



REPORT OF WHO CONSULTATION ON SALMONELLOSIS
CONTROL IN AGRICULTURE

Orvieto, Italy, 9 - 12 April 1990

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INTRODUCTION

In 1987, the World Health Organization in cooperation with the Food and Agriculture Organization of the United Nations initiated activities focusing on the control of sub-clinical infections which lead to contamination of animal products, including harvesting and slaughter processes. They concern salmonellosis, campylobacteriosis, listeriosis, toxoplasmosis, Q-fever, anisakiasis and other human diseases due to pathogens which may not be detected in the live animal and which escape routine meat and fish inspection. A number of WHO consultations have dealt with such agents.^{1, 2, 3, 4, 5, 6} The WHO Expert Committee on Salmonellosis Control in 1988 stressed the role of animal and animal product hygiene.⁷ New epidemiological developments, particularly the spread of *Salmonella enteritidis*, stimulated increased activities in a number of countries. This has been reflected in the WHO Consultation on Epidemiological Emergency in Poultry and Egg Salmonellosis (Geneva, March 1989);¹ a WHO Working Group on Salmonella Immunization (Jena, March 1989);⁸ and a WHO Consultation on Research on New Slaughter Technologies to reduce Cross-contamination, (Roskilde, February 1990).⁹

Several international groups were created during these meetings mainly to promote further research, activities which are in line with a resolution of the World Health Assembly, 1989 (WHA42.40)¹⁰ urging Member States to intensify their efforts for the prevention and control of salmonellosis, in the spirit of intersectoral collaboration.

The analysis of present technology and potential for development in the production of animals free of invasive salmonellae in microbiologically monitored farms has encouraged the research teams and national authorities to promote breeding hygiene, including proper feed safety and the zoo-sanitary measures. New approaches and rapid scientific advances called for the meeting which is the subject of this report. The participants (see Annex 1) paid particular attention to the production of animals free of infection, use of vaccines, elimination of infection from infected herds and on improved slaughter hygiene.

The meeting was opened in the name of the Region of Umbria and the Municipality of Orvieto by Professor Casasola and Dr Pacioni. They welcomed the representatives of international organizations and the participants and emphasized the great importance of collaboration among the consumers, local authorities and farmers in salmonellosis control.

Professor A. Mantovani greeted the participants on behalf of the Italian Government, and the WHO Collaborating Centre for Research and Training in Veterinary Public Health, Istituto Superiore di Sanità, Rome.

Dr K. Bögel expressed gratitude for the support and cooperation received from the Umbria Region and the Municipality of Orvieto. He conveyed greetings and good wishes for a successful meeting from Dr H. Nakajima, Director-General of the World Health Organization, and officially opened the meeting on his behalf.

Professor A. Mantovani was elected Chairman and Professor B. Smith Vice-Chairman; Dr C. Wray agreed to act as Rapporteur. The meeting started with a tribute to the late Dr R. Olds who had contributed to the programme as Officer in the Food and Agriculture Organization of the United Nations.

1. GENERAL ASPECTS

The number of human cases of salmonellosis has increased markedly in recent years in many countries, in some, the number of cases is increasing annually by more than 30%. The true incidence of human salmonellosis is, however, probably much higher than the reported figures because not all cases of diarrhoeal disease are investigated bacteriologically. Comparison of data from different countries is difficult because of the variety of surveillance systems in operation.

In recent years, *S. enteritidis* has become a major human pathogen in a number of countries and eggs appear to be an important vehicle of infection.

Poultry and poultry products appear to be the commonest cause of human salmonellosis, although eating habits such as the consumption of raw red meat may be important in some countries. Some outbreaks of salmonellosis, such as those caused by contaminated milk can be extensive and affect whole communities.

Changing practices in farm management have been associated with an increased incidence of salmonellosis in animals. The rearing of animals in intensive livestock production systems facilitates the spread of pathogenic organisms including salmonella. The economic production of animals may also require cheap sources of dietary protein, which are frequently contaminated with salmonella. The disposal of manure and effluents from these production systems creates difficulties and often results in environmental contamination. Many production units rely on the use of antimicrobial agents, not only for the treatment of infection but also for growth promotion and the prevention of disease. As a consequence, some bacteria in livestock are becoming increasingly resistant to antimicrobial agents. These resistant bacteria may also affect the human population and create public health problems. In particular, systemic invasion of pathogenic bacteria, such as salmonella are becoming increasingly difficult to treat. The presence of symptomless salmonella carriers may result in the contamination of many other carcasses during slaughter.

Salmonella control requires surveillance systems which:

- i) allow the rapid recognition of current trends;
- ii) monitor control programmes; and
- iii) allow a rapid response to emergency situations.

The complex nature of the epidemiology of salmonellosis necessitates integrated and multidisciplinary approach for its control. In addition, community participation involving both the producer and the consumer is essential, whether at the local or national level. Salmonella outbreaks may also result from the movement of food or animal products from one country to another and international technical cooperation is essential to reduce these incidents.

Salmonella control in humans requires a reduced incidence in farm animals and improved hygiene during slaughter to reduce cross-contamination.

Experience generated in national schemes and results of experimental approaches indicate that it is possible to reduce the incidence of infection in herds and the contamination rate in raw animal products by more than 95%. Different approaches may increase the effectiveness further.

Since measures in animal production and slaughter hygiene applied to salmonella affect a wide range of pathogens, it is reasonable to assume that a reduction of the pathogenic load by a number of factors would reduce foodborne infections by 50-70%.

The cost of hygienic measures for animal production described below is relatively low, but these measures do require considerable input from surveillance services. In addition, zoo-sanitary codes and measures based on close cooperation between producers, consumers, and national animal product and health services, are needed for national control schemes.

In general, improved strategies for animal production offer the greatest potential for effective prevention of the majority of foodborne infections in humans.

Besides sterilization and pasteurization, the group sees at present no better procedure in this respect than the combined measures discussed in this report.

2. SALMONELLA- AND PATHOGEN-FREE POULTRY AND LIVESTOCK PRODUCTION

2.1 Salmonella-free rearing of animals

Meat from domestic animals, and eggs, play a very important role in the epidemiology of human salmonellosis. Salmonella in poultry still remains the predominant source of human salmonellosis in most countries. Pork plays an important role in some countries, whereas beef and mutton are only of minor importance.

The predominant serotypes in man in many countries are *S. typhimurium* and *S. enteritidis*. Although these are not usually found in animal feeds, in some countries *S. typhimurium* has been shown to be foodborne, and this serotype has also been detected in environmental sources.

With the exception of *S. typhimurium* and *S. enteritidis* the predominant serotypes in pigs and poultry originate from the feed. The serotypes vary from country to country depending upon their prevalence in imported feeds and in particular the use and the source of meat, bonemeal and fishmeal.

2.2 Pathogen-free raising of animals

2.2.1 Pigs. The raising of specific pathogen-free (SPF) herds has been introduced, not to protect the consumer but to reduce or eliminate the incidence of certain animal diseases causing serious economic losses to the farmer. The SPF system for pigs is aimed at six well-defined contagious diseases affecting particularly the respiratory tracts of the animal and infestation with mange, mites and lice, but it does not cover zoonotic diseases in pigs. The prevalence of salmonella carriers among pigs coming from SPF herds is usually nearly as high as for pigs coming from conventional farms. An SPF system does, however, offer a possibility to minimise the prevalence of salmonellae provided that some simple hygienic measures are employed and that the feed is guaranteed to be salmonella-free.

Clinical salmonellosis in pigs is uncommon and the prevalence of healthy salmonella carriers among pigs varies from country to country and is related, for example, to its incidence in the feed.

2.2.2 Poultry. The SPF system in poultry should also include salmonellae other than *S. pullorum/gallinarum*. Infection of poultry flocks with *S. typhimurium* may cause great losses in the first 1-2 weeks in the life of chickens. Chickens which survive become excretors and may remain so for the rest of their short lives. Broilers raised according to the SPF principle are free of clinical salmonellosis but the flocks may harbour and excrete zoonotic serotypes of salmonellae.

The approach to eliminate salmonellae in poultry differs according to the serotypes involved, each having its own epidemiological pattern. The serotypes of the invasive kind, e.g. *S. enteritidis*, *S. typhimurium*, establish themselves in the ovary or oviduct, and are transmitted vertically in the poultry farming system.

Elimination of the invasive serotypes has to be attacked from the very beginning of the poultry raising programme, starting with the grandparent flocks and appropriate preventive measures have to be taken in all the subsequent steps of raising parent flocks, layer flocks and broiler flocks. A comprehensive description was provided in the report of the WHO Consultation on Epidemiological Emergency in Poultry and Egg Salmonellosis, Geneva, 1989.

Exotic serotypes are to a large extent feed-borne in poultry, but their presence in final dressed poultry may have other sources such as the slaughterhouse. The presence of salmonellae in compounded feed is dealt with separately (see 2.2.4).

2.2.3 Campylobacteriosis in poultry. Poultry are considered to be one of the most important sources of campylobacteriosis in man. If campylobacter is present in a flock, 100% of the birds will harbour the organism in the intestinal tract. Not all flocks will excrete campylobacter but the geographical distribution of campylobacter species in poultry flocks in different countries is not fully known. Investigations in Sweden on the possibility of eliminating bacteria from eggs used for hatching, supported by some simple preventive measures on the farm, e.g. (1) provision of an ante-room in each poultry house to exclude environmental sources of recontamination of the flocks, (2) the SPF system in the raising of poultry, seem to offer good possibilities for the elimination of campylobacter infection from poultry. The presence of campylobacter in poultry causes no loss at the production level so that farmers have to be motivated to eradicate the bacteria in order to protect human health.

2.2.4 Prevention or elimination of salmonella in feedstuffs. Compound feedstuffs play an important role in the transmission of certain salmonella serotypes in the food chain. The most important source of salmonella in compound feeds is meat and bone meal, fishmeal and vegetable feeds in oil cakes which are subjected to oil or fat extraction procedures, e.g., cotton cake and soya-bean meal.

The current technology for pelleting feed produces feed which is convenient for feeding to animals but does not eliminate salmonellae or other zoonotic agents. Feeds may be made salmonella-free by improving procedures for pelletization, by addition of organic acids or by irradiation. However, the pelleting process itself offers numerous possibilities for recontamination of the final product, especially with salmonellae, through fat coating the equipment.

Pelletization will produce salmonella-free feed provided that strict hygiene at the feed compounder premises is monitored regularly throughout the working day, in particular the temperature of the Cascade mixers and pellets coming out of the pelleting matrix, and the prevention of recontamination after heat treatment.

In general, feed compounders do not seem to be well informed of the need for good manufacturing practices (GMP) as far as hygiene is concerned. "Good hygiene practices" need to be elaborated for the production of compounded feeds and the construction of equipment which offers better possibilities of securing a salmonella-free product.

The addition of organic acids such as propionic acid and formic acid at the level of 2.3% may add to the security and may also have an effect on the presence of salmonella in the intestinal tract of the animals. The addition of 1.2% of organic acid may protect the feed against recontamination and subsequent growth of salmonellae after the delivery of the feed from the compounders.

3. METHODS FOR THE DETECTION AND CONTROL OF SALMONELLA INFECTION IN ANIMALS

3.1 Methods of detection

Bacteriological culturing is still the most widely applied detection technique. Alternative sensitive immunological detection assays (e.g. ELISA) are becoming increasingly available. In the foreseeable future DNA techniques may also become more important.

3.1.1. Cultural techniques. A very wide range of techniques for the isolation of salmonella has been published. Many have been developed for special situations or even specific serotypes. When bacteriological culturing is used for detection and control purposes examinations should be carried out in accordance with the International Standards Organization (ISO) standard 6575 or equivalent recognized methodology.

The ISO standard is not specific for certain serotypes but detects a wide range of salmonella serotypes. However, large-scale testing is time consuming, labour intensive and therefore expensive. The advantage of the method is that it is standardized and can be easily applied even in laboratories with limited facilities.

3.1.2. Immunological tests. Immunological tests can be applied for testing of serum, milk, egg yolk, etc. for the presence of either antigens or antibodies. However, antigen detection techniques are often serotype-specific, have limited sensitivity, and therefore limited application in large-scale screening.

Antibody detection using sensitive immunological techniques can be designed as serotype specific, group specific or even general salmonella specific test methods. The presence of antibodies does not in itself prove current infection; a positive result only indicates either previous exposure or, in the case of very young animals, previous exposure of the parents. Where antibody techniques are used as a screening method for the detection of infected herds or flocks, a positive antibody response must be confirmed subsequently by cultural techniques.

When a negative serological result is obtained care must be exercised in interpreting such results as evidence of the absence of current infection. Sequential antibody screening may overcome some of these problems. Care must also be exercised in the interpretation of the result of serological screening when vaccination is practised. However, systems can be developed to distinguish between vaccination and natural infection. When salmonella infection is limited to intestinal colonization, it is unlikely that current antibody detection techniques can be applied.

3.1.3 DNA techniques. The DNA technology is developing rapidly and some products for detection of salmonella are already commercially available. However, experience with these products is too limited to allow a comprehensive evaluation of their use. Initial work has indicated that such technology may well have an important role in the detection of infected animals and potentially for rapid and specific screening of a large number of samples.

Further research is needed on immunological detection methods and DNA technology with particular reference to their application for large-scale screening purposes and the detection of infected herds and flocks.

3.1.4 Application of a detection technique for campylobacter. Bacterial culture according to the ISO standard method 6575 is at present the most reliable technique to detect campylobacter. However, this is not as widely applied as in salmonella studies. As indicated above, for salmonella it is further recommended that research on immunological methods and DNA techniques for campylobacter is intensified.

3.2 Application of methods

Selection of the appropriate type and quantity of samples to be examined and the frequency of sampling are critical to the efficiency of any screening technique. For example, while rectal swabs are convenient and may be acceptable in cattle and pigs, cloacal swabs in poultry are not considered satisfactory, but faeces samples are acceptable for salmonella detection. For the detection of campylobacter in poultry, the preferred sample is fresh cloacal dropping. Faecal excretion of salmonella is intermittent, and the frequency of sampling is, therefore, important. Moreover, a herd or flock may be infected with more than one serotype of salmonella and the sensitivity of the screening technique is dependent on the number of samples cultured individually.

In the design of any routine screening method for the detection of infected herds or flocks proper attention should be given to the principles of the Hazard Analysis Critical Control Point (HACCP) concept.¹¹

3.3 Control strategies

3.3.1. Priorities for control strategies in food animals. It is recommended that priorities in the detection and control of salmonella in infected food animals must be given to: (a) poultry production including both eggs and poultry meat; (b) the production of milk.

3.3.2. Control in poultry. This area of control was considered in the WHO Consultation on Epidemiological Emergency in Poultry and Egg Salmonellosis (Geneva, March 1989), and is summarized below.

With regard to poultry, control strategies are urgently required to eliminate salmonella types which can be transmitted vertically in the breeding sector in order to ensure the supply of clean unenforced stock to the production level. A strategy is also needed for commercial production flocks to ensure continuing freedom from infection.

Control strategies require active and continuous bacteriological monitoring of breeding flocks and hatcheries in order to establish the salmonella-free status. The hatchery is one of the major critical control points in poultry production. The frequency of bacteriological monitoring will be determined by the epidemiological characteristics of the serotypes involved and the history of infection of such serotypes in the breeding population. One of the major advantages of bacterial monitoring in a hatchery (where the samples of choice are meconium, dead in shell, or culled chicks) is that the monitoring not only indicates the status of the parent flock but, at the same time, that of the progeny.

Sampling for bacteriological monitoring must have a sound statistical base. For example, a commonly used statistical sample is one designed to give a 95% probability of detecting one infected animal within a herd or flock given that the prevalence of infection in that flock is at the 5% level. This statistical sample procedure requires a maximum of 60 samples selected at random from any one population.

When bacteriological monitoring of herds or flocks is carried out and a positive result is obtained it is essential that further investigations are carried out within the herd or flock to identify the animals currently infected. In poultry these further investigations should include bacteriological examination of tissues from birds (such as oviduct, ovary, liver and intestines). All these additional investigations and sampling should also be carried out using statistical sampling procedures.

At present, when invasive salmonella such as *S. enteritidis* or *S. typhimurium* are confirmed in a poultry breeding flock (either grandparent or parent), slaughter, removal of the flock and destruction of any existing hatching eggs from those flocks, are necessary.

Appropriate measures should also be taken immediately to ensure that the risk to man from the products of these flocks is minimized (e.g. appropriate heat treatment - for example pasteurization of egg products and precautionary procedures at the slaughter house).

When salmonella infection is detected in a broiler flock, the use of antimicrobial treatment as a technique has been practised to minimize the risk to human health. While such treatment may be effective in relation to the products from that individual flock, it is considered that widespread or continuous application of such treatments as a routine procedure will present problems in the development of antimicrobial resistant strains and a consequent public health concern. Antimicrobial treatment in this area is, therefore, not recommended.

3.3.3 Control of salmonella in milk. On farms where milk is produced a control strategy including routine regular bacteriological monitoring of both animals and milk should be carried out.

When infection is confirmed, the milk from infected animals should be heat treated before supply to other animals on the premises. Heat treatment should be continued until such time as it can be established that infection is no longer present in the herd.

3.3.4 General. In all strategies every effort should be made to determine the source of infection in order to avoid microbial resistance. Strategies in relation to campylobacter are not proposed at this time, but more research is recommended for development of control strategies similar to those for salmonella.

Team leaders from the WHO working group on salmonella immunization in animals met prior to the consultation to report on developments in their respective field. A summary of that meeting report will be found in Annex 2.

4. SALMONELLA VACCINES

In the United Kingdom a number of groups have been investigating the use of both killed and live vaccines to control *S. enteritidis* infection in poultry. Several thousand doses of aromatic deficient auxotrophic living *S. dublin* and *S. typhimurium* have been used parenterally in the United States. The effect of vaccination looks promising in field trials. The major problem encountered has been interference with vaccination from antimicrobial use by farmers.

In the German Democratic Republic the nationwide control programme against salmonella infections, including widespread application (i.e. complete vaccination in territories and production chains) of the live salmonella vaccines against *S. dublin*, *S. typhimurium* and *S. choleraesuis*, has resulted in a decrease of about 80% of the officially confirmed outbreaks of salmonellosis since 1980. The total number of salmonella isolations in the Veterinary Investigation Centres and especially the bacteriological findings of salmonellae during meat inspection in this period were reduced by an average of 30-50%.

Apart from zoo-sanitary and organizational procedures, immunization with live *S. typhimurium* vaccine is currently being investigated in the GDR as a means of control of *S. enteritidis*. Chickens (including goslings, turkey poults) are immunised orally via drinking water three times in the first 12-14 weeks of life. Preliminary results have shown protection against *S. typhimurium* and cross-protection against *S. enteritidis*.

Double vaccination of breeder goose and duck flocks (shortly before the laying period) is also being practised.

Preliminary results of oral vaccination of young poultry flocks are encouraging. Further epidemiological monitoring and widespread application, combined with other sanitary procedures, are necessary.

4.1 Salmonella virulence

Attention has been paid to the development of avirulent strains of salmonella, because of the cumulative evidence that live attenuated strains of salmonella are more effective than killed or subunit vaccines in inducing a protective immune response against infection by different species of salmonella in humans and animals.

Modern technologies such as transposon-insertion mutagenesis permit the construction of many kinds of salmonella mutants irrespective of the fact that their virulence-related characters are determined by plasmids, prophages or by chromosomal genes. Some mutants cannot survive within the macrophages but can confer protective immunity on host organisms.

Salmonella mutants may be useful, not only for better understanding of intracellular parasitism but also as vaccine candidates. Mutants are also needed for research and collaborative studies on salmonella virulence.

4.2 Improved serological diagnosis tests

One of the greatest obstacles in salmonella control has been the lack of a serological test to detect latent carriers.

The ELISA test developed by Smith et al at Davis, California, USA, for the serological diagnosis of *S. dublin* in dairy cattle has been found to be more appropriate than culture for the detection of 'latent' carriers (see Annex 2). The test can also be used for milk samples. ELISA can be automated and many samples can be rapidly analysed.

In the United Kingdom, ELISA tests are being developed for the serological diagnosis of *S. enteritidis* infection in poultry. ELISA tests are also being developed for the detection of antibody in egg yolk and the test is being evaluated for detection of carriers in infected flocks.

5. RESEARCH ON NEW SLAUGHTER TECHNOLOGIES TO REDUCE CROSS-CONTAMINATION

The overall quality of meat and edible by-products depends heavily on its microbiological quality. This is very dependent on the contamination level of the production equipment, as well as on the skin and intestinal microflora of animals and the technology and techniques used in slaughtering and processing.

The microflora of the skin is divided between (1) the "transient" flora dependent on handling hygiene and environmental conditions, and (2) the resident microflora consisting of microorganisms which are more or less constantly present.

In general, pathogenic bacteria on slaughtered animals are either part of the indigenous microflora of the animal, or the result of propagation on equipment, with subsequent cross-contamination.

The faecal microflora of animals for slaughter is by far the predominant source of pathogens present on the final dressed carcass. The pharyngeal microflora is another very important source. More than 20 species of bacteria, pathogenic to man and originating in pharynx of carcasses, can easily be listed.

Cross-contamination with pathogenic bacteria during slaughter is much less pronounced with carcasses of beef and sheep than with pork and poultry carcasses.

Spoilage bacteria on dressed carcasses will not be of human origin, but due to cross-contamination from environmental sources, e.g. contact with surfaces such as tables and tools.

Modern slaughter lines offer many risks of cross-contamination. Many risks could be eliminated by application of known techniques or the development or modification of techniques which are possible or at least manageable.

In addition to consumer expectations of nutritional and organoleptic properties, hygiene and "safety" have become important aspects of meat quality.

A system to ensure quality and safety must be designed for the entire chain of operations from the farm to the consumer. This includes transport of animals, holding the animals before slaughter, slaughtering of pigs, cattle, sheep, goats and poultry, and the unit operations in slaughtering, butchering, and processing.

Quality assurance means that potential microbiological problems are identified and control measures taken at all stages of the entire meat production chain.

Different types of microorganisms have to be taken into account in the identification of risks. Hazards may differ from country to country and vary with animal health, slaughter procedures, the types of meat products and the system of storage and distribution.

Taking the above into account, this evaluation deals mainly with three areas of activity: slaughter processes, monitoring key parameters and disinfection of tools and equipment.

A clear objective of the slaughter process is to produce meat from the carcass in such a way that the transfer of material and bacteria from the unclean parts of the live animal onto the final carcass is avoided.

The unclean parts of the animal are the outer surfaces of the live animal (skin or feathers) and the contents of the alimentary tract. Animals suffering from certain diseases may also contribute to microbial contamination.

Improved hygienic procedures at all stages of production and processing are an urgent priority to reduce the occurrence on carcasses and meat products of pathogenic microorganisms, e.g. Salmonella spp, Campylobacter jejuni, Listeria monocytogenes, Yersina enterocolitica and Staphylococcus aureus.

Slaughterline equipment plays a very important role in cross-contamination. Most spoilage bacteria propagate readily in the environment present in the slaughterhouse and constitute a constant, never-failing source of contamination of both carcass and organs.

Cross-contamination of carcasses with biological aerosols also play a role, e.g. high-pressure cleaning during processing leads to formation of aerosols, which carry contamination to meat surfaces.

The role of disinfection of tools and equipment in prevention of cross-contamination is extremely important. Disinfection should involve two steps, namely - cleaning and then disinfection itself. These procedures are defined in the Guidelines on Prevention and Control of Salmonellosis.^{1,2}

Some of the slaughter technologies, including those under development, may not decrease, but even carry increased risks of cross-contamination. Therefore, further research for improved procedures for decontamination of tools and equipment is important.

Improvements in the design and construction of slaughter equipment can make cleaning and disinfection easier and more effective. At present, machines and equipment are not always easy to clean and disinfect, e.g. polishing machine for pigs, evisceration machine for poultry, plucking machine, etc. Therefore, engineering and design of new slaughter equipment should take into account aspects of cleaning and disinfection.

Since the human factor has much influence on the effectiveness of cleaning and disinfection it is necessary to mechanize and automate the cleaning and disinfection procedures. Automated procedures would also permit more frequent cleaning and in a more standardized manner.

Disinfection of equipment and tools with hot water (82°C) is less effective than generally believed because protein denatures and the time elapsed is often too short to sanitize effectively. Although this temperature will kill most non-spore forming pathogenic and spoilage bacteria, bacteria are protected by organic matter and thus the effect of the disinfection is significantly diminished. More efficient disinfection methods are needed. Cleaning before disinfection with water at 60°C is necessary.

The list of chemicals used for cleaning and disinfection is long and varies from country to country. Further research is necessary on the most effective use of chemicals, as well as to develop more effective compounds.

The long-term solution is to design equipment that can be effectively "cleaned in place".

Due to the differences in slaughter technology, a distinction will be necessary between slaughter lines for pigs, cattle and poultry with respect to critical points of cleaning and disinfection.

Long-term research and its potentials for reduction of cross-contamination during the slaughter processes were described at a WHO Consultation held in Roskilde in February 1990. The initiative of WHO to form international teams for research on equipment, disinfection and monitoring during slaughter processes was endorsed.

5.1 Decontamination

Decontamination of carcasses (chemical or physical or a combination) and other methods for improving slaughter technology and hygiene, offers good prospects for the prevention of transmission of disease-causing microorganisms from animal to man. Chlorines, organic acids and alkalines in combination with chlorination may not lead to total elimination of the pathogens but marked reductions in microbial numbers (100-1000 fold or higher) can be obtained by these methods applied singly or in combination.

Decontamination procedures have been widely applied in poultry slaughter. Based on practical experience, the following procedures are recommended:

- (a) Chlorine in the chill systems at 10-50ppm. Higher levels may result in objectionable environmental conditions.
- (b) Alkalisng of scald tank waters to pH9 reduces the D_{52°C} of salmonellae from 35 minutes to 1.3 minutes.
- (c) Treatment of carcasses with 1.2% lactic acid at pH 2.0 before chilling (air chilling) reduces enterobacteriaceae counts.

A combination of alkaline and chlorine treatment decreases the salmonella count to a "zero level" in poultry carcasses.

6. INTERNATIONAL TECHNICAL COOPERATION

Many factors contribute to the increasing need for strengthened food hygiene programmes. However, the complexity of these factors and their interactions have multiplied to such an extent that it is almost impossible to design a generally valid system.

The control of a single zoonosis such as salmonellosis may fail or be hampered because many countries lack the resources to cope with all the components of the disease and to control the many factors involved from growth or production of primary food supplies to gathering, processing, transport, storage and distribution. Therefore, an intersectoral approach, international cooperation, and technical support become indispensable.

6.1 The role of international organizations

6.1.1 Food and Agriculture Organization of the United Nations (FAO). FAO's major function is to ensure the global provision of food of adequate quantity and quality. Different disciplines and services and divisions are involved in ensuring food safety from its production to its consumption.

6.1.2 International Office of Epizootics (OIE). The primary responsibility OIE is exchange of information about animal health between countries and the adoption of the International Zoo-sanitary Code.

6.1.3 United Nations Environment Programme (UNEP). This organization is involved in activities for prevention of food shortage and food contamination.

6.1.4 World Health Organization (WHO). WHO fosters a wide range of research activities, as described in this report. Comprehensive approaches along the food chain, education and training, and harmonization of legislation are areas dealt with jointly with FAO. Animal production hygiene, including slaughter technologies and the whole complex of food safety, e.g. processing industries, the retail trade and final food preparation, including consumer education, receive WHO's attention. The Organization provides advice on various aspects of food hygiene and publishes many documents on intersectoral and interprofessional cooperation.

WHO/FAO Collaborating Centres. These centres provide education and training, reference services, consultation, expertise, exchange of information, etc.

WHO regional and sub-regional VPH centres. These are the Pan American Zoonoses Center (Buenos Aires, Argentina) and the Mediterranean Zoonoses Centre in Athens, Greece. Their main objectives are: coordination of surveillance and control of zoonoses and foodborne diseases; collaboration in elaboration and implementation of national programmes; preparation of teaching material; establishment of panels of experts; cooperation with international organizations, especially with WHO/HQ (Veterinary Public Health) and its Regional Offices for the Americas (AMRO), for Europe (EURO) and for the Eastern Mediterranean (EMRO), FAO, the United Nations Development Programme (UNDP), UNEP, OIE and other governmental and nongovernmental institutions.

6.1.5 Other organizations and institutions. These include:

- World Veterinary Association; and
- World Association of Veterinary Food Hygienists.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

7.1.1 Salmonella-free feed may be produced with strict hygiene at feed compounders' premises, including the maintenance of the required temperatures in the various phases of pelletizing processes and the avoidance of recontamination of the pellets.

7.1.2 Animal husbandry systems designed for rearing pathogen-free animals may require transition towards confinement from extensive free-range husbandry systems.

7.1.3 Since many measures for salmonella control apply to other foodborne pathogens, such as campylobacters, they may reduce the risk of infection by these organisms. However, further research is urgently needed on the elimination of campylobacter species from animals, since this infection is one of the most frequent causes of acute enteritis in humans in many countries.

7.1.4 Immunization of young animals with living auxotrophic Salmonella vaccines under controlled conditions can significantly reduce the number of infected animals and faecal shedding of virulent salmonella. Vaccination of laying hens as young chicks shows great promise in reducing the number of contaminated and infected eggs. Vaccine strains can be readily differentiated from field strains.

7.1.5 A combination of strict sanitation and immunization with live vaccine reduced outbreaks of salmonellosis in livestock to 20% of the former levels between 1980 and 1988 in the GDR. The total number of isolations at slaughter were reduced to 33% of the former levels in calves and to 48% in pigs. In many cases salmonella serotypes important for man and/or animals were successfully eliminated from herds or flocks.

7.1.6 The identification of active and latent carriers for removal is possible through use of enzyme linked immunosorbent assay (ELISA) which detects persistently elevated serum antibody levels for S. dublin in cattle. Milk antibody levels can be used for initial screening for S. dublin carriers in dairy cattle. Latent carriers (not shedding salmonella in faeces or in milk) usually occur with host-adapted serotypes and can only be detected by serology.

7.1.7 Immunization and removal of carriers must be accompanied by environmental decontamination and application of sanitary measures.

7.1.8 Reintroduction of carrier cattle must be prevented. New livestock should be tested by faecal culture and serology for persistently elevated salmonella antibody levels before introduction into a herd.

7.1.9 Continuous epidemiological monitoring and surveillance is essential to identify infected herds/flocks and to evaluate the long-term efficacy of the above control steps.

7.1.10 Further studies are needed regarding the fundamental mechanisms of salmonella virulence and immunity. The antigens responsible for eliciting protective immunity must be defined; the ideal immunizing agent may be an inactivated sub-unit vaccine.

7.1.11 Although it appears unlikely that the measures available today will result in the complete elimination of salmonella infection in livestock in the near future, the combination of various interventions along the food chain will markedly reduce the occurrence of salmonella and thus the incidence of human infection. The impact of various measures taken in animal production are summarized in Table 1.

7.2 Recommendations

7.2.1 Presently available animal health schemes should be carefully appraised for identification, protection, and maintenance of salmonella-free animal husbandry.

Specific pathogen-free (SPF) systems should be applied to establish salmonella-free swine and poultry production. Repopulation with SPF animals can contribute to development of salmonella-free herds and flocks as a basis of salmonella-free livestock production and salmonellosis control.

Other alternative methods, such as colostrum-deprived, isolated animal rearing systems and related technologies are also recommended in order to attain salmonella-free animal husbandry.

7.2.2 Since raw materials and feedstuffs play an important role in the epidemiology of salmonella infection in agricultural animals, it is recommended that WHO, FAO, UNEP and OIE elaborate international requirements (guidelines) for hygienic production of feedstuffs, their storage, transportation and microbiological control.

7.2.3 All feed for poultry should be pelletized, using hygienic procedures and adequate temperatures.

7.2.4 To prevent the re-introduction of salmonella in herds and flocks requirements should be specified for physical protection, disinfection and non-specific measures such as competitive exclusion (the Nurmi concept - see Annex IV of document WHO/CDS/VPH/89.82),¹ the use of probiotics, acidification of feed, addition of sand to chicken feed, and use of lactose and other sugars.

7.2.5 The economic and social costs of raising salmonella-free animals should be estimated more accurately.

7.2.6 The existing requirements for hygienic slaughter of animals, reflected in the documents of the Codex Alimentarius Commission (GMP for fresh meat, processing meat and poultry) will not lead to total elimination of salmonella in the final product and/or fully preclude a cross-contamination of carcasses. Proposals should therefore be elaborated for new hygienic requirements and regulations.

7.2.7 In addition to the requirements existing in the GMP marked reduction in the number of viable microorganisms can be achieved in poultry by the following procedures:

- chlorine in the chill systems at 10-50 ppm. Higher levels may result in objectionable environmental conditions;
- alkalisng of scald tank waters to pH9 can reduce the D52°C of salmonellae from 35 minutes to 1.3 minutes;
- treatment of carcasses with 1.2% lactic acid at pH 2.0 before chilling (air chilling) reduces enterobacteriaceae counts.

National authorities are urged to investigate the effects of alkaline treatment in combination with chlorination which was reported to decrease counts of vegetative forms of pathogenic organisms to zero level.

7.2.8 Future research is needed to develop rapid methods suitable for early detection of salmonella on-line, as well as new technological approaches to minimize cross-contamination of carcasses (e.g. during evisceration of animals, scalding, plucking and chilling of poultry, etc.).

7.2.9 FAO and WHO should elaborate international guidelines on cleaning and disinfection (including heat and steam treatment) for slaughterhouses and meat processing plants to harmonise the knowledge and experience in different countries. These guidelines might include both general and specific aspects, e.g. the frequency of cleaning and disinfection, recommendations on the best disinfectants, control of disinfection efficiency, etc.

7.2.10 Slaughter of animals from farms identified as salmonellosis-free should be carried out separately in space and time from other animals. Care should be taken that the separation applies along the whole production and slaughter line. This requires certification of origin and former status as well as identification of animals along the slaughter processes.

7.2.11 National authorities and industries are recommended to support research as specified by the WHO Consultation on Research on New Slaughter Technologies to Reduce Cross-Contamination, February 1990 in Roskilde, Denmark.⁹

7.2.12 Systems and procedures should be investigated for licensing hygienic aspects of slaughterhouse equipment, including automatic machinery.

7.2.13 WHO should gather a group of experts to elaborate harmonized procedures of specimen collection, certification, laboratory tests and reporting. This may preferably be done in conjunction with the establishment of zoo-sanitary requirements (OIE), and requirements for trade with raw food of animal origin (Codex Alimentarius).

TABLE 1. Reduction of Salmonella Incidence and Contamination Rates in Animal Production by Presently Available Procedures⁽¹⁾

	Poultry		Pigs	Cattle	Sheep
	broilers	eggs			
Breeding	95%, 5-10 years countrywide	>95%, 2-3 years countryw.	95%, 5-10 years countryw.	20%, countryw.	
Control (test and slaughter)	95%, 5-10 years Countryw.	95%, 2-3 years Countryw.		Carrier control ⁽²⁾	
Vaccinology (2-3 sero- types only) years		100%, 1 year 2 years per district (in humans)	90%, countryw. per farm	66%, countryw., > 5 years	> 5
Slaughter	100% 99% immediate				

- 1) Expressed by percent reduction.
- 2) Control in feedstuff only.

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- ¹⁰Forty-second World Health Assembly, 1989: Prevention and control of salmonellosis, WHO42.40.
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- ¹²Guidelines on Prevention and Control of Salmonellosis (WHO document VPH/83.42).

ANNEX 1

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ANNEX 2

SUMMARY OF REPORT OF THE WHO WORKING GROUP MEETING ON SALMONELLOSIS
IMMUNIZATION IN ANIMALS, Orvieto, Italy, 8 April 1990.

The heads of the working groups met to review the work completed and to plan further collaborative studies. Plans were also made for the next scientific meeting on Salmonellosis Control in Agriculture, to take place in Jena, German Democratic Republic, June 1991.

1. Salmonella virulence and basic mechanisms of immunity to Salmonella

Using transposon-insertion mutagenesis, two genetic regions for serum resistance and mouse lethality had been identified in an 80kb virulence plasmid of *S. dublin*. The plasmid gene for serum resistance was found to be closely involved with the expression of the neutral sugar composition of the *S. dublin* lipopolysaccharide. A strain of *S. dublin* with both serum resistance and mouse lethality genes inactivated by transposon mutagenesis that shows reduced virulence may be an appropriate live candidate vaccine.

Epidemiological investigations on pathogenicity factors in salmonella had been undertaken and mannose-resistant fimbriae had been detected in 10 and 20% of strains isolated from swine and cattle respectively.

2. Improved serological diagnostic tests

The ELISA test, developed at Davis, USA, for the serological diagnosis of *S. dublin* in dairy cattle has been found to be more appropriate for the detection of 'latent' carriers than culture. The test can also be used for milk samples and automation will allow the rapid analysis of many samples. Members of the team at Davis, USA, will visit Jena in late 1990 to oversee the test evaluation and the Jena team and WHO will organize a training course in June 1991.

In the United Kingdom, ELISA tests are being developed for the serological diagnosis of *S. enteritidis* infection in poultry. ELISA tests are also being developed for the detection of antibody in egg yolk and it is hoped that this test could be used to detect infected flocks.

3. Salmonella vaccines

Results of studies with a live oral auxotrophic vaccine for the control of salmonellosis in poultry were reported. The results had been encouraging and in one district its use in all young laying birds was associated with a decrease in the prevalence of human infection. It is currently mandatory to immunize all young laying birds in the GDR.

In the UK, a number of groups are investigating the use of vaccines to control *S. enteritidis* infection and at the Central Veterinary Laboratory both killed and live vaccines are being evaluated.

4. Information exchange

A summary of the groups' work and pertinent references had been circulated. It was agreed that an annual review should be produced by the Central Veterinary Laboratory, Weybridge, UK.

5. Surveillance

Bearing in mind that surveillance and epidemiological data are needed to actively monitor control programmes, it appears essential that:

- (i) regulations are produced which ensure the collaboration of the farmer and reward him for salmonella control, and
- (ii) practices are established which would include worker training and education programmes.
