteins (OMP) from gram-negative bacteria as potential vaccine candidates. Majority of studies on *Salmonella* OMP for potential vaccines have been done in mice. Several studies have demonstrated that OMP from *Salmonella* possess antigenic determinants for the stimulation of both humoral and cell mediated immunity and provide long lasting protection against *Salmonella* infection. The use of cellular proteins from *S. gallinarum* for prevention of fowl typhoid infection in chickens has been reported. In this study it was concluded that cellular proteins can be used as vaccines in chickens to protect against a lethal challenge. These vaccines were capable of inducing an immune response that cleared *S. gallinarum* from the ovary and thus reducing egg transmission.

Outer membrane proteins isolated from both smooth and rough strains of *S. typhimurium* are known to be good protective antigens in mice. It has been demonstrated that OMPs were valuable immunogens for the prevention of typhoid fever, and the cross protection observed between *S. typhi* and *S. typhimurium* indicate that they share common epitopes. The *Salmonella* OMPs can induce both humoral as well as cellular immunity to mediate protection. This presentation will focus on what is known on the use of killed and subunit vaccines for the control of salmonellosis in poultry.

The Use of Live, Attenuated Vaccines against Salmonella in Poultry

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Although under some circumstances it is possible to rear poultry in the absence of Salmonella and other zoonotic bacterial pathogens by strict adherence to hygiene and management procedures, the costs of doing so would put any country's poultry industry at a great economic disadvantage. Biological control methods are thus becoming increasingly used. Amongst these there is a recognition that live, attenuated vaccines have a considerable role to play and are seen to be increasingly acceptable to the industry. The protective ability of attenuated mutants of *S. gallinarum* has been demonstrated over a number of years and improved mutants are now available. Most work on the food-poisoning serotypes has been done with *S. typhimurium* and *S. enteritidis*. There is evidence that vaccines can increase the resistance of birds to Salmonella infection. A few attentuated vaccines are already available and more will come on to the market within a few years. One of the big questions that yet needs to be addressed is that of avirulence for man.

Intervention Strategies in the Processing Plant

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Salmonella and *E. coli* are two organisms that poultry processors are faced with reducing/eliminating. There seem to be seasonal trends for *Salmonella* on broilers. Although the conditions for *E. coli* are fairly understood, *Salmonella* seems to be ubiquitous on the live animal and, until heat is sufficiently applied, on the raw production. Slaughter provides opportunities for setting the *Salmonella* on the bird. *Salmonella* seems to be imbedded in feather follicles before and during slaughter. Scalders, conventional and countercurrent, establish ideal conditions for contamination of the carcasses; pickers beat the bacteria into the follicles and, warm temperatures as the bird goes from live animal to meat, set opportunities for bacterial presence on raw chicken. In addition croppers, neck breakers, and bird washers affect contamination of carcasses. Recent evisceration equipment has been developed to minimize contact of the viscera with the external product.

Since the advent of "Mega regulations" and "HACCP," processing plants have greatly increased volume of water on the bird during the process. Effective tools in minimizing contamination are water and chlorine, both are affected by pH. Bacteria has a hard time attaching on a wet bird. A number of new bird wash sprays and cabinets have been added to evisceration lines-increasing volume of water to as much as 9 g/b (gallons per bird). Systems exist to control chlorine as well as pH of the chlorine going into the chillers. At least one new technology to reduce bacteria involves application of an antimicrobial compound in a low pressure spray cabinet before the chillers. Irradiation has not proved to be a process adaptable to the slaughter plant, but new applications after "birds" leave the plant may find a niche.

Unfortunately, there is no magic bullet. Processors must be attentive to multiple hurdles as we seek to reduce contamination and minimize bacteria on birds in the poultry process.

Preharvest Crop Contamination and *Salmonella* Recovery from Broiler Carcasses at Processing

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Published and unpublished data from our laboratories indicate that leakage of crop contents during processing is sometimes a major source of carcass contamination with *Salmonella*. Also, we have observed marked increases in the frequency of *Salmonella* (and *Campylobacter*) recovery from crops following feed withdrawal, with 2- to 3-fold increases in incidence, probably due to ingestion of contaminated litter and feces during the feed withdrawal period. Very recent data indicates that litter and feces consumption behavior during feed withdrawal is strongly influenced by light intensity, with low photo intensities resulting in greatly reduced litter/feces ingestion. Data collected at commercial processing indicates that the site of crop removal may be a major critical control point for contamination of carcasses, as carcass contamination increases markedly at this point of evisceration. By sampling and culture of several known *Salmonella*-negative and -positive flocks at multiple points through processing, we have confirmed that the point of crop removal is associated with the primary increase in *Salmonella*-positive carcasses in some plants. These data also indicated that *Salmonella*-negative flocks (ante mortem), when entering the processing plant as the first flock following plant clean-up (first flock of the day), remain negative throughout processing. Carcasses from *Salmonella*-positive flocks become maximally contaminated at or immediately following crop removal and remain positive following immersion chilling. Carcasses from *Salmonella*-negative flocks which immediately followed *Salmonella*-positive flocks at commercial processing were contaminated with a low frequency prior to chill immersion. These data indicate that carcass contamination with crop contents may be at least as important as fecal contamination for *Salmonella* control, and support the premise that antemortem *Salmonella* reduction strategies may be useful for attaining reduced carcass contamination frequencies following processing.

The effect of management practices (collection, processing, storage, and distribution) on the growth of Salmonella enteritidis in naturally contaminated shell eggs.

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ABSTRACT Getting a shell egg from the production facility tot he consumer's table in as fresh and as sterile a condition as society demands, 100 percent of the time, can be a daunting task. How the shell egg's shelf life and temperature is managed throughout the journey can be either the saving grace or the downfall relative to both quality and safety.

It's also true, however, that managing shell egg temperature and shelf-life is a dynamic process involving many variables, a process in which managerial actions can bring unintended and sometimes unwanted results. It might be helpful for managers to view the individual stages (collection, processing, storage and distribution) as a complete system whose individual elements must interface well to preserve the quality and manage shelf-life of the shell egg.

Studies have shown that internal temperatures of eggs washed at 46° C increase at a rate of .2°C/second during washing. Studies have indicated that *Salmonella enteritidis* in naturally infected laying hens occurs in low numbers of cells and the principle site of contamination may be the vitelline membrane or the albumen adjacent to the vitelline membrane, however, some of the same studies also indicate that contamination can occur in the yolk. In addition, studies have shown that holding eggs at 4 to 8° C reduced the heat resistance of certain strains of *Salmonella enteritidis*. Some studies conclude that there may be a lag phase of growth of in naturally contaminated eggs of at least 21 days and that this lag phase may be dependent on the effect of time and temperature on the physiochemical properties of the intact egg. Studies observed a change in permeability of the vitelline membrane which may be highly dependent on holding temperatures and time of storage.

Irradiation Pasteurization: Efficacy, Marketability, Consumer Acceptance

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Irradiation pasteurization of foods with low doses of gamma rays, X-rays, and electrons will effectively control foodborne pathogens on beef, pork, lamb, and fish. This process destroys pathogenic non-spore forming foodborne bacteria and parasitic organisms including *Salmonella, Escherichia coli* O157:H7, *Vibrio vulnificus, Listeria monocytogenes, Campylobacteria,* and trichina. Microbiological safety is consumers' most frequently volunteered food safety concern and the level of concern has increased in recent years. This suggests consumers are receptive to processes that reduce or destroy harmful bacteria. For years most consumers have expressed less concern about food irradiation than other food processing technologies. National surveys indicate consumer interest in purchasing irradiated food has steadily increased reaching 80% in 1998. Attitude studies have demonstrated that when given science-based information, up to 90% of consumers prefer the advantages irradiation provides. When information is accompanied by samples, acceptance may increase to 99%. Consumers have purchased irradiated fruits and poultry when they have been available. Information on irradiation should include product benefits, safety and wholesomeness, address environmental safety issues, and include endorsements by recognized health authorities. Educational and marketing programs should be directed toward retailers and processors. Given the opportunity, consumers will buy high quality, safety-enhanced irradiated food.

Presentations

Abstracts of Poster Presentations

(1)

EFFECT OF A PROBIOTIC LACTIC ACID BACTERIUM ON CHICKS CHALLENGED WITH SALMONELLA ENTERITIDIS

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Our country has many problems with *Salmonella* in poultry. In the search for a natural solution for this problem and keeping in mind the concept of "competitive exclusion" (Nurmi & Rantala 1973), strains with antimicrobial properties were isolated from the intestinal tract of healthy young chickens in order to devise a probiotic food supplement. A strain of *Enterococcus faecium* was selected because of its probiotic features (Audisio *et al.* 1997) and tested *in vivo.*

We worked with 30 hour-old chicks which were vaccinated on the day of hatch against Gumboro and New Castle diseases. A cell-suspension of *E. faecilim* J96 (ca $1x10^9$ cell/chick) was orally administered twice a day for three consecutive days with a period of 12 h between each dose. Chicks were orally infected with about $1x10^5$ *S. enteritidis* cells per chick the following day after the protective dose was administered. They were killed by cervical dislocation on days 2, 5 and 8 after challenge. Ceca epithelia and contents and crops were aseptically removed and their microflora was analyzed. Also, the pH of the crop and cecal suspensions was registered.

The flock that was protected for 3 days with *E. faecium* J96, and that was challenged on the 4th day with *S. enteritidis,* showed a lower number of enterobacteria both in the cecum epithelium (ca $1x10^5$ cfug-1) and in its content ($1x10^7$ cfug-1) when it was compared with the number of enterobacteria found in the control group. This number was respectively $1x10^7$ and $1x10^8$ cfug-1. Lactobacilli and enterococci remained almost constant. When the crops were analyzed, substantial differences were not observed between control and challenged groups. Enterococci were predominant, with lactobacilli in second place while enterobacteria remained low ($< 10^5$ cfug-1).

These results suggest that E *faecium* J96 might have a beneficial effect on the birds challenged with *S. enteritidis* and that it might be used successfully in a probiotic supplement-feed which would help young chicks.

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(2)

PREVENTION OF SALMONELLA INFECTION BY CONTACT USING INTESTINAL FLORA OF ADULT BIRDS

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The study was done to assess the ability of competitive exclusion to prevent *Salmonella* infection by contact between newly hatched chicks. One bird infected with *Salmonella* was placed in a box with non-infected birds previously treated with broth culture of faeces from adult birds (CE). CE was prepared by adding faeces from adult birds into BHI broth. The broth culture was incubated for 24 hours at 37°C. From this, 0.1 ml was inoculated into the crop of the bird. Twenty-four hours later, infected birds were placed into the boxes where the non-infected birds were. Three birds were killed at 4 and 8 days later to estimate the amount of *Salmonella* in the caecal contents. The study was done with *S. typhimurium* (STM) and *S. infantis* (INF). The experimental design had 5 repetitions for STM and 3 for INF. As a control in each trial and in the same condition, groups were added with no previous pre-treatment with CE. The same approach was repeated with STM, but in this case, the birds received feed containing 0.8% of a mixture of formic acid (70%) and propionic acid (30%). While the birds from the control groups were fully contaminated by *Salmonella*, those pre-treated with CE had less than 10^2 viable cells per gram of caecal contents. It was observed that the protection did persist against STM when a mixture of organic acids was added into the feed given to birds pre-treated with CE.

Financial support for this research was provided by CNPq, FAPESP and FUNDUNESP.

(3)

COMPETITIVE EXCLUSION CONTROL IN CHICKENS AGAINST AN INVASIVE SALMONELLA ENTERITIDIS CARRYING 54KB VIRULENCE - ASSOCIATED PLASMID

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Plasmid profiles of highly invasive S. enteritidis strains were used as epidemiologic markers to trace the source of infection in 11 broilers flocks. The predominant plasmid profiles, in decreasing order of frequency, followed by bracketted percent of infected flocks were: 12.8 and 54 kb (81.8%); 1.8, 12.8, and 54 kb (9.1%); 12.8 kb (9.1%). The 12.8 and 54 kb plasmid profile was present in four S. enteritidis recovered from individual oviducts of a breeder flock, the source of day-old chicks to the 11 broiler flocks.

The application of Competitive Exclusion Microflora (CEM) by spray at one-day of age to broiler chicks, followed by a controlled challenge at three days of age with the predominant plasmid profile of S. enteritidis (12.8 and 54 kb) (2.85×10^5 (cfu/bird in the mouth) reduced the mortality by 3.93%, in comparison to untreated challenged birds. The average percent of spleens and livers infected with the challenged organism at 45 days of age was reduced to zero in the CEM - treated birds compared to 15.6% infectivity of spleens or livers in the untreated-challenged birds. The cecal infection with the challenged organism was 25% in the CEM - treated birds compared to 59.4% in untreated - challenged birds.

(4)

CONTROL OF EXPERIMENTAL INFECTION OF BROILERS BY *SALMONELLA ENTERITIDIS* AND *S. TYPHIMURIUM* WITH THE USE OF ORGANIC COMPOSITES AND ANAEROBIC CECAL MICROFLORA.

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ABSTRACT

The efects of organic composites and anaerobic cecal microflora (ACM) for the control of experimental infection of broilers by *Salmonella enteritidis* and *S. typhimurium* was investigated. One day-old chicks were submitted to different treatments with organic acids and for carbohydrates in the drinking water and / or ACM intra-esophagically daily for five days. At the third day of treatment, the chickens were infected intra-esophagically with *S. enteritidis* or *S. typhimurium*. The chickens were weighed and three chickens were slaughtered in each experimental group at 24, 96, 240 and 432 hours after inoculation. At each of these periods, the bacterial samples were quantified in the liver and faeces, and also colonization of the esophagus, crop, proventriculus, duodenum, jejunum, caeca and rectum was evaluated. The pH was also measured in the caeca. The infections were more persistents in the caeca, followed by the rectum and crop. Except with the use of formic acid, the treatments did not interfere in the body weight. The infections were self limited, as the treated and the control showed the same degree of infection at the end of the study. The use of acetic, propionic and formic acids separately, did not affect either the presence of the challenge samples in the faeces or the colonization of the digestive system, especially with *S. enteritidis*, however, they reduced the presence of *Salmonella* in the liver, as well as reduced the caecum pH during the treatment period. The use of ACM separately or associated with lactose, significantly reduced the colonization of the chicks's digestive system by *S. enteritidis* and *S. typhimurium*. Lactose, in opposition to previous reports, did not have a synergic significative effect when associated with the ACM. The use of lactose separately reduced the colonization of chicks's caeca by *S. typhimurium*. The use of sucrose or mannitol did not produce any difference when compared to the control group. The use of carbohydrates and ACM only produced a slight reduction of the caecum pH, 24 hours after inoculation, as seen with the organics acids, although only the treatment with ACM reduced the bacterial quantity in the faeces. The *S. enteritidis* was much more invasive than the *S. typhimurium* and, the use of carbohydrates, organic acids and ACM, had little effect on reduction of the sistemic infection. The prevention of the enteric colonization produced by *Salmonella* spp. may be explained, by the inter-relation of physiological, microbiological and imunological factors, and also possible changes of the caecum pH.

(5)

An Attenuated *Salmonella gallinarum* Live Vaccine Induces Long-Term Protection Against *Salmonella enteritidis* Challenge in Chickens. T.Z. Tan, B. Nay, J.M. Bricker*, H. Hughes, F. Sterner and R. Hein. Intervet Inc., 405 State Street, Millsboro, Delaware, 19966.

Salmonella enteritidis is the most prevalent *Salmonella* serotype associated with human foodborne illness in the United States, being mainly attributable to the consumption of contaminated table eggs, egg-containing foods, or poultry meat. Both *S. enteritidis* and *S. gallinarum* belong to serogroup D1 of the Kaufmann-White serotyping system and therefore, share some common surface antigens. However, *S. enteritidis* is a zoonotic pathogen, while *S. gallinarum* is only avian specific. An attenuated *S. gallinarum* strain 9R has been used as a live vaccine for many years in European, Asian, South American and African countries to control fowl typhoid disease, and currently is being registered in Europe for use in layer and breeder chickens. The objective of this study was to investigate duration of immunity of the 9R vaccine against *S. enteritidis* infection in chickens. Forty layer chickens were vaccinated subcutaneously with *S. gallinarum* 9R [10^7 Colony Forming Units (CFU) per bird] at 6 weeks of age, and boosted orally 4 weeks later. Another group of 40 birds remained unvaccinated as controls. Six months post second vaccination, all chickens received a severe challenge of *S. enteritidis* (10^6 CFU per bird) by a combination of intramuscular and oral routes. Birds were sacrificed 7 days post challenge and the liver, spleen, ovary, oviduct, and cecal tissue were collected to determine the degree of bacterial colonization in the tissues. Reisolation rates in the individual tissues were significantly lower ($P<0.001$) in the vaccinates (mean 6% tissue infection) than in the challenged controls (mean 58% tissue infection). The preventable fractions against *S. enteritidis* infection were 91%, 90%, 95% and 81% for liver, spleen, combined ovaries and oviduct, and intestine, respectively. When the number of *S. enteritidis* was quantitated in the above tissues, significant reductions were also observed in vaccinated chickens (79% to 96%; $P<0.05$). Four control birds (10%) died from *Salmonella* infection post challenge, while no mortality occurred in vaccinated birds. This study indicates that vaccination with the live *S. gallinarum* 9R vaccine provides a minimum of 6 months protection against *S. enteritidis* colonization of tissues, including reproductive tract and intestine, and against mortality caused by *S. enteritidis*.

(6)

Salmonella live vaccine strains Zoosaloral H, Bovisaloral and Suisaloral: molecular characterization and differentiation from field isolates

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Attenuated auxotrophic mutants of *Salmonella enterica* subsp. *enterica* (*S.*) serovar Typhimurium, Dublin and Choleraesuis isolates serve as live vaccine strains to prevent domestic animals from infections with *Salmonella* field isolates of the same serovar. The *S.* Typhimurium live vaccine strain Zoosaloral H (IDT-Impfstoffwerk Dessau Tornau GmbH) has been widely used in chickens in reunified Germany since 1992. The *S.* Choleraesuis and *S.* Dublin live vaccine strains, Suisaloral and Bovisaloral (both IDT-Impfstoffwerk Dessau Tornau GmbH), had been admitted in 1992 and 1994 to use in pigs and cattle, respectively. The widespread use of these live vaccine strains requires methods for differentiating the vaccine strains from homologous field isolates and for monitoring their genetic stability during animal and environmental passages. In this regard, molecular techniques proved to be most powerful tools. Therefore, the live vaccine strains Zoosaloral H, Bovisaloral and Suisaloral as well as a representative pool of epidemiologically unrelated field isolates of *S.* Typhimurium from chickens, *S.* Dublin from cattle and *S.* Choleraesuis from pigs, were investigated by plasmid analyses, ribotyping, IS*200* typing and macrorestriction analyses.

The Zoosaloral H strain showed a unique *Hin*dIII-restriction pattern of its virulence plasmid and differed from *S.* Typhimurium field isolates by its unique *Spe*I- and *Xba*I-macrorestriction patterns as well as by its unique rRNA- and IS*200*-hybridization patterns. *Bln*I- and *Xho*I-macrorestriction analysis revealed the presence of unique fragment patterns of the Bovisaloral live vaccine strain. The Suisaloral live vaccine strain was differentiated from homologous field isolates by its plasmid profile and a unique *Xba*I-macrorestriction pattern. These molecular techniques enabled a highly reliable identification of all three live vaccine strains and also differentiated them from field isolates of the same serovar. Moreover, the reisolated vaccine strains showed the same molecular characteristics suggesting genomic stability of these live vaccine strains after animal and environmental passages.

(7)

Salmonella Enteritidis live vaccine candidates and their genetic relationship to *Salmonella* Enteritidis field isolates of the same and other phage types

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So far no live vaccine strain to specifically protect domestic animals from infections with *Salmonella enterica* subsp. *enterica* (*S.*) serovar Enteritidis has been admitted to use in poultry or other animals in Germany. In 1996, Martin and coworkers (BgVV, Jena, Germany) produced eleven auxotrophic attenuated live vaccine candidates by chemical mutagenesis from a virulent *S.* Enteritidis phage type (PT) 4 isolate. Phage typing showed that seven mutants still exhibited the lytic pattern specific for PT4 while two mutants changed to PT7 and each one mutant displayed the lytic pattern of PT1 and PT9.

In order to provide a detailed characterization of the live vaccine candidates suitable for differentiating them from *S.* Enteritidis field isolates of the same phage types and for monitoring their genetic stability under in-vitro and in-vivo conditions, the eleven vaccine candidates and another 65 *S.* Enteritidis isolates of 14 phage types but also of RDNC types were comparatively investigated by molecular typing methods. All live vaccine candidates were plasmidfree and so differed from most of the *S.* Enteritidis field isolates which usually carried virulence plasmids of 55, 60 or 95 kbp alone or in addition to smaller cryptic plasmids. Ribotyping revealed two and IS*200*-typing three hybridization patterns with the vaccine candidates exhibiting the same hybridization patterns as the majority of the field isolates. However, macrorestriction analysis as performed with the three suitable restriction endonucleases, *Xba*I, *Spe*I and *Not*I, proved to be the method of choice for the differentiation of *S.* Enteritidis. Based on the results obtained from macrorestriction analysis and plasmid profiling, the 76 *S.* Enteritidis isolates were assigned to 26 different genomic groups. The eleven live vaccine candidates represented three genomic groups, the characteristics of which were not shared by any of the *S.* Enteritidis field isolates. In addition to the auxotrophic markers, molecular analysis proved to provide valuable information for a reliable differentiation of the vaccine candidates from *S.* Enteritidis field isolates of the same phage types.

(8)

Live Vaccines for *Salmonella* Control in Poultry

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Optimization of hygiene, decontamination of feed and immunization are important parts of a successful Salmonella control program in breeders and layers. Especially live vaccines induce cell-mediated immunity and reduce shedding of field strains. The result is a lower infection pressure and the interruption of the chain of infection.

Extensive laboratory and field trials with live culture vaccines, based on *S.typhimurium* and *S. enteritidis*, were carried out in SPF leghorn birds and broiler breeders. The results are demonstrating that these vaccines are strongly inhibiting the shedding and persistence of the homologous wild type in comparison with unvaccinated birds. Breeder birds vaccinated during rearing time were protected for the whole production period. A combined vaccination schedule using consecutively live and inactivated vaccine provides a similar protection. Inactivated vaccine alone could not inhibit the shedding and peristence of the SE challenge strain.

(9)

CONTROL OF S. ENTERITIDIS IN REPRODUCING CHICKENS

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In The Netherlands control of *S. enteritidis* has been effective since 1989. This includes compulsory monitoring of all reproduction stock, elimination or treatment of infected flocks and a biosecurity improvement programme. Recently vaccination has been added as an option to reduce the number of infections.

Monitoring of selective breeding flocks (GPS) consists of monthly bacteriological testing of 150 faecel samples per house. Testing of layer or broiler parents flocks (PS) is done bimonthly with a specific Elisa on 60 serum samples per house. In addition a fluff sample is examined from every machine at every hatch for *Salmonella* isolation. Monitoring results, in numbers of infected (and percentages of tested) flocks, were:

in the years	in layer GPS and PS		in broiler GPS and PS	
1989	9	(2.8)	18	(1.7)
1990	4	(1.4)	22	(1.1)
1991	0	(0)	15	(0.8)
1992	2	(0.6)	22	(1.1)
1993	14	(4.3)	27	(1.0)
1994	4	(1.2)	27	(1.1)
1995	3	(0.8)	36	(1.8)
1996	0	(0)	35	(2.0)
1997	0	(0)	23	(1.4)

Flocks infected with any serotype of S. enteritidis are excluded from further reproduction, either by killing them (GPS and layer PS) or by a combination of antibacterial treatment and administration of competitive exclusion flora, developed in 1992. The percentage of flocks that remained free till the end of the regular production period was, over the last 5 years, 71, 81, 83, 85 and 88 respectively.

Biosecurity was improved by ordering measures, based on case-control study of risk-factors. These include all-in-all-out management, cleaning and disinfection of houses and their surroundings between batches, separate equipment per house, regular cleaning of feed silos, reduction of poultry house visits, e. g. by allowing no uncontrolled entrance to the farm, changing of clothes and boots at the house entrance, etc.

Vaccination during the rearing period by i.m. application of inactivated *S. enteritidis* vaccine appeared to reduce considerably the rise of infection in flocks on contaminated farms. It was the most evident explanation for the decrease in the infection rate last year.

(10)

Induction of cross-protection against *S. enteritidis* by the *S. gallinarum* 9R vaccine.

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Since its development in the 1950s by Williams Smith, the *S. gallinarum* 9R vaccine strain has been used extensively by the poultry industry, mainly in subtropical countries for the induction of protection against fowl typhoid.
We have tested the efficacy of the 9R strain (Nobilis SG 9R vaccine) against *S. enteritidis*, another member of the D1 serogroup, in chickens. In laboratory studies, chickens vaccinated subcutaneously with Nobilis SG 9R at 6 or at 6 and 18 weeks of age and unvaccinated controls were challenged with a virulent *S. enteritidis* PT4 strain. Challenge at 10 weeks of age resulted in 85% mortality in the controls compared to only 10% for the vaccinated chickens. When challenged at 23 weeks of age, mortality in the control group was 12% and there were no deaths in the vaccinated group. At necropsy 14 days post challenge, a significantly greater proportion of the controls suffered from follicular atresia as a result of the challenge. Reisolation rates of the challenge organism from cloaca swabs and cecum contents were also significantly lower in the vaccinated group.
Alternative vaccination schedules combining drinking water vaccination at 6 weeks of age with a subcutaneous or an intramuscular vaccination at 18 weeks of age were at least equally effective. Significant reduction of intestinal colonization was obtained after *S. enteritidis* infection at 23, 32 or 45 weeks of age. In addition, all vaccination regimens induced protection against a lethal *S. gallinarum* challenge at 58 weeks of age.
The efficacy of Nobilis SG 9R against *S. enteritidis* under field conditions is being evaluated in a trial in laying hens in The Netherlands. Over 2,200,000 chickens on high-risk farms were vaccinated twice during the rearing period. Of the 79 flocks included in the trial, 18 have come to the end of production up till the present (February 1998), and all have stayed *S. enteritidis* negative. No outbreaks of *S. enteritidis* have occurred in the 61 remaining flocks either, whereas the normal incidence of *S. enteritidis* in Dutch laying flocks is estimated to be approximately 15%.
An advantage of the use of Nobilis SG 9R for *S. enteritidis* control is that serological surveillance based on antibodies to the *S. enteritidis* flagellin (GM-DAS blocking ELISA, IDEXX, France) is still possible after vaccination as *S. gallinarum* is non-flagellated. Furthermore, as the host range of *S. gallinarum* is limited to poultry, there are no public health risks associated with the use of the 9R strain.

(11)

Safety Studies and Risk Analysis of an Attenuated *Salmonella gallinarum* Live Vaccine for Layer Chickens. T.Z. Tan[1], B. Nay[1], J.M. Bricker[1,*], M. Witvliet[2], H. Hughes[1] F. Sterner[1] and R. Hein[1]. [1]Intervet Inc., 405 State Street, Millsboro, Delaware, 19966, [2]Intervet International b.v., P.O. Box 31, 5830AA Boxmeer, The Netherlands.

An attenuated *Salmonella gallinarum* 9R live vaccine has been used in many countries to control fowl typhoid disease for over 40 years. The 9R vaccine has also been shown to be immunogenic against *S. enteritidis* infection in chickens. To address the safety of the 9R vaccine further, we have conducted a series of experiments to examine the use of Intervet's 9R vaccine. These experiments included overdose vaccinations in different breeds of chickens, turkeys and cattle, investigation of reversion to virulence, and horizontal and vertical transmission of the vaccine organism. In the chicken safety studies, 415 white leghorn, Hyline-brown, Isa-brown, and Dekalb-brown chickens at one day to 10 weeks of age were vaccinated twice (2- to 12-week intervals) with up to 100 times of the recommended dose by different routes (subcutaneous, wing-web, spray and oral). Mortality occurred only in Isa-brown birds (2 of 19) when vaccinated at one day of age with 10 times above normal dose by spray (Note: this use is contraindicated by the label: Intervet's vaccine is intended to be used in chickens at least 6 weeks of age subcutaneously). No clinical signs, postmortem tissue lesions and mortality were found in any other vaccinated birds. In fact, the average weight gain of one of the vaccinated groups was significantly higher ($P<0.05$) than that of unvaccinated controls. Ten turkeys were also inoculated with 10 chicken doses intramuscularly, subcutaneously and orally. No safety problems occurred. In another study, 8 calves (3 to 5 weeks old) were inoculated twice (2- to 3-week intervals) subcutaneously (50 chicken doses) or orally (500 chickens doses) with the 9R vaccine. No abnormalities were observed except a transient elevated temperature occurred within 3 days post vaccination. The biological stability of the 9R vaccine organism following five *in vivo* passages in chickens was also investigated. Ten day-old chickens were orally inoculated with 100 doses of the 9R vaccine. Four days post inoculation, the livers and spleens were homogenized and used to inoculate another group of chickens. This was continued for a total of 5 continuous passages. The vaccine organism was only recovered from the first passage (10/10) and second passage (3/10) groups. No clinical signs or gross lesions were seen in any inoculated chickens. Five "shed and spread" studies were conducted to determine the horizontal transmission of the 9R strain. Five to 20 sentinel chickens were housed with various numbers of vaccinated birds (ranging from 5 to 10,000) for up to 22 weeks and shared the same feeders and waterers. No 9R strain was isolated from and no seroconversion was found in any of the sentinel birds. To investigate vertical transmission of the vaccine organism, 137 layer chickens were vaccinated with the 9R strain and 335 eggs were collected for *Salmonella* isolation. No *Salmonella* was found from any of the eggs. Results of the above experiments along with available literature information were analyzed using an USDA APHIS' Veterinary Biologics Risk Analysis Process, which is a multifactorial approach to assess risks to animals, public health, and the environment. It indicates that Intervet's *S. gallinarum* 9R vaccine is safe for chickens vaccinated at 2 weeks of age or older and it does not revert to virulence. The likelihood of horizontal and vertical spreadings is low. Furthermore, the 9R strain can be differentiated from wild-type *S. gallinarum*. The vaccine is intended for use in commercial egg layers only; therefore, it should not interfere with pullorum/typhoid testing of breeders. Unlike *S. enteritidis* and *S. typhimurium*, *S. gallinarum* is not a zoonotic pathogen. Intervet's 9R strain has been used for several decades in many countries without any adverse effects or hazards to public health.

Expression and characterization of *Salmonella enteritidis* attachment proteins

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Egg-borne *Salmonella enteritidis* (SE) infection has emerged as a major public health problem in the United States as well as several European countries during the past decade. An essential event in the pathogenesis of SE is the attachment to the host intestinal mucosa through specific receptors on the host cell surface. Identification and characterization of SE specific attachment proteins would help to design an effective control measure.

We have studied the interaction of SE with epithelial cells using the ^{35}S-methionine labeling system. After incubation of SE with the mammalian cells, bacterial adherence to the epithelial cells were observed. New SE proteins induced by epithelial cells were expressed during the incubation period. The size of these various attachment proteins were about 150, 60, 50, 40, 22, and 18 Kilodaltons. Other proteins, whose synthesis is increased in response to incubation with epithelial cells, was also observed. In comparison to salmonella broth media, similar proteins were also expressed in tissue culture media. The expressed SE attachment proteins were further characterized by Two-Dimension SDS-PAGE and analyzed with Western-Blot using SE specific antibodies. Inoculation of chicken with purified or recombinant SE attachment proteins is expected to inhibit the binding of the organisms to chicken intestinal mucosal cells and therefore, it may protect the chicken to harbor SE organisms in their intestinal tract. Reduction of SE colonization in intestinal epithelial cells will lead to reduction of contaminated eggs consumed by humans.

(13)

Difference in virulence in mice or chickens of attenuated *S. enteritidis* miniTn5*lacZ*1 insertion mutants

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Balb/c mice are commonly used as a model to study the *in vivo Salmonella* infection process. We report a different virulent phenotype for a number of *S. enteritidis* transposon insertion mutants in Balb/c mice and one day old chickens and the identification of two new *Salmonella* genes involved in virulence in mice and chickens.

Attenuated mutants of *S. enteritidis* PT4 were isolated through transposon insertion mutagenesis with miniTn*5lacZ*1, a minitransposon generating transcriptional fusions to the β-galactosidase gene *lacZ*. Mutants were tested for β-galactosidase activity under different inducing conditions e.g. iron depriviation, osmotic stress, ... Mutants with inducible β-galactosidase activity were tested for virulence in Balb/c mice by oral infection with 10^5 LD_{50}. Several attenuated mutants were identified and six induced protective immunity against a successive oral challenge with the virulent parental strain. The mutants are still invasive to epithelial cell lines but have a reduced capacity to infect liver, spleen and kidneys of orally infected Balb/c mice.

Sequence analysis revealed that for two mutants the transposon insertion occured in unknown *Salmonella* genes while the other four were insertions in the *Salmonella* plasmid virulence (*spv)* operon.

These six mutants were given orally to one-day-old SPF chickens. Groups of 12 chickens each were orally infected with a dose of 10^9 cfu/chicken of a mutant or parental strain. The chickens were observed for mortality and clinical symptoms. After 28 days the surviving chickens were killed for autopsy and serum was taken for serological testing.

The four *spv* mutants showed no significant difference in virulence with the wild type parental strain: the death rate lies between 42 % and 75 % compared with 83 % for the wild type strain.

Mutant EZ1188 seemed to be hypervirulent. The infected chickens showed severe disease symptoms and growth retardation and were all killed within 6 days. The transposon insertion in this mutant occured in a gene having homology to the *E. coli nfrC* gene, involved in adsorption of bacteriophage N4 to *E. coli* K12.

Only one mutant EZ1263 confirmed it's attenuated phenotype in chickens. Eleven chickens survived the infection with EZ1263 showing less severe diarrhoea and little growth retardation.

(14)

Antibody responses to *Salmonella enteritidis* in sera, intestinal secretions and bile from four chicken lines presenting differences in cecal carrier state.

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Previously, we observed that L2 and B13 chicken lines presented higher frequency and level, as well as longer duration of cecal carriage of *Salmonella enteritidis* (SE) than PA12 and Y11 lines. Y11 line was the most resistant. We studied the antibody responses to SE in sera, intestinal secretions and bile from the chickens belonging to the four lines and orally inoculated at one week of age with 5 x 10^4 colony forming units of one strain of SE PT4. IgM, IgG and IgA against the whole cell of bacteria were quantified using an indirect ELISA in the three types of samples, once a week for 12 weeks postinoculation (pi).
Bacteriological data confirmed differences in SE levels in ceca between the four lines, with the highest ones in B13 and L2 lines and the lowest in PA12 and Y11 lines. SE infection of spleens little differed according to the chicken line.
In sera, the production of IgM antibodies occurred early and the levels were maximum from the 2nd to the 5th week pi and then decreased. The levels of IgM antibodies were higher in sera of B13 and L2 chickens. The IgG antibodies were produced later than the IgM ones and maximum levels occurred from the 3rd to the 6th week pi, and then decreased. These levels were similar and high in the four chicken lines. IgA antibody levels were low in all the sera.
In intestinal secretions, the productions of IgM and IgG antibodies were weak, except in L2 and B13 animals, during weeks 3 and 4 pi respectively. The levels of IgA antibodies increased from the 2nd week pi to reach a maximum during the 3th week pi in L2 chickens and one week later in the other lines. Then, those levels persisted in intestinal secretions of L2 and B13 lines which presented the strongest SE colonization in ceca, but not in the most resistant PA12 and Y11 lines. The lowest production was always observed in Y11 line.
In bile, IgM and IgG antibody levels were very low in each chicken line, in contrast to IgA ones. In this latter case, the levels were maximum during the 2nd week pi in L2 and PA12 lines, the 3rd one in Y11 line and the 4th one in B13 line. So, in each line except the B13 one, IgA antibody levels reached their maximum earlier in bile than in intestinal secretions. In bile, the levels of IgA antibodies remained maximum throughout the experiment in most animals.
In conclusion, the production of IgM and IgG antibodies in sera and of IgA antibodies in bile were not related to SE carriage in ceca. In contrast, the IgA antibody levels in intestinal secretions sounded to be in relation to the levels of the challenge strain in ceca, but may not contribute to explain the resistance to SE carrier state in these organs.

SEROLOGICAL AND BACTERIOLOGICAL INVESTIGATIONS OF POULTRY FLOCKS EXPERIMENTALLY INFECTED WITH *SALMONELLA* TYPHIMURIUM AND *SALMONELLA* ENTERITIDIS

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In Denmark the number of human cases of salmonellosis has been steadily increasing during the last decade, with *Salmonella* (*S.*) Enteritidis and *S.* Typhimurium as the most frequently isolated serotypes, and with especially eggs, pork and poultry products being documented as major sources of human infection.

Several investigations have pointed at vertical transmission as the most important route of infection for these serotypes in eggs and broiler flocks.

Based on these observations three groups of chickens (White Leghorns) were experimentally infected and followed by bacteriological and serological examinations for salmonella through a one-year period to compare and evaluate the ability of the methods to identify infected flocks and birds.

Birds were infected as day-olds by crop instillation. One group received 1.9×10^8 CFU of a rifampicillin resistant *S.* Enteritidis strain (phage type 1), another group received 9.2×10^7 CFU of a rifampicillin resistant *S.* Typhimurium strain (phage type 110), and the last group being non-infected served as a control group.

One hundred individually numbered birds were included in each group, but due to chickens being sacrificed for the bacteriological examination of organs, and due to natural mortality, the groups included approximately 35 birds throughout the investigation period of one year.

Cloacal swabs and blood samples were obtained from each animal at set intervals. Within the first six weeks sampling was done weekly, the following three months every second week, and for the remaining period every third to fourth week. From the point-of-lay, eggs produced the day before sampling were collected for serological analysis as well.

Serological examinations were performed by use of a lipopolysaccharide (LPS) based enzyme-linked immunosorbent assay (ELISA) analysis of blood and egg yolk samples. Reactions were recorded as relative values (OD%), based on comparison of optical densities of samples to a strongly positive control.

Bacteriological examinations were carried out according to the method recommended by the Nordic Committee on Food Analysis (NMKL No. 71, 1991)

Birds in the control group remained negative to salmonella throughout the experimental period, as judged by bacteriological examinations of cloacal swabs and organs (cecal tonsils, spleen, liver, lungs, and ovary + oviduct/testicles) from killed or dead birds.

In the infected groups, the number of birds testing positive by bacteriology declined with the time elapsed since infection. Within the first 16 weeks a decline in the prevalence of positive birds (from 100 % to 6 %) in the *S.* Typhimurium group was observed. In the *S.*

Enteritidis group a similar decline was observed within the first eight weeks, but with relapses (up to 40 % positive birds) at the onset of egg production and at the time when the production was at its highest.

In the *S.* Enteritidis group two birds showed positive by bacteriological examination (one bird in the spleen, liver, and cloacal swab and the other bird only in the cecal tonsils) at the end of the study. One of these had been shedding throughout the whole period, whereas the other had not produced positive samples since it was 20 weeks of age. The other birds in the group had been shedding intermittently or nearly not at all.

Intermittent shedding was observed in the *S.* Typhimurium group, but no birds were shown to shed throughout the whole period of investigation. Two birds were positive by cloacal swabs and in caecum, but not in other organ samples at the end of the study. These birds had not produced positive samples since the twelfth and fourteenth week, respectively.

Antibodies against salmonella were detected in blood samples from the infected groups three weeks after infection, and in egg yolk from some of the first eggs produced. For the control group the 95 % confidence interval of mean value (mean value ± 1.96 x standard error) of OD % for blood samples varied between 0.3 and 27.5 OD % at different times during the investigation. Corresponding figures for egg samples were found to be between 4.7 and 28.4 OD %. The results showed that an OD % ≥ 40 may be used as cut-off value (i.e. birds with OD % values above this limit being regarded as serologically positive). Based on this cut-off value the infected groups were found to be serologically positive from week three and throughout the rest of the investigation, although some birds or eggs within the groups were found to be negative at every time of sampling.

In conclusion, we suggest that birds with OD % values ≥ 40 may be regarded serologically positive, and we conclude that the test is reliable and may point out infected flocks. However, the LPS based ELISA may not be used for identifying individually infected birds and nor flocks in the initial phase of the infection due to the lag of serological response. It is furthermore concluded that due to the intermittent pattern of fecal shedding of salmonella bacteria from infected birds, the ELISA may be a tool for the screening of poultry flocks for salmonella.

(16)

Factors Contributing to the Virulence of Avian *Salmonella* Isolates

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In previous work, phenotypic characteristics of 12 paired, *Salmonella* serotypes isolated from healthy and ill chickens were compared. The two groups differed significantly in their carbon utilization patterns, ability to hemagglutinate erythrocytes, and invasiveness in primary chick kidney cell cultures with the two groups being best delineated by their degree of invasiveness (1). When this difference in invasiveness was examined in greater detail using one (*Salmonella typhimurium* var Copenhagen isolates) of these 12 pairs, it was found that the difference in invasiveness could be ascribed to intracellular survival (2). When the disease-associated member of this pair was mutated by replacement of its *fkpA* gene with the inactive *fkpA*1::Ω-Cm gene from *Escherichia coli* to create *S. typhimurium* var Copenhagen KY32H1, it was found that although the wildtype and its mutant could invade cells equally well, the mutant survived less well than the wildtype during the first 6 hours post-infection. Complementation of the mutant with the wild-type gene restored the mutant to wild-type levels of intracellular survival (3). Overall, intracellular survival appears to be an important contributor to the virulence of avian salmonellae, and expression of the *fkpA* gene may play an important role in the virulence and/or intracellular growth of these organisms.

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Infection of Chicks with *Salmonella enteritidis* at One Day of Age Results in Long-term Persistent Infection and Immunological Hyporesponsiveness in the Challenged Individuals. P.S. HOLT*[1], R.K. GAST[1], R.E. PORTER, Jr[2], and H.D. STONE[1]. [1]USDA/ARS Southeast Poultry Research Laboratory, Athens, GA 30605; [2]Purdue University, West Lafayette, IN 47907-1175.

Very young poultry are acutely susceptible to infections by intestinal pathogens such as *Salmonella enteritidis* (SE). As exposure to these organisms in the hatchery is a very real possibility, a study was conducted which followed the course of infection and development of systemic and intestinal immunity in chicks challenged with SE at 1 day of age. A high percentage of livers, spleens, and ceca were culture positive for SE at 1 and 4 weeks post challenge. Thereafter, no SE could be detected in livers or spleens but 40%-50% of the birds were cecal or fecal positive out to the termination of the experiment at week 24. In one trial, one bird did produce eggs contaminated with SE. Little immune responsiveness to SE was observed at one week post challenge and the percentage of birds responsive immunologically in the serum or intestine seldom exceeded 60% throughout the trials. Many birds that were organ positive for SE possessed no detectable immune response to the challenge organism indicating the inability of the immune system to respond to the challenge. Birds infected at this time period also experienced reduced ability to respond to vaccination. Depressed responsiveness to an SE bacterin was observed one and two weeks post administration while those individuals receiving an inactivated Newcastle disease vaccine experienced a reduced response 4 and 6 weeks post vaccination, indicating that the persistent infection affected the ability of the immune system to respond to homologous and heterologous antigens. Histological examination of tissues revealed relative lymphoid depletion in the spleen of infected birds which was prevalent at 1 week post infection but less obvious at 4 weeks. These results indicate that exposure of birds to SE early in life compromises the ability of these individuals to immunologically respond to and ultimately clear the SE challenge. The hatchery is therefore an important critical control point for reducing future SE problems within a flock.

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Molecular Epidemiology of Indigenous *Salmonella enterica* subsp. *enterica* Serovar *albany* by Ribotyping and Pulsed-Field Gel Electrophoresis

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ABSTRACT

A high isolation prevalence of *Salmonella enterica* subsp *enterica* serovar *albany* (*S. albany*) in the Northern Punjab area of Pakistan, provided the opportunity to investigate the molecular epidemiology of this less frequently reported serovar. A total of 63 unrelated isolates of *S. albany* collected during 3- years period from 12 different sources were investigated by different molecular typing methods. Ribotyping using *BamH*1 digested whole cell DNA with *5S-16S-23S* rRNA gene probe showed complete degree of homogeneity within *albany* serovar. Such data suggest that there is a single clonal line widely disseminated within the studied area. The results obtained from macrorestriction analysis strongly depend on the choice of restriction enzyme. *Spe*1 exhibited typical macrorestriction pattern, while two different fragment pattern were observed with *Xba*1. Macrorestriction analysis with *Xba*1 proved to be the most discriminatory method for the epidemiological typing of *S. albany.*

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THE QUALITY OF FEEDSTUFFS AND RATIONS IN THE AVIAN SETTING

SANTOS, E. J.; CARVALHO, E. P.; SANCHES, R. L.; BARRIOS-BARRIOS, B. E.; DIONIZIO, F. L.*

The hygienic and sanitary quality of feed rations is a control point to prevent spreading of pathogens, since feed is an integral part of the food chain extending from the animal production system to the consumer. Because it is very important to control microbiological adulteration of rations and raw materials, the present study aimed to evaluate the quality of these products by detecting contamination due to mesophiles, fungi, fecal coliforms and the presence of *Salmonella*. Also, a microscopic analysis was performed to detect possible adulteration of nutrient meat and bone meals.

As a general rule, the methods reported by the American Public Health Association (APHA) (Speck, 1984) were utilized for the microbiological analyses. Microscopy was done according to the methodology described by A. A. F. M., 1992. Meat and bone meals contaminated by *Salmonella* (90%) were the chief sources for transfer of this pathogen to the rations (36.36%) for the consequent spread to birds. Mesophiles, fungi and yeasts were found in 100% of the animal and plant meals of high quality. The count of fungi and yeasts in 27.27% of rations of poor quality exceeded 10^6 FU/g. The presence of fecal coliforms in meat and bone meals (20%) and rations (44.45%) was associated with the lack of general hygiene in the handling and storage of the product. Adulterants such as hoofs/horns (40%) and hide (60%) in meat showed these feeds were of poor quality.

Key words: *Salmonella*, poultry feeds, quality.

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EXPERIMENTAL INVESTIGATION OF MEAT AND BONE MEALS AS A SOURCE OF *SALMONELLA* IN LAYING HEN HOUSES

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Salmonellosis has worried the public and the consumption of eggs or raw egg based products has harmed consumers. Salmonellae spreads to eggs, and vertical transmission to the egg is a predominating factor and a reason for conducting the present work. Laying hens were given feed containing naturally contaminated meat and bone meals. Following infection after three weeks, 9 birds were necropsied and samples of the intestine and ovaries were collected for culture of Salmonella. The birds were kept according to the feed group in the cages, and three from each group were submitted for necropsy. Whole egg, samples of meat and bone meals (6), rations (8) and eight pools of eggs were analysed, with albumen, yolk and shell analyzed every week. For selective enrichment, tetrathionate Muller Kauffman and Rappaport Vassiliades broths were utilized. For further selection, Rambach and SS (*Salmonella Shigella*) agar was used for culture at 37° and 43°. A few bacterial strains from the meals of animal origin and rations were identified by the commercial systems API 20E and Bac Tray (I and II) and sent for serotyping by the Bacterial Zoonosis Laboratory of Fundação Oswaldo Cruz. There was no isolation of Salmonella from any sample of albumen, yolk or whole eggs. There was isolation of suspect colonies from the intestine, oviduct and egg shells obtained from the avian setting, which could extend to consumers in meat and eggs.

Key words: meat and bone meal, *Salmonella*, poultry house.

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Survival of Salmonella in chicken litter during small vessel composting

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To study variables that could influence the killing of pathogens during composting, litter used by chickens that had been infected with a nalidixic acid resistant strain of Salmonella typhimurium was mixed with peat or hay and water and was composted in plastic barrels that had aeration holes. Each barrel contained about 45 kg of the biomass and thermocouples were situated in the bottom, middle and top levels of the mass. In Expt. 1, 3 of the 4 barrels were insulated and the contents of each barrel was mixed before sampling. Maximum temperatures ranged from 60 to 70 C in the insulated barrels and from 31 to 60 C in the non-insulated barrel. On day 0 there were 4000 colony forming units (CFU) of S. typhimurium per g of biomass. On day 4, Salmonella was recovered from the non-insulated barrel and 2 of the 3 insulated barrels, whereas on days 10 and 35 the organism was not recovered from any of the barrels. For comparative purposes, survival of fecal streptococci was also studied. There were 6-7 million CFU of fecal streptococci per g in the non-insulated barrel on days 0, 10 and 35. Yet those organisms were not detectable in the insulated barrels on days 10 and 35. Thus the lower temperatures in the non-insulated barrel were sufficient for killing of Salmonella but not fecal streptococci.

In Expt. 2, all 4 barrels were insulated and 3 dead chickens were placed in the middle layer of each of 2 barrels. Thermocouples were placed as above in the biomass and also in the birds. Following initial preparation, the contents of barrels were not mixed. Maximum temperatures were from 63 to 71 C. There was some variation between replicates in the rate of temperature increase. For example in one of the two barrels that did not contain dead birds, a temperature of 55 C was reached at all 10 points between days 8 and 9. In the replicate it took 14 to 22 days to reach 55 C. Temperatures in the chickens were similar to that in adjacent portions of the mass. In this study there were about 3000 CFU of S. typhimurium per g of litter to be composted. On day 0, S. typhimurium was recovered from deep swabs from the centre of the mass in each barrel but the organism was not recovered from swabs similarly collected on days 7, 19 and 40. However, on day 40, S. typhimurium was recovered from the internal walls of the barrel, above the level of the compost.

(22)

STUDIES ON BACTERIA CAUSING LOW HATCHABILITY AND LOW FERTILITY IN TURKEYS

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A turkey farm suffering from low fertility and low hatchability as well as reduction in body weight gain was examined to determine the causative agents. Bacteriological samples included heart blood, fertile eggs, semen, cloacal swabs, newly hatched chicks, intestinal and cecal contents, and bone marrow. In addition, rations and water were cultured.

Salmonella enterica serovar Typhimurium was isolated from semen, fertile eggs and unhatched eggs. *Pseudomonas* spp. were isolated from semen, eggs and cloacal swabs. *Mycoplasma* spp. were isolated from eggs and unhatched eggs. Coliform bacilli were isolated from eggs, semen and cloacal swabs. Tube agglutination tests prooved that blood samples were positive for *S.* Typhimurium.

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EPIDEMIOLOGICAL TRACKING OF SEROTYPES OF SALMONELLA IN BROILER CHICKENS

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Birds infect themselves orally, and contaminated feed is an important vehicle. The antigenic characterization of *Salmonella* is an important method for classifying the salmonellae into different serotypes. Serotyping was used to identify *Salmonella* circulating in the avian setting. The present work investigated if there was a link between a high infection rate in birds and the intake of contaminated rations. Sixty-seven (67) samples in all were cultured, which consisted of meat and bone meals (10), soybean meal (1), corn meal (1), rations (11), cloacal swab (11), bile vesicle (11), intestine (11) and carcasses. The samples of bile vesicle, intestine and carcasses came from 33 birds collected according to their feeding. Tetrathionate Muller Kaufman and Rappaport Vassiliadis broths were used for selective enrichment, and Rambach and SS (*Salmonella Shigella*) agars were used for further selection at culture temperatures of 37° and 43°. Suspect bacterial colonies were identified as salmonellae by the commercial systems API 20 E and Bac Tray (I and II) and later sent for serotyping to the Bacterial Zoonosis Laboratory of Fundação Oswaldo Cruz. The following serotypes were isolated from bird feed: *S. senftenberg*, *S. infantis*, *S. give* and *S. fyris*. From birds, *S. infantis*, *S. bredeney*, *S. senftenberg* and *S. give* were isolated. In 72.73% of birds, there was a close relationship between the serotypes found in the rations and feeds and those found in the birds eating the rations. The presence of the serumvar *S. bredeney* in birds was not associated with the intake of contaminated feed. In these several segments, the existence of multiple sources of contamination was confirmed both during production and during slaughter of birds for broiler meat.

Key words: *Salmonella*, serotype, poultry.

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Epidemiology of *Salmonella* in chickens in Zambia. 1. Occurrence of *Salmonella* Enteritidis in hatcheries, table eggs and chicken meat in Zambia.

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Our two surveys (1976 - 84 and 1985 - 94) in Zambia had demonstrated that *Salmonella* infections had been a major problem in the poultry industry. In recent years, breeding farms and hatcheries in Zambia have been experiencing losses between 15% and 31% through death of in shell embryos. Bacteriological investigation of specimens from three big hatcheries revealed the majority of isolates were enteric bacteria. *Salmonella enteritidis* was recorded from (34) 8.87% of isolates. Of 34 *Salmonella enteritidis* cultures, the majority (58%) were phage type 4 (PT4), followed by PT1 and PTlb (18%), and PT7 and PT7a (9%).

Presuming wide spread dissemination of *Salmonella enteritidis* in the Zambian poultry population through contaminated breeding birds, further study was undertaken on its occurrence in human food of chicken origin. On Bacteriological examination of chicken table eggs and chicken meat, out of 2400 table eggs and 382 broilers destined for human consumption, 3.75% of eggs and 4.7% of broiler meat were found positive for *Salmonella enteritidis*. The majority of *Salmonella enteritidis* isolates from eggs and meat belonged to pathogenic phage type 4 (PT4). The isolation of *Salmonella enteritidis* PT4 for the first time in Zambia confirms its occurrence on the African continent and opens a new page in the epidemiology of *Salmonella* in this country.

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Using an hygienic score as a forecasting tool for the *Salmonella* status of broiler houses after cleansing and disinfection

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Aim

A fast and quantitative method using fecal streptococci counts, and a visual cleansing inspection grid were performed in this study in order to investigate if the residual contamination appreciated by these methods was related to the *Salmonella* status of the house before entering day-old chicks.

Material and methods

Eighty five flocks of commercial broiler chickens were included in the study and each house was visited before entering day-old chicks. A visual inspection grid was filled out and 12 ENTEROCOUNT count plates (AES Laboratoire, Combourg, France) were applicated per house on the base of walls. The *Salmonella* status of the house was assessed by means of a gauze swab-based procedure including 13 swabs per house sampled from the walls, the feeders, the new litter and the ventilation systems.

Results and Discussion

A bacteriological score was built with the plate counts, with a correspondance analysis followed by a hierarchical clustering (table I). The visual mark, out of 200, obtained for each house, was clustered in four quartiles (table I) leading to a visual score out of 4. An hygienic score (ranging from 2 to 8) was built adding the bacteriological score (out of 4) to the visual score. The results given by the hygienic score were highly correlated with the *Salmonella* status of the house (table II). A significant relative risk was obtained, meaning that beyond the hygienic score 4, the risk of contamination of the house by *Salmonella* was about 4 times greater.

This method could be used in a quality assurance program performed at the farm level, in order to assess the risk of introducing day-old chicks in a *Salmonella* contaminated environment. This real-time method would allow sufficient time (result in 24 hours) for surfaces to be recleaned before placing day-old chicks in the house.

Table I : Description of the bacteriological and visual scores

Bacteriological		Visual	
Score	Description (% of the counts) [cfu/plate]	Score	Description [visual mark]
1 (n = 43)	> 88.8% [0]	1 (n = 19)	[175 - 200]
2 (n = 16)	> 38.5% [1-5[and > 23.4% [5-50[	2 (n = 24)	[147.5 - 175[
3 (n = 11)	> 55.9% [5-50[	3 (n = 21)	[111.3 - 147.5[
4 (n = 15)	> 73% [50- ∞[	4 (n = 21)	< 111.3

n = broiler houses frequencies

Table II : Hygienic score and *Salmonella* status of the house

		Salmonella status	
		positive	négative
Hygienic score	> 4	25	12
	≤ 4	8	40

$\chi^2 = 22.8$, $p < 1‰$
Relative risk = 4.05
Confidence Interval = [2.20 - 7.67]

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Antimicrobial resistance in *Salmonella enterica subsp. enterica* serovars Enteritidis, Typhimurium, Hadar and Saintpaul from poultry

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Emerging antimicrobial resistance in bacteria is regarded as an inevitable genetic response to the selective pressure imposed by the widespread use of antimicrobials for therapeutic, prophylactic or nutritive purposes. The observation that many resistance genes have been detected on mobile genetic elements may explain the rapid development of antimicrobial resistance by the exchange of resistance genes between bacteria of different species and genera which share the same gene pool. In this regard, isolates of *Salmonella enterica* share a common resistance gene pool with many bacteria of the family *Enterobacteriaceae*.

In this study, we investigated 173 *Salmonella enterica* isolates obtained from chickens in Northern Germany between 1993 and 1997. These isolates included 59 *S.* Typhimurium, 43 *S.* Enteritidis, 59 *S.* Hadar and 12 *S.* Saintpaul. In addition 16 *S.* Typhimurium var. Copenhagen isolates from pigeons were also studied. All *Salmonella* isolates were investigated for resistance to tetracycline, ampicillin, kanamycin, chloramphenicol, gentamicin, streptomycin, nalidixic acid, and sulfamethoxazole/trimethoprim by the agar diffusion method. Isolates of the different serovars varied distinctly in their resistance patterns. In *S.* Enteritidis, streptomycin resistance as detected in 20 (46.5 %) isolates was the only resistance property. The majority (78.0 %) of the *S.* Typhimurium isolates also proved to be sensitive to all antimicrobial agents tested and multiple antibiotic resistance was detected rarely. In contrast, all *S.* Typhimurium var. Copenhagen isolates proved to be resistant to two to four different antibiotics. Multiple resistance to tetracycline, streptomycin, sulfamethoxazole/trimethoprim and/or nalidixic acid was seen in all *S.* Hadar isolates. All *S.* Saintpaul isolates proved to be resistant to tetracycline and streptomycin while additional resistance to kanamycin was observed in 83.3 % of the *S.* Saintpaul isolates.

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EFFECT OF FEED WITHDRAWAL ON THE INCIDENCE OF *SALMONELLA* AND *CAMPYLOBACTER* IN THE CROPS AND CECA OF MARKET AGE BROILER CHICKENS. J.A. Byrd[1]*, D. E. Corrier[1], M.E. Hume[1], R.H. Bailey[1], B.M. Hargis[1,2], and L.H. Stanker[1]. [1]Food Animal Protection Research Laboratory, USDA-ARS, College Station, Texas, 77845, [2]Departments of Veterinary Pathobiology and Poultry Sciences, Texas Agriculture Experiment Station, College Station, Texas, 77843.

Previously published research has focused predominately on cecal and intestinal contents as the major source of *Salmonella* and *Campylobacter* contamination of poultry carcasses. Recent studies have suggested that the crop contents may serve as an important source of *Salmonella* carcass contamination. During the present study, we evaluated the effect of preslaughter feed withdrawal on the incidence of *Salmonella* and *Campylobacter* in the crops of market age broiler chickens prior to transport. To determine whether crops may serve as a reservoir of field-derived *Salmonella* and *Campylobacter* prior to transport and processing, crops were collected from broilers in 9 commercial broiler houses. Ceca were collected from broilers in 6 of the same houses for comparison. The incidence of *Salmonella* in crop contents increased significantly ($P < 0.05$) in five of nine flocks during feed withdrawal. The total number of *Salmonella* contaminated crops from all nine flocks increased significantly ($P < 0.005$) from 7/360 (1.9%) before feed withdrawal to 36/359 (10.0%) at the end of feed withdrawal. The incidence of *Campylobacter* contaminated crops increased significantly ($P < 0.005$) from 90/360 (25%) before feed removal to 224/359 (62%) at the end of feed withdrawal. However, the incidence of *Salmonella* and *Campylobacter* in the ceca of broilers was not significantly higher ($P < 0.05$) following feed withdrawal than in samples obtained immediately prior to feed withdrawal. These studies indicate that feed withdrawal increases the incidence of *Salmonella* and *Campylobacter* in broiler crops prior to transport and may be an important critical control point for reducing *Salmonella* and *Campylobacter* entry into the processing plant and contamination of broiler carcasses.

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Salmonellae concentrations in broiler scald water. J. A. Cason, A. Hinton, Jr., and K. D. Ingram. Poultry Processing and Meat Quality Research Unit, Russell Research Center, USDA/ARS, P.O. Box 5677, Athens, GA 30604-5677.

Broiler scald water samples from a commercial processing plant were tested for salmonellae to evaluate the extent to which cross-contamination of salmonellae may occur between carcasses during scalding and defeathering. Scald water samples were taken from each of three tanks on four different days after six-week old broilers had been processed for a full eight-hour shift. Mean scald water temperature was 56.0 C. The Most Probable Number (MPN) of salmonellae was determined by enriching serial dilutions of samples in Selenite Cysteine Broth followed by plating on XLD and Bismuth Sulfite agar. Isolates were confirmed by biochemical and serological tests. Eight of 12 scald water samples were positive for salmonellae with a mean MPN/100 ml of 38.9 in the positive samples. The highest observed concentration in positive samples was 89.9 MPN/100 ml and the lowest was 4.1 MPN/100 ml. There would have been minimal probability of cross-contamination via scald water when salmonellae could not be recovered, but in salmonellae-positive samples there would have been some possibility of cross-contamination. With wet carcasses carrying 175-200 ml of scald water into the defeathering equipment, whether these concentrations increase the percentage of salmonellae-positive carcasses would depend on the numbers of salmonellae already present on the skin and the extent to which bacteria carried in wet feathers are transferred to the skin during defeathering.

Key words: Salmonellae, Scald water, Cross-contamination, Poultry

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Recovery of *Salmonella typhimurium* from Media Containing Propionic Acid and Potassium Chloride or Sodium Chloride

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Three agar media were used to enumerate *Salmonella typhimurium* in cultures grown in Brain Heart Infusion (BHI) supplemented with propionic acid and potassium chloride (KCl) and/or sodium chloride (NaCl). BHI was supplemented with 0, 250, 500, or 750 mM of either KCl or NaCl or with 0, 125, 250, or 375 mM of both KCl and NaCl. Either 0, 25, 50, or 75 mM of propionic acid were added to the BHI-salts mixture. The final pH of the medium was 6.5. The supplemented BHI was inoculated with 10^3 *S. typhimurium*/ml and incubated at 37°C for 48 h. After incubation, serial dilutions of the cultures were spread on Plate Count Agar (PCA), BG Sulfa Agar (BGS), and Bismuth Sulfite Agar (BS) and incubated at 37°C for 24 h. There was no significant ($p < 0.05$) difference in the number of *S. typhimurium* recovered from BHI supplemented with various concentrations of propionic acid, KCl, or NaCl only or mixtures of KCl and NaCl. However, combining 25 mM of propionic acid and 750 mM KCl did cause significant ($p < 0.05$) reductions in *S. typhimurium* growth. Additionally, significantly ($p < 0.05$) fewer *S. typhimurium* were recovered from BHI supplemented with 50 or 75 mM propionic acid and 500 mM KCl or NaCl or a mixture of 250 mM KCl and NaCl. Differences in the numbers of *S. typhimurium* recovered on PCA, BGS, or BS were not significant ($p < 0.05$). Findings indicate that a synergistic reaction between propionic acid and KCl or NaCl may inhibit *S. typhimurium* growth in BHI. Mixtures of the salts and propionic acid produced no cellular injury that reduced the ability of the bacterium to grow on selective media, such as BGS or BS.

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Salmonella enterica Serovar Enteritidis Attachment to and Invasion of Avian Ovary Granulosa Cells In Vitro May Explain the Mechanism of Transovarian Transmission that Leads to Salmonella-Contaminated Eggs

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Most strains *of Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) that were isolated from human cases of food poisoning attached to and invaded the granulosa cells prepared from adult hen's ovary in vitro. Several groups of adult laying Leghorn hens were experimentally infected with invasive and noninvasive strains of *S.* Enteritidis. The challenge *Salmonella* strains were isolated from a significant number of eggs laid during the first 2 weeks posinfection. Additionally, the same organisms were isolated from the different developmental stages of the preovulatory follicles of the hens at necropsy, particularly the membranes of the follicles. Histopathological studies demonstrated the *Salmonella* organisms in the granulosa cell layer of the preovulatory follicles in a close proximity to the yolk content, particularly with F1, the most advanced stage of the follicles. These findings may explain the mechanism of transovarian transmission of the *S.* Enteritidis that leads to the contamination of laid eggs with these pathogens. The noninvasive *Salmonella* strains were less likely to cause severe ovarian infection in the inoculated hens. Analysis of the data suggests that *S.* Enteritidis that in vitro attaches to and invades the hens' ovarian granulosa cells have a high pathogenicity in layers.

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The Use of Commercially Available Molecular Methods to Accurately Detect, Identify and Characterize Strains of *Salmonella* at an Integrated Poultry Production Facility

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A large integrated poultry company utilized the BAX™ for Screening system, which uses polymerase chain reaction (PCR), to routinely assay for the presence of *Salmonella*. Up to 800 samples per month were tested. Sample types tested included ceca, litter, feed, environmental swabs, eggshells, chick pad swabs, water, whole bird feather rinses, carcass rinses, and finished product. This AOAC Performance Tested Method^{SM} system provides next-day results that are equivalent to or surpass the conventional BAM methods in sensitivity and specificity. Strains from positive BAX™ samples were isolated and further characterized by a DNA fingerprinting method based on rRNA/DNA. Using the automated RiboPrinter® Microbial Characterization System, more than 200 samples were characterized by generating normalized DNA patterns, called RiboPrint® patterns. The system software automatically analyzed each DNA pattern generated and compared the sample pattern to the system reference database patterns and to custom reference database patterns. This provides characterization and identification of *Salmonella* strains in monitoring and epidemiological studies. These molecular tools allowed for a very sensitive, accurate method to rapidly screen for the presence of *Salmonella* and the subsequent characterization and identification of the *Salmonella* strains.