



REPORT OF THE WHO WORKING GROUP ON SALMONELLA  
IMMUNIZATION IN ANIMALS

Jena, Germany, 17 - 21 May 1991

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Les opinions exprimées dans les documents par des auteurs cités nommément n'engagent que lesdits auteurs.

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## 1. INTRODUCTION

Animal production hygiene has, in recent years, become an area of research and practice with new orientations. Remarkable influences, stimulating a whole series of national and international activities, originate from two major sources, namely increasing incidences of human infection related to food of animal origin and the demand for new managerial processes of food quality assurance.

The occurrence of salmonella infection in people and its control can be considered as the dominant problem in animal production hygiene, although other infections may have acquired similar significance in some countries. A number of WHO consultations have dealt with such human diseases due to pathogens which may not be detected easily in the live animal and escape meat and fish inspection. In 1988 the WHO Expert Committee on Salmonellosis Control stressed the role of animal product hygiene, and a WHO consultation dealt with the specific aspects of systems research and approaches in this difficult field of intersectoral and interdisciplinary cooperation. Guiding principles for the management of surveillance and control activities in such complex systems have been issued to assist national veterinary public health services.

Following a resolution of the World Health Assembly from 1989 (WHA 42.40) member states provided support to several international research teams for the development of guidelines for the prevention of infection in humans and animals. The significance of international cooperation was underlined by a special meeting of Chief Veterinary and Public Health Officers of international organizations, who met in Ottawa in 1990.

The rapid progress in immunization research was discussed at the meeting which is the subject of the following report. The participants (ANNEX I) paid particular attention to the planning of further research and of comprehensive pilot projects integrating vaccination in a system of other measures of hygiene and specific disease control.

Dr K. Bögel welcomed the participants on behalf of Dr H. Nakajima, Director General of the World Health Organization, and he expressed his gratitude for the continuing cooperation of leading institutions in so many countries. In particular he thanked the representatives of FAO and OIE for their cooperation and he congratulated Prof. H. Meyer on the recent designation of his institute as WHO Collaborating Centre for Reference and Research on Bacterial Zoonoses. In this connection Dr. Bögel thanked the representative of the German government Dr H. Pittler, for the invaluable support the research teams and WHO received over so many years from this country.

Professor H. Meyer was elected Chairman and Professor B.P. Smith, Vice-Chairman; Dr T. Blaha and Dr A. Koulikovskiy agreed to act as Rapporteurs.

## 2. ADVANCES IN RESEARCH

The following summaries of papers read by 17 scientists from seven countries (Czechoslovakia, Germany, Japan, Poland, United Kingdom, USA and USSR) and the results of the discussions at the 3rd meeting of the WHO Working Group on Salmonella Immunization in Animals provide an overview on the advances in salmonellosis research in the fields of salmonella virulence, diagnostics and vaccination.

## 2.1. Vaccine development for poultry

*Salmonella enteritidis* Aro A vaccines have been shown to be safe in chickens when injected by the intravenous route. Oral vaccination of chickens at seven-day intervals until 21 days of age conferred significant protection against intravenous challenge and reduced faecal shedding following oral challenge. Serum antibodies to lipopolysaccharide antigen were detected by the ELISA test only in those birds immunized with the higher dose.

Heat shock proteins are produced as a protective response against stress. Recent work on *S. enteritidis* mutants with altered heat shock protein genes indicates that the oral administration of the candidate vaccine strain confers considerable protection against oral challenge but not against intravenous challenge.

An experimentally inactivated *S. enteritidis* vaccine containing  $10^{11}$  organisms in an oil adjuvant showed that it provided good protection against intravenous and intramuscular challenge with large doses of a virulent field strain. However, *S. enteritidis* was recovered from the internal organs, including the ovaries of a number of immunized birds. Birds on a number of poultry farms have been vaccinated at day 1 of age and 4 weeks later. They were then transported to the laboratory at 8, 12 and 16 weeks of age and challenged intravenously with  $10^8$  *S. enteritidis*. Preliminary results indicate that protection levels of 56-70% have been achieved, although in some cases much lower levels were apparent.

## 2.2 Advances in prevention and control of salmonellosis in cattle and poultry in Czechoslovakia

Safe and immunogenic attenuated vaccines from defective mutants of *S. typhimurium* have been developed for active immunization of calves and poultry. The characteristics of vaccine strains obtained by induced mutagenesis with nitrosoguanidin are the following: absence of galactose utilization, auxotrophy, resistance to streptomycin (500 - 1000 u/ml), extension of generation time and low virulence for C57BL/6 mice after i.p. administration. Both vaccine strains contain one 90-kb plasmid. Peroral immunization with  $10^7$  cells per ml induced local immunity and systemic cell-mediated immunity (CMI) detectable after 2 - 5 days. Cross immunity was demonstrated with the antigens of *S. enteritidis* and *S. dublin*. The vaccination increased the resistance of animals, reduced the development of infection and accelerated elimination of salmonellae from tissues. No significant increase of specific agglutinin levels in blood was recorded. The strains did not penetrate into organs and were detectable only sporadically in feces after concentration.

For passive protection in young calves, a biological substance has been developed consisting of serum and ultrafiltrate of disintegrated bovine lymphocytes collected from cattle immunized with selected inactivated salmonella strains in complete Freund's adjuvant. Parenteral administration of the biological preparation transfers specific humoral and CMI persisting for one month. The protective immunity increases phagocytosis, values of the leucocyte migration inhibition (LMI) test, and prevents death in calves challenged with  $LD_{50}$  salmonellae.

A constant problem in the control of salmonella infection is the lack of available immunological methods for checking the postinfection and postvaccination immunity. For detection of specific CMI the delayed type hypersensitivity test (DTH) has been used. An antigen prepared from a purified and concentrated mixture of porin proteins showed lowest toxicity and highest specificity in DTH in mice, guinea pigs, hens and calves. A high degree of correlation was found in calves between the values of the intradermal test and the LMI test.

### 2.3 systematic control of salmonella infections in livestock using a complex immunization programme

Intensive control measures from 1980 to 1989 resulted in an 85% reduction of the incidence of salmonellosis in livestock of the former GDR, from 1007 outbreaks in 1980 to 150 outbreaks in 1989. Simultaneously, the number of salmonella isolations from carcasses, varying between the different animal species decreased to approximately 50%. These good results were obtained by means of sophisticated control programmes, the inseparable components of which are hygienic, organizational and immunoprophylactic measures.

The immunoprophylaxis is carried out with three live vaccines based on attenuated, auxotrophic mutants against *S. dublin*, *S. choleraesuis* and *S. typhimurium* and, in adult cattle, with two formalin-inactivated adjuvant-adsorbed vaccines.

However, during the same period salmonella infections in man increased considerably, in spite of the fact that infections of animals by *S. typhimurium*, *S. dublin* and *S. choleraesuis* decreased. This increase was mainly due to *S. enteritidis*. Analysis of the salmonella isolations from livestock showed that *S. enteritidis* also increased markedly in animals. For *S. enteritidis* infections, contaminated eggs and chicken carcasses played an important role.

The basic conclusion drawn from all results obtained from 1980 to 1989 in the former GDR is that it is possible to decrease the prevalence of host-adapted salmonella serovars such as *S. dublin* in cattle and *S. choleraesuis* in pigs, when hygienic, organizational and proper immunoprophylactic measures are combined. By means of these measures it is even possible to reduce the prevalence of salmonella serovars (e.g. *S. typhimurium* and *S. enteritidis*) which occur in several animal species and in man.

It has to be emphasized that these favourable results in cattle and pigs were obtained under the conditions of a government-controlled agriculture and a centralized veterinary service of the former GDR. The present task is now to evaluate to what extent these experiences can be made use of within the market economy.

Since salmonellosis of man can be caused by various salmonella serovars, e.g. *S. typhimurium* and *S. enteritidis*, the question of cross immunities is of very great interest. Orally administered live vaccines show great promise of offering cross protection.

In calves, the orally applied *S. typhimurium* live vaccine shows a very good effect against an oral *S. dublin* challenge and the orally applied *S. dublin* vaccine against an oral challenge with *S. enteritidis*.

However, none of the possible cross-protections can be accurately theoretically predicted. Every combination has to be experimentally considered.

The question of whether the shedding of salmonellae in animals can be decreased (number of bacteria and duration of shedding) via immunization is of great importance in the framework of the control of salmonellosis, especially in poultry and calves. In some trials it could be demonstrated that the excretion of *S. dublin* and *S. typhimurium* by calves immunized orally with the live vaccines was lower and of shorter duration than those by the non-immunized animals and, in most cases, than those by calves immunized parenterally with the inactivated vaccine.

The results presented show that live vaccines not only ameliorate the course of salmonellosis in calves, but they also decrease the amount and duration of salmonella excretion. This explains why live vaccines, in contrast to inactivated vaccines, are able to contribute to a real decrease in prevalence of salmonellae in livestock.

The influence of the 60-MD virulence plasmid in the vaccine strain of the *S. typhimurium* live vaccine was examined. In an immunization and challenge experiment with calves, it could be shown that the immunogenicity (efficacy) of the vaccine strain remained after its 60-MD plasmid had been deleted.

#### 2.4 Evaluation of the efficacy of an *S. typhimurium* live vaccine in poultry

Chickens were immunized orally with the *S. typhimurium* live vaccine at the 4th, 6th and 7th week of age. The vaccine recipients, together with a control group, were parenterally (i.m.) challenged during the 3rd, 7th and 12th weeks after the last immunization with virulent *S. typhimurium* and *S. enteritidis* strains.

The course of infection was monitored by:

- (i) Clinical examination (morbidity and mortality)
- (ii) Bacteriological examination:
  - (a) cloacal swabs
  - (b) Organs from dead and euthanized chickens.

Results:

- (1) In comparison to the control group, immunized chickens were protected against a homologous challenge until the 12th week after the last immunization.
- (2) Cross-protection against *S. enteritidis* was seen until the 7th week after immunization. At the 12th week post vaccination, no differences between immunized and control groups were found.
- (3) Oral immunization reduced the faecal excretion and the persistence of the parenterally administered challenge strain. Using a *S. typhimurium* live vaccine, it is possible to induce a cross protection against *S. enteritidis*. The reduction of faecal salmonella shedding leads to a reduction of the wild strains in the flock.

## 2.5 Experimental oral infection of chickens with salmonellae as a model for testing efficacy of immunization

Models for the experimental oral infection of chickens with epidemiologically relevant salmonella serovars were developed. Criteria for the evaluation of the experimental infection were the examination of the numbers of salmonellae in liver, spleen and caecum at weekly intervals after infection. Using virulent *S. typhimurium* and *S. enteritidis* strains a challenge dose of  $10^2$  cfu in 1-day-old chickens,  $10^9$  cfu in 8-day-old chickens and approximately  $10^{10}$  cfu in 4-week old chickens is suitable to induce a pronounced infection. The course of infection with various salmonella serovars and strains showed reproducible characteristics.

Differences in the ability of several salmonella strains to colonize the caecum and to invade liver and spleen can be demonstrated by the infection of 1-day-old chickens with  $10^2$  cfu.

The immunization of broiler chickens using a *S. typhimurium* his<sup>-</sup>pur<sup>-</sup> mutant live vaccine leads to a limited and short-lasting protection against homologous and heterologous (*S. enteritidis*) challenges.

## 2.6 Specific prophylaxis of salmonella in poultry

In the USSR wide-scale trials were conducted with a live bivalent vaccine for *S. typhimurium*/*S. dublin*. For this purpose, the ribosomal mutants of these bacteria were used on the infected poultry production unit. For reasons of comparison, simultaneously, inactivated formalin aluminium hydroxide adjuvanted bivalent vaccine (*S. enteritidis*, *S. typhimurium*) have been used. The total number of vaccinated birds was around 65 000 and non-immunized (control) around 38 000. The results of these experiments have revealed that live vaccine significantly decreased the shedding of salmonella by infected poultry (by 6 times, in comparison with unvaccinated birds, and by 4 times in comparison with inactivated vaccine).

## 2.7 Genetic approach for the construction of salmonella vaccines

In the USSR a genetic approach for vaccine construction was developed. Good results under laboratory conditions have been achieved with the mutants of *S. typhimurium* generated by insertion of transposon Tn-10 from *E. coli* pTH-18. One of them, with a defect in the outer membrane of the cell wall, was studied in detail (immunogenicity, virulence, resistance to antibiotics, etc.). It differed significantly from the parent strain in virulence (4 to 5 log order less), and it was sensitive to different antibiotics. It persisted in mice for 10 days. The mutant could not actively develop biofilms which could play an important role in protection of the bacterial population from different abiotic factors and antibiotics. This mutant is considered a good candidate for vaccine production. However, further research including field trials is necessary.

## 2.8 Mammary gland vaccination against calf salmonellosis: Immunological memory, detectability of specific Ig-classes in serum and faecal samples of calves, and the effect of an adjuvant on the cow's antibody response

The necessity to protect calves by colostral antibodies is based on three facts:

- (1) Calves are born without immunoglobulin (Ig);
- (2) Neonatal calves are not completely immunocompetent for about 3 weeks
- (3) Calf mortality is highest within 2 weeks after birth and about 80% of calves which die during this period have low Ig-concentrations.

Mammary gland vaccination at ablation (dry period) was introduced to provoke specific salmonella IgG, and additionally IgA, and IgM antibodies.

In a previous study it was shown in challenge infected calves from vaccinated dams that a good correlation existed between G-fixing specific IgG1 and protection. Cows having been vaccinated in this way produced a long lasting immunological memory.

In contrast to non-specific Igs, specific Igs persisted in the calf for considerably longer periods of time. This applied to serum antibodies and to fecal antibodies. The application of an emulsion of the vaccine with an oily antibiotic preparation as it is normally used for intracisternal infusion at ablation provided a higher antibody response.

#### 2.9 ELISA for the detection of S. typhimurium antibodies in poultry

An ELISA for measuring the IgG and IgM antibody response in chickens which had been vaccinated and infected with S. typhimurium has been established. Only IgG (or IgY) antibodies were detected in dried egg preparations from hens which had been vaccinated against salmonellosis, and the same was true for chicks from immunized hens. In these chicks, antibodies declined to non-detectability between day 9 and 15 after hatching.

Birds vaccinated with the live S. typhimurium vaccine in different ways produced high anti-IgG and IgM antibody levels over prolonged periods. A differentiation between infected and vaccinated chickens on the one hand, and non-vaccinated, but infected ones on the other, was not possible by the test system applied.

The detection rate of infected chickens by the IgG ELISA was high and makes this technique a valuable diagnostic tool. The crude S. typhimurium used did not allow for a differentiation between S. typhimurium and S. enteritidis antibodies. In this context, the question about practical requirements regarding serovar-specificity must be posed.

#### 2.10 Characterization of the virulence regions in the plasmids of three salmonella live vaccine strains

The virulence plasmids of three live salmonella vaccines (S. typhimurium, S. dublin and S. choleraesuis) were analyzed in comparison to their parental strains. The complete agreement of all features of the strains examined indicates that no structural or functional changes in the virulence-encoding region of the plasmid of these vaccine strains could be found, and that these plasmids might be functionally fully intact. Taking into account the potential risks linked with the presence of virulence plasmids in salmonella strains, a requirement for future live salmonella vaccines should be freedom from such plasmids.

### 2.11 Toxicity of salmonella strains isolated from swine, calf and chicken

Many of the investigated salmonella strains isolated from swine and calf showed toxic activity in dermatotoxicity tests and rabbit ileal loop tests. When using suckling mouse tests no salmonella strain was found to produce the thermostable enterotoxin.

The chicken fibroblast test was found to be useless for the detection of toxin-positive strains. Several *S. enteritidis* strains isolated from chickens produced substances which exerted toxic effects on Vero and Chinese hamster ovary (CHO) cells.

### 2.12 Prevalence of salmonella virulence plasmids in isolates of defined sources

It is well accepted that in certain salmonella serovars large plasmids are necessary to cause disease in mice. The role of these plasmids for man and livestock, however, is still unclear.

An epidemiological study on salmonella isolates from defined sources was performed, using DNA hybridization with a virulence plasmid-derived gene probe. The data showed that almost all isolates from organs of infected animals contained the virulence plasmid whereas only 50% of the strains from asymptomatic excretors were virulence plasmid carriers. The data may indicate the importance of the virulence plasmid for causing systemic infections in man and livestock.

### 2.13 Some aspects of salmonella virulence and diagnostics

Virulence factors of salmonella:

- invasiveness and intracellular parasitism
- endo- and enterotoxins
- adhesion by fimbriae and OMP
- iron binding
- serum resistance.

The mechanisms of adhesion, invasiveness and intracellular parasitism (fimbriae, OMP, flagellae) should be studied. Type 1 fimbriae are common in many serovars and strains. Little information is available about mannose-resistant hemagglutination.

Heat-labile enterotoxins are also common, but we should compare their prevalence in host-adapted and non-adapted serovars and differentiate between cytotoxic and cytotoxic factors.

The host cell-mediated expression of bacterial virulence factors (enterotoxin production and release, invasive proteins) remains to be elucidated.

### 2.14 Virulence of *S. choleraesuis* mediated by a 50-Kb plasmid

- (1) The 50-Kb plasmid carried by *S. choleraesuis* is its virulence plasmid.
- (2) The 6.4 Kb portion of the 50-Kb plasmid is essential for host bacteria to cause bacteremia in mice.

- (3) Five open reading frames were found within the 6.4-Kb region.
- (4) Positive and negative regulator genes (mba 2 and mba R, respectively) were identified.
- (5) Four proteins were identified within the 6.4-Kb region (32 K, 70 K, 29 K) by SDS-PAGE.
- (6) The function of these proteins is not known yet. Some of them play an important role to protect the organism from killing by defensin or lysosomal enzymes of macrophages.

#### 2.15 Detection of enterotoxigenic activities among salmonella strains of bovine origin

Most salmonella strains investigated were found to be able to produce and release substances which induced positive reactions in the various test systems for E.coli heat-labile toxins. However, identical reaction patterns in the different assays induced by salmonella supernatants and E.coli preparations do not mean that the toxins produced are identical.

#### 2.16 Assessment of the importance of the virulence plasmid for the pathogenesis of salmonellosis in calves

The oral application of a mixture of equal quantities of two salmonella strains provides the possibility to compare their invasiveness in calves under identical host conditions. The comparison of isogenic *S. typhimurium* strains revealed that the presence of the virulence plasmid was accompanied by higher invasiveness. A comparison of isogenic *S. enteritidis* strains, however, showed that the presence of the plasmid is not always an inevitable condition for virulence of salmonellae in calves.

#### 2.17 Vaccination against salmonella in poultry and the use of ELISA technology for serological detection of infection

##### Vaccination

Strong immunity against oral infection with *S. typhimurium* was produced by oral or intramuscular infection with a fully virulent strain of the same serovar. Two mutants, one rough and an aro A strain were also prepared and tested. Neither strain was very effective in preventing faecal excretion of the parent challenge strain when injected intramuscularly. The rough strain but not the aro A strain produced good protection when administered orally. Both strains were not virulent for chickens or man and were not excreted extensively in the faeces.

An aro A rough mutant of *S. enteritidis* and the 9R *S. gallinarum* vaccine were tested for protection against systemic infection of laying hens with a phage type 4 strain of *S. enteritidis*. The 9R strain but not the aro A rough mutant reduced colonization of the liver, spleen and ovary. Both vaccines reduce the isolation rate of the challenge strain from laid eggs.

A virulence-plasmid cured mutant of *S. gallinarum* proved to be an effective vaccine against fowl typhoid. It was less virulent than the standard 9R strain.

## ELISA

An indirect ELISA has been developed for *S. typhimurium*, *S. enteritidis* and *S. gallinarum* based on LPS and flagellar antigens. The ELISA can detect infection in the absence of bacteriological excretion. Using different antigens, infection with different strains can be differentiated. Sera or blood can be collected and dried on paper. Such preparations can also be treated with disinfectants such as phenol vapour. Good correlation has been obtained with the ELISA between serological and bacteriological results for field samples. The ELISA is proving to be a valuable screening tool for these serovars with many advantages over current serological procedures.

### 2.18 Monoclonal antibodies as a potential tool for the epidemiology, diagnosis and prevention of salmonellosis

28 cell lines producing different monoclonal antibodies against various salmonella antigens have been prepared. Among them are antibody preparations which cover the LPS determined O-antigens of *S. typhimurium*, *S. enteritidis*, *S. dublin* and *S. infantis* which are the predominating serovars in many countries. Other antibodies are directed against the lipid A portion of the LPS or against outer membrane proteins. One antibody seems to be specific against the genus *Salmonella* and does not react with other Enterobacteriaceae.

These monoclonal antibodies can be used for the following purposes:

- (1) Speed up diagnostic procedures by making use of the unique binding characteristics of the monoclonal antibodies, e.g. ELISA, with magnetic Latex particles.
- (2) Subgrouping of frequently encountered serovars using antibodies which detect variable minor antigens on some of the strains.
- (3) Passive protection of livestock in the first days of life.

### 2.19 Serological detection of Salmonella dublin infection in cattle

Observation and sampling of uninfected, acutely infected, vaccinated, and mammary and faecal *S. dublin* carriers over extended periods of time, followed by extensive culture of tissues at necropsy, has shown culture to be less sensitive than ELISA for antibody at detecting mammary or faecal carriers. Carriers shed *S. dublin* in milk from 2.2% to 45.7% of samples, and in feces from 3.4% to 17.6% of samples. ELISA titers for IgG antibodies remained consistently positive in carriers and negative in uninfected controls. Previous beliefs that positive serological results were false positives were based on very limited numbers of cultures, and negative cultures were actually false negatives.

### 3. MAJOR CONCLUSIONS AND RECOMMENDATIONS

#### 3.1 Salmonella vaccines

##### CONCLUSIONS

- (1) It is possible to decrease decisively host-adapted serovars such as *S. dublin* and *S. choleraesuis* by use of vaccines in combination with other methods, such as improved hygiene and management.
- (2) It is probably not possible to eradicate non-host-adapted serovars by use of vaccines, although vaccines may be used to reduce the level of infection in flocks/herds.
- (3) Poultry vaccines may be effective in breeder and layer flocks, but are less likely to be effective in broilers at the present time.
  - a) Vaccines can reduce the transmission of infectious agents from breeder to hatchery.
  - b) Maternal immunity to progeny may also decrease salmonella shedding after oral challenge.
- (4) In animals, vaccines will reduce faecal shedding, reduce the incidence of carriers and infection of internal organs. Better results are being obtained with live vaccines.
- (5) The use of live vaccines has been experimentally shown to reduce salmonella contamination of eggs.
- (6) Oral immunization of calves with attenuated vaccines will decrease mortality, morbidity and salmonella shedding.
- (7) Live salmonella vaccines can provide cross protection against other serovars.
- (8) Millions of doses of live salmonella vaccines have been used in a number of countries for many years without adverse effects to animals and human health.
- (9) Laboratory tests have shown that mammary gland vaccination of cows can protect calves against an early infection and that long lasting immunological memory is provoked by this vaccination regime.
- (10) Recently, monoclonal antibodies have been used to protect mice against salmonellosis and domestic farm animals against other bacterial diseases.

##### RECOMMENDATIONS

- (11) Further work to develop tests to determine the efficacy of salmonella vaccines should be supported.
- (12) Further studies to develop experimental challenge models for poultry should be supported.

- (13) Salmonella vaccination programmes in poultry must be accompanied by proper flock monitoring which includes serological testing and antigen detection and must also be compatible with control schemes for other diseases.
- (14) Vaccination programmes should be compatible with government control schemes.
- (15) Vaccine strains should include markers to distinguish them from field strains and possibly to distinguish their serological response from natural infection.
- (16) Work on the protective effects of monoclonal antibodies in livestock should be initiated. (Drs Schwarzer and Helmuth will cooperate in this field).
- (17) Experiments should be undertaken to determine the efficacy of intramammary vaccination against salmonellosis in infected farms.
- (18) Work should be carried out to determine the efficacy of combined competitive exclusion (probiotics) and vaccination.
- (19) Mutations to reduce virulence in live vaccine strains should be deletions with a low reversion to virulence.
- (20) Potential candidates for live vaccines should be tested for susceptibility against growth promoters and other prophylactic feed additives.
- (21) Vaccines should be checked for protection against other serovars, including those which are less invasive.
- (22) Further research efforts on subunit and synthetic vaccines should include the identification of the antigens which stimulate immunity and protection as well as using an empirical approach.
- (23) Further work should be undertaken on adjuvants and immuno-regulators.

### 3.2 Salmonella virulence

#### CONCLUSIONS

- (1) Various salmonella serovars exhibit biological activities in assays developed for detection of Escherichia coli and Vibrio cholerae toxins.
- (2) The serovar-specific plasmids in *S. typhimurium*, *S. enteritidis*, and *S. choleraesuis* are one virulence determinant to cause systemic infections in livestock.
- (3) Three live vaccines (against *S. typhimurium*, *S. dublin*, *S. choleraesuis*) used in the territory of eastern Germany have proved to contain the virulence region on their plasmids.
- (4) A system to assay salmonella virulence factors under identical host conditions in livestock (strains are given to one individual) has been established.

## RECOMMENDATIONS

- (5) Biological activities in assays developed for the detection of E. coli or Vibrio cholerae toxins were also detected in various salmonella serovars. Further research should be performed to investigate if these activities correlate with the pathogenicity of salmonella and to investigate their genetic background.
- (6) The composition and structure of the bacterial envelope and on its interaction with host cells should be studied further. The role of biofilms formed by bacterial populations for the resistance, survival, virulence, adhesion, pathogenicity and immunogenicity of salmonellae should be clarified.
- (7) The importance of the serovar-specific plasmids for virulence in livestock and man, and their role in various serovar-host combinations should be studied.
- (8) The use of DNA probes for monitoring salmonella serovars and their virulence plasmids should be intensified.
- (9) More research on the chromosomally encoded virulence genes is necessary. (Dr Helmuth will prepare a protocol on further processing for WHO).

### 3.3 Salmonella diagnosis

#### CONCLUSIONS

- (1) Of the serological tests evaluated, ELISA appears to have the greatest advantages and fewest disadvantages.
- (2) Monoclonal antibodies against important O antigens, proteins and other components of salmonellae have been developed. They can be used for diagnostic purposes by subgrouping frequently encountered serovars or using them in latex agglutination; coupled to magnetic beads they might be the basis for new diagnostic methods.
- (3) Disadvantages of serological tests compared to culture are that they may not detect some infected animals which have not yet produced antibodies. There may be false-positive results in some animals which have previously been infected but are no longer infected, and there may be some cross-reactivity with other salmonella serovars. In spite of these problems, serological tests such as ELISA are useful in control programs because they can be made sensitive and specific, ELISA can detect animals not shedding enough salmonella to be detected by culture at the time of sampling, and ELISA can be automated so that many samples can be run rapidly and economically.
- (4) Serological tests such as IgG-ELISA can be used for detection of infected flocks using serum or egg yolk. The number of sera to be examined should be according to standard epidemiological charts taking into consideration the level of infection considered significant in the particular control programme.

- (5) The use of paper disc tests with a spot of dried blood should be encouraged as a means of collecting, transporting and storing blood for serological tests.
- (6) Egg yolk contains anti-salmonella antibodies detectable with anti-IgG sera. Yolk can be used instead of serum.
- (7) ELISA for IgG antibodies in milk or serum of cows taken at 60-day intervals can be used to detect S. dublin carrier cows which maintain elevated titres. Sensitive ELISA is more reliable than culture, but somewhat less specific at titres near the cutoff between + and -.
- (8) S. dublin carrier cattle may only sporadically (3.5% of samples) shed salmonella in faeces and milk in numbers which can be detected by usual methods of culture enrichment.

#### RECOMMENDATIONS

- (9) The effect of vaccination on ELISA should be studied. If possible, vaccinal antibodies should be distinguishable from those due to natural response.
- (10) Priority should be given to ELISA research which will help to differentiate important serovars of salmonella in each species:

Poultry:	S. typhimurium, S. enteritidis, S. pullorum, S. gallinarum and further serovars of potential significance;
Cattle:	S. typhimurium, S. dublin, and further serovars of potential significance;
Pigs:	S. typhimurium, <u>S. choleraesuis</u> , S. typhisuis and further serovars of potential significance.
- (11) Monoclonal antibodies should be developed for the differential diagnosis of salmonella serovars, especially to design new diagnostic tests specific to S. enteritidis, S. gallinarum/pullorum and S. typhimurium in poultry and for S. dublin and S. typhimurium in cattle.
- (12) Conventional bacteriological techniques to detect infected and carrier animals should be replaced by serological techniques, such as ELISA. However guidelines regarding their use should be developed according to the type of production concerned and the scope of the control programme.
- (13) Skin testing to determine carrier status in cattle should continue to be explored.
- (14) Research for further evaluating and standardizing ELISA for detection of infection of cattle by S. dublin and other serovars, as well as of poultry by S. enteritidis and other serovars, should be supported by the WHO.
- (15) The working group should continue its efforts to establish the usefulness of PCR and DNA probes and other new techniques for diagnostics and research on salmonellosis.

#### 4. RECOMMENDED COOPERATION

The WHO secretariat should assist the working group on salmonella immunization in providing the information available from the working group on enzyme immunoassay for brucellosis diagnostics and research, and should coordinate the activities of both groups whenever required. The following groups working with salmonella ELISA should try to standardize the test with regard to antigens, positive and negative control sera, and interpretation of results.

4.1 Dr Paul Barrow, Institute for Animal Health, Houghton, UK, will send the following items to labs participating in poultry ELISA for *S. enteritidis*:

- a) 2 strong positive, 2 weak positive, and 2 negative sera for *S. enteritidis*, all from SPF chickens with known history.
- b) Antigen recipe
- c) ELISA protocol

Laboratories participating in poultry ELISA development:

- (1) Houghton, UK: Institute for Animal Health (P. Barrow)
- (2) Weybridge, UK: Central Veterinary Laboratory (C. Wray)
- (3) Berlin, Germany: Federal Health Office, (C. Staak)
- (4) Grub, Germany: Bavarian Central Animal Health Laboratories (C. Schwarzer)
- (5) Jena, Germany: Research Institute for Bacterial Animal Diseases (G. Steinbach)
- (6) St. Paul, Minn, USA: Univ. of Minn. (Dr Nagaraja)
- (7) USDA Center, USA:
- (8) Lelystad, Netherlands: Animal Health Institute (Van den Wyngaard)
- (9) Brno, Czechoslovakia: Veterinary Research Institute (F. Sisak)
- (10) Ploufragan, France: Central Research Laboratory (Dr Michèle Guittet)

4.2 Dr Bradford P. Smith, Univ. of Calif., Davis, California, USA, will send the following items to labs participating in cattle ELISA for *S. dublin*:

- a) 2 strong positive, 2 weak positive and 2 negative sera for *S. dublin* cattle;
- b) antigen recipe;
- c) ELISA protocol.

Laboratories participating in cattle ELISA development:

- (1) Davis, Calif., USA: Univ. of California (B. Smith)
- (2) Jena, Germany: Research Institute for Bacterial Animal Diseases (G. Steinbach)
- (3) Weybridge, UK: Central Veterinary Laboratory (C. Wray)
- (4) Berlin, Germany: Federal Health Office (C. Staak)
- (5) Brno, Czechoslovakia: Veterinary Research Institute (F. Sisak)

4.3 Results from the sera will be run on 3 different days in triplicate ( 9 times in total), and reported back to P. Barrow or B. Smith as:

- a) Nine individual optical densities (blank subtracted);
- b) Mean O.D.± S.D.;
- c) O.D. of positive and negative sera used routinely by each laboratory.

4.4 When the results 4.1-4.3 above are received by Barrow and Smith, they will compile them and send to all participating laboratories along with 20 chicken or cattle sera to be run blindly by participating laboratories in triplicate.

Each laboratory will then report the results and their interpretation (positive or negative) to Barrow and Smith, who will again compile results and distribute to all laboratories.

Following this and depending on the amount of inter-laboratory variation, participants will decide on whether there is a need to standardize antigens and techniques. If there is such a need a meeting should be arranged by WHO to decide on specific points of standardization.

4.5 The group should develop an ELISA kit for salmonellosis diagnostics in poultry and cattle with special interest to *S. enteritidis* and *S. dublin* infections by the end of 1992. The kit should be distributed to interested institutions aiming at the assessment of the kit in comparison with other conventional diagnostic methods. The kit can be introduced as herd/ flock basis of diagnostics and monitoring.

4.6 Skin test antigen available in Brno will be provided to the institutes in Jena and Davis for further collaborative application and assessment of cattle infected with salmonella at various levels. This activities can be implemented by the end of 1991.

ANNEX I

List of Participants

- Dr P.A. Barrow, Institute for Animal Health, Houghton Laboratory, Houghton, Huntingdon, Cambridgeshire, PE17 2DA, UK
- Dr H. Danbara, Department of Bacteriology, The Kitasato Institute, 5-9-1, Shirokane, Minato-ku, Tokyo 108, Japan
- Dr A. Koulikovskiy, All-Union Research Institute for Veterinary Sanitation, Hygiene and Ecology, Zvenigorodskoe Shosse H. 5, Moscow H 123022, USSR (Rapporteur)
- Professor H. Meyer, Research Institute for Bacterial Animal Diseases Jena, Naumburger Str. 96a, D-0-6900 Jena, Germany (Chairman)
- Dr H. Pittler, Bundesministerium für Ernährung, Landwirtschaft und Forsten, Rochusstraße 1; PF 140 270, D-W-5300 Bonn, Germany
- Dr Ch. Schwarzer, Zentralinstitut des Tiergesundheitsdienstes Bayern, Senator-Gerauer-Str. 23, D-W-8011 Grub, Germany
- Dr B. Shuster, All-Union State Institute for Control of Veterinary Biologics, Zvenigorodskoe Shosse H. 5, 123022 Moscow, USSR
- Dr F. Sisak, Veterinary Research Institute, Hudcova 70, 621 32 Brno, Czechoslovakia
- Professor B.P. Smith, University of California, School of Veterinary Medicine, Department of Medicine, Davis, California 95616, USA (Vice-Chairman)
- Dr Chr. Staak, Institute of Veterinary Medicine, (Robert von Ostertag Institute), Federal Health Office, Thielallee 88-92, PF 330013, D-W-1000 Berlin 33, Germany
- Professor M. Truszczyński, Veterinary Research Institute, Al Partyzantow 55, Pulawy, Poland
- Dr C. Wray, Central Veterinary Laboratory, New Haw, Weybridge, Surrey KT15 3NB, United Kingdom

Representatives from other organizations

International Office of Epizootics, Paris, France:

Dr Michèle Guittet, Directeur adjoint du Laboratoire central de recherches avicole et porcine, CNEVA, B.P. 53, 22440 Ploufragan, France

Food and Agriculture Organization of the United Nations, Rome, Italy:

Dr J. De Rycke, Animal Health Officer (Bacteriology)

Secretariat

Dr K. Bögel, Chief, Veterinary Public Health, Division of Communicable Diseases, WHO,  
1211 Geneva 27, Switzerland (Secretary)

Dr T. Fujikura, Scientist, Veterinary Public Health, Division of Communicable  
Diseases, WHO, 1211 Geneva 27, Switzerland

Dr T. Blaha, Research Institute for Bacterial Animal Diseases Jena,  
Naumburger Str. 96a, D-O-6909 Jena, Germany

Additional Participants

Dr W. Beyer, Institute of Epizootiology and Control of Animal Diseases,  
Seestr. 55, D-O-1903 Wusterhausen, Germany

Dr Ute Dinjus, Research Institute for Bacterial Animal Diseases Jena,  
Naumburger Str. 96a, D-O-6909 Jena, Germany

Dr Ilka Hahn, Research Institute of Veterinary Ecomicrobiology and Immunology,  
Jahnstr. 8, PF 26, D-O-4500 Dessau, Germany

Dr R. Helmuth, Institute of Veterinary Medicine, (Robert von Ostertag-Institute),  
Federal Health Office, Thielallee 88-92, PF 330013, D-W-1000 Berlin 33, Germany

Dr H. Kühn, Robert-Koch-Institut of the Federal Health Office, Burgstr. 37,  
D-O-3700 Wernigerode, Germany

G. Martin, Research Institute for Bacterial Animal Diseases Jena,  
Naumburger Str. 96a, D-O-6909 Jena, Germany

U. Methner, Research Institute for Bacterial Animal Diseases Jena,  
Naumburger Str. 96a, D-O-6909 Jena, Germany

Dr Christine Rudolph, Research Institute for Bacterial Animal Diseases Jena,  
Naumburger Str. 96a, D-O-6909 Jena, Germany

Dr H. Schlüter, Institute of Epizootiology and Control of Animal Diseases,  
Seestr. 55, D-O-1903 Wusterhausen, Germany

Professor H.-J. Selbitz, Institute for Microbiology, Faculty of Veterinary  
Medicine, Margarete-Blank-Str. 8, D-O-7010 Leipzig, Germany

Professor G. Steinbach, Research Institute for Bacterial Animal Diseases Jena,  
Naumburger Str. 96a, D-O-6909 Jena, Germany

ANNEX II

Immunoprophylaxis against salmonella infections in animals -  
an overview 1988 -1991 \*

In comparison to the last report in March 1989 the following changes can be noticed:

1. In addition to the well-known efficacy of auxotrophic mutants also pho P mutants showed a good efficacy against *S. typhimurium* (Galan et al., 1989) and *ts*-mutants against *S. enteritidis* (Cerquetti et al., 1990) as well as reverse mutants for streptomycin susceptibility against *S. abortusovis* (Pardon et al., 1990).
2. Whole cell preparations, extracts (detoxified LPS, outer membrane proteins) and a synthetic lipopeptide were used as inactivated vaccines. The synthetic lipopeptide was effective only together with acetone-killed bacteria. But it was possible to reduce the number of the acetone-killed bacteria by 90% compared with the number of acetone-killed bacteria which were necessary to have a good protection without synthetic lipopeptide (Schlecht et al., 1989).
3. The number of papers about salmonellosis in poultry has increased. Most of them reported about vaccine development against *S. typhimurium* and *S. enteritidis*.
4. In poultry most of the vaccines were live vaccines. In other animal species, live vaccines were used as well as inactivated ones.
5. The aim of immunization in poultry is to reduce the excretion of *Salmonellae* in order to reduce the concentration of *Salmonellae* under the minimal infection dose in the flock.
6. The better understanding of pathogenicity of *Salmonella* infections will result not only in the elucidation of virulence determinants of *Salmonella* but also allow the construction of avirulent highly immunogenic vaccine strains (Wilson et al., 1990). Interesting investigations in this field came from Miller et al. (1990) about the pho P locus, which is the major regulator of coordinated gene expression in virulent *Salmonella*. The development of a balanced lethal host-vector system has been reported by Nakayama et al. (1988), Curtiss III et al. (1990), and Galan et al. (1990). The basis of the system is the requirement of *asd* mutants of *S. typhimurium* for diaminopimelic acid (DAP). The reintroduction of the *asd* gene with a vector containing more cloned genes results in recombinant avirulent vaccine strains with stable, high-level expression of foreign colonization or virulence antigen. The tendency to express foreign antigenic determinants in attenuated *Salmonella* strains in order to construct live oral multivalent vaccines has continued (Clements and Cardenas, 1990) especially also in human medical problems (Levine et al., 1990; Sjöstedt et al., 1990; Pistor and Hobom, 1990).

\* Prepared by Drs Ch. Rudolph, G. Martin and U. Methner, Research Institute for Bacterial Animal Diseases, D-0-6909 Jena, Germany, and presented at the meeting.

The attached table has been prepared to provide further information.

In the next few years decisive breakthroughs are to be expected in the area of genetic engineering.

Abbreviations in the table

aro <sup>-</sup>	aromatic compounds requiring salmonellae (auxotrophy)
bw	body weight
cfu	colony forming units
DTH	delayed type hypersensitivity
galE	galactose-epimerase-defect
his <sup>-</sup>	histidine auxotrophy
im.	intramuscular
imm.	immunization
inact.	inactivated
ip.	intraperitoneal
iv.	intravenous
LPS	lipopolysaccharide
n.t.	not tested
Nal <sup>r</sup>	nalidixic acid-resistant
OMP	outer membrane protein
pe.	parenteral
pho P	no acid phosphatase activity
pur <sup>-</sup>	purin auxotrophy
R	rough strain
S	Salmonella
Sao	Salmonella abortusovis
Scs	Salmonella choleraesuis
Sd	Salmonella dublin
Se	Salmonella enteritidis
Sg	Salmonella gallinarum
Sp	Salmonella pullorum
Spc <sup>r</sup>	spectinomycin resistant
Stm	Salmonella typhimurium
Stmvc	Salmonella typhimurium variation copenhagen
sc.	subcutaneous
thia <sup>-</sup>	thiamine auxotrophy
ts	temperature sensitive
VP <sup>- r</sup>	virulence plasmid cured, rough strain
cya cyp	adenylate cyclase, cyclic AMP receptor protein deletion mutant

Immunoprophylaxis against Salmonella infections in animals - an overview 1988 - 1991 (references are given at end of table)

author, year	animal species	type of vaccine	dose/application	challenge	protection	additional information
<u>L. mice, rats</u>						
Cerquetti et al. (1990)	mice	Se ts-mutant	10 <sup>7</sup> cfu ip.	10 <sup>3</sup> cfu ip.	good	
Ding et al. (1990)	mice	inact. a) LPS b) detoxified LPS	a) 50ug + 25ug + 25ug b) 300ug + 150ug + 150ug	a) b) St# 2x10 <sup>5</sup> -2x10 <sup>4</sup> cfu	good	detoxified-LPS is 10 <sup>3</sup> fold less toxic than untreated LPS
Galan et al. (1989)	mice	St# pho P mutants	a) 2x10 <sup>8</sup> or 2x10 <sup>9</sup> oral b) 2x10 <sup>4</sup> , 2x10 <sup>5</sup> or 2x10 <sup>6</sup> ip.	a) St# oral b) St# ip.	a), b) good	DTH 30 days after oral imm. positive
Hornasche et al. (1990)	mice	a) St# aro A mutant b) Sd aro A mutant c) Se aro A mutant	10 <sup>6</sup> cfu iv.	a) St# iv. or oral Se iv. or oral b) Sd oral St# oral	good no low no	

author, year	animal species	type of vaccine	dose/application	challenge	protection	additional information
				c) Se oral Stm oral	low no	
Hougen et al. (1990)	rats	a) Stm formalin-killed bacteria b) Stm sublethal infection c) passive (plasma or spleen cells of imm. animals from b)	a) 10 <sup>8</sup> cells twice a week for 4 weeks, ip. b) 10 <sup>6</sup> cfu/animal ip. c) 3ml plasma or 3x10 <sup>8</sup> spleen cells ip.	a), b), c) Stm ip.	a) no b) good c) plasma no, spleen cells good	
[Sibasi et al. (1988)	mice	S.typhi outer membrane protein	30 ug two times, interval 2 weeks, ip.	S.typhi 20-1000 x LD <sub>50</sub> ip.	good	
Mnalue (1990)	mice	a) Stm aro A mutant b) Scs aro A mutant	2x10 <sup>6</sup> to 5x10 <sup>6</sup> cfu ip. two times	a) Scs ip. Stm ip. b) Scs ip. Stm ip.	no good no good	

author, year	animal species	type of vaccine	dose/application	challenge	protection	additional information
Schlecht et al. (1989)	mice	Stm a) synth. lipopeptide + acetone-killed bact. b) acetone-killed bact.	a), b) 0,2ml two times ip.	a) Stm b) Stm	good good	lipopeptide alone not effective, a) 90% reduction of acetone-killed bacteria possible compared to b)
Sigwart et al. (1989)	mice	Sd a) aro A mutants b) pur A mutants	a), b) 10 <sup>10</sup> cfu oral	n.t.		only a) colonized, invaded, persisted and serum + mucosal antibodies were produced b) may be not effective as a vaccine strain

author, year	animal species	type of vaccine	dose/application	challenge	protection	additional information
<u>2. Calves</u>						
Anderson et al. (1991)	calves	a) Stm bacterin	a) two times 2 and 4 weeks of age, sc.	a) Stm UCD 108-11 ( $3 \times 10^{11}$ cfu) at 6 weeks of age, oral	no protection	
		b) Stm oil-in-water emulsion with LPS	b) two times 2 and 4 weeks of age, sc.	b) Stm UCD 108-11 ( $1.5 \times 10^{11}$ cfu) at 6 weeks of age, oral	no protection	
		c) Stm oil-in-water emulsion with Lipid A	c) two times 2 and 4 weeks of age, sc.	c) Stm UCD 108-11 ( $3 \times 10^{11}$ cfu) at 6 weeks of age, oral	no protection	
		d) Stm oil-in-water emulsion with Lipid A	d) two times 2 and 6 weeks of age, sc.	d) Stm UCD 108-11 ( $3 \times 10^{11}$ cfu) at 8 weeks of age, oral	no protection	
Jones et al. (1988)	calves	Stm, inact.	vaccination of cows, 7 and 2 weeks before parturition sc., passive imm. of calves via colostrum	Stm oral $10^8$ cfu	good	no correlation between protection and antibody titer (serum)
Masalski et al. (1989)	calves	Sd live	oral, dose not published	Sd	good	

author, year	animal species	type of vaccine	dose/application	challenge	protection	additional information
Meyer et al. (1983a)	calves	a) Sd pur <sup>-</sup> /thia <sup>-</sup> mutant b) St <sup>+</sup> his <sup>-</sup> /pur <sup>-</sup> mutant	a) 1,5x10 <sup>10</sup> - 5x10 <sup>11</sup> oral, once b) 1x10 <sup>9</sup> - 1x10 <sup>10</sup> oral, once	a) Sd oral b) St <sup>+</sup> oral	a) good b) good	
Meyer et al. (1983b)	calves	St <sup>+</sup> his <sup>-</sup> /pur <sup>-</sup> mutant	1x10 <sup>9</sup> - 5x10 <sup>10</sup> oral, once	Sd oral	good	cross-protection
Nikula et al. (1989)	calves	St <sup>+</sup> a) aro <sup>-</sup> mutant b) galE mutant c) inact. whole cells	a,b) 4x10 <sup>7</sup> - 1x10 <sup>10</sup> oral, two or three times c) 1x10 <sup>10</sup> inact. bacteria two or three times, im.	a) n.t., calves died due to vaccination b) St <sup>+</sup> 10 <sup>6</sup> or 10 <sup>9</sup> cfu oral c) St <sup>+</sup> 10 <sup>6</sup> cfu oral	b) good c) good	b) mild illness, due to vaccination
Staak et al. (1989, 1990)	calves	St <sup>+</sup> or Sd inact.	4x5ml (5x10 <sup>9</sup> cfu/ml inact.) into the mammary gland of pregnant cows, passive im. of calves via colostrum	St <sup>+</sup> or Sd 10 <sup>7</sup> cfu, oral	good	excretion rate of salmonellae in the faeces lower, duration of excretion not influenced

author, year	animal species	type of vaccine	dose/application	challenge	protection	additional information
<u>3. sheep</u>						
Begg et al. (1950)	sheep	Stm, aro <sup>-</sup> mutant	a) 1x10 <sup>10</sup> cfu ia. b) 1x10 <sup>8</sup> cfu ia., after 2 weeks 1x10 <sup>10</sup> cfu ia. c) 1x10 <sup>10</sup> cfu sc. d) 1x10 <sup>8</sup> cfu sc., after 2 weeks 1x10 <sup>10</sup> cfu sc. e) 1x10 <sup>10</sup> cfu oral f) 1x10 <sup>8</sup> cfu oral, after 2 weeks 1x10 <sup>10</sup> cfu oral	Stm 1x10 <sup>10</sup> cfu oral	c) no protection a), b), d), e) and f) good protection	work was done in order to modify vaccine the vaccination modus of Mukkur et al. (1987)
Islanov et al. (1990)	sheep	Sao a) inact. b) live	a) not published b) 2ml sc.	field trial	a) no b) good	spontaneous infection in some herds
Pardon et al. (1990)	sheep	Sao reverse mutant for streptomycin susceptibility	10 <sup>8</sup> cfu sc., once	Sao 2 x 10 <sup>9</sup> cfu sc.	good	

author, year	animal species	type of vaccine	dose/application	challenge	protection	additional information
<u>4. swine</u>						
Meyer et al. (1989a)	swine	Scs R/pur <sup>-</sup> mutant	1x10 <sup>8</sup> - 1x10 <sup>9</sup> cfu oral or pe.	Scs oral	good	
Ninow et al. (1988)	swine	Scs, live	2x10 <sup>8</sup> , appl. not published		n.t.	antibody titer high after immunization
Schwartz (1991)	swine	a) inact. Scs b) inact. polyvalent (Scs, Stx, Pasteu- rella, Bordetella)	a) 2al pe. b) 3al pe.	field trial, spontaneous infection	low, a) better than b)	
<u>5. poultry</u>						
Barrow (1990)	chicken	plasmid-cured Sg	two times 1 day of age (10 <sup>6</sup> cfu) 14 days of age (10 <sup>8</sup> cfu), in.	10 <sup>8</sup> cfu Sg 9 at 28 days of age, oral	signif. reduction in mortality	
	chicken	plasmid-cured Sg	two times 1 day of age (10 <sup>6</sup> cfu) 14 days of age (10 <sup>8</sup> cfu), in.	10 <sup>6</sup> cfu Sg 9 at 28 days of age, oral	signif. reduction in mortality	

author, year	animal species	type of vaccine	dose/application	challenge	protection	additional information
	chicken	plasmid-cured Sp	two times 1 day of age ( $10^6$ cfu) 14 days of age ( $10^8$ cfu), i.v.	$10^6$ cfu Sg 9 at 28 days of age, oral	not signif. reduction in mortality	
	chicken	plasmid-cured Sg	two times 1 day of age ( $10^6$ cfu) 14 days of age ( $10^8$ cfu), i.v.	Sg 9 at 28 days of age, i.v.	high	
	chicken	plasmid-cured Sg	two times 21 days of age ( $10^7$ cfu) 35 days of age ( $10^7$ cfu), i.v.	Sg 9 at 48 days of age, i.v.	high	
	chicken	plasmid-cured Sp	two times 1 day of age ( $10^5$ cfu) 14 days of age ( $10^8$ cfu), i.v.	Sg 9 at 28 days of age, i.v.	good	

author, year animal species type of vaccine dose/application challenge protection additional information

chicken	Sg 9 VP-φ rNa1r	single 3 weeks of age (3x10 <sup>8</sup> cfu), oral	3x10 <sup>8</sup> cfu Sg 9 at 6 weeks of age, oral	low protection	
chicken	Sg SR Na1r	single 3 weeks of age (3x10 <sup>8</sup> cfu), oral	3x10 <sup>8</sup> cfu Sg 9 at 6 weeks of age, oral	high protection	
chicken	Sg 9 VP-φ rNa1r	single 3 weeks of age (10 <sup>8</sup> cfu), ia. or sc.	3x10 <sup>8</sup> cfu Sg 9 at 6 weeks of age, oral	lower protection than by oral immunization	
chicken	Sq 9 VP-φ rNa1r	single 21 days of age (10 <sup>7</sup> cfu), ia.	Sq 9 at 42 days of age, ia.	high protection	
chicken	Sg SR Na1r rNa1r	single 21 days of age (10 <sup>7</sup> cfu), ia.	Sg 9 at 42 days of age, ia.	high protection	

author, year	animal species	type of vaccine	dose/application	challenge	protection	additional information
Cooper et al. (1990)	chicken	Se 795 aro A	single	10 <sup>9</sup> cfu Se 109 Na1 <sup>r</sup> at 30 days of age, oral	significant re- duction in shedding of chal- lenge strain	
			1 day of age (10 <sup>9</sup> cfu), oral			
	chicken	Se 795 aro A	two times	10 <sup>9</sup> cfu Se 109 Na1 <sup>r</sup> at 30 days of age, oral	significant re- duction in shedding of chal- lenge strain	
			1 day of age (10 <sup>7</sup> cfu), 14 days of age (10 <sup>7</sup> cfu), oral			
	chicken	Se 795 aro A	four times	10 <sup>9</sup> cfu Se 109 Na1 <sup>r</sup> at 39 days of age, oral	significant re- duction in shedding of chal- lenge strain	
			1 and 7 days of age (10 <sup>5</sup> cfu), 14 and 21 days of age (10 <sup>9</sup> cfu), oral			
	chicken	Se 795 aro A	single	10 <sup>9</sup> cfu Se 109 Na1 <sup>r</sup> at 30 days of age, oral	no protection	
			1 day of age (10 <sup>9</sup> cfu), im.			

author, year	animal species	type of vaccine	dose/application	challenge	protection	additional information
Barrow et al. (1990c)	laying hens	Sq 9 K	two times in two week interval ( $10^8$ cfu), in.	$10^8$ cfu Se NaI <sup>r</sup> two weeks after in., oral	good	cross protection
	laying hens	Se aro A rough	two times in two week interval ( $10^8$ cfu), oral and in.	$10^8$ cfu Se NaI <sup>r</sup> two weeks after in., oral	low	
Barrow et al. (1990b)	chicken	St <sub>a</sub> aroA	single 4 days of age ( $10^8$ cfu), oral	St <sub>a</sub> after several weeks, oral	low	
	chicken	St <sub>a</sub> rough	single 4 days of age ( $10^8$ cfu), oral	St <sub>a</sub> after several weeks, oral	high	
	chicken	St <sub>a</sub> aroA	single 4 days of age ( $10^8$ cfu), in.	St <sub>a</sub> after several weeks, oral	low	

author, year	animal species	type of vaccine	dose/application	challenge	protection	additional information
Barrow et al. (1990a)	chicken	St# F98 Spc <sup>r</sup>	single 4 days of age (10 <sup>8</sup> cfu), oral	10 <sup>8</sup> cfu St# F98 Na1 <sup>r</sup> at 32 days of age, oral	significant re- duction in shedding of chal- lenge strain	
	chicken	St# F98 Spc <sup>r</sup>	single 4 days of age (10 <sup>5</sup> cfu), im.	10 <sup>8</sup> cfu St# F98 Na1 <sup>r</sup> at 32 days of age, oral	significant re- duction in shedding of chal- lenge strain	
	chicken	St# F98 Spc <sup>r</sup> (inactivated)	daily 4-42 days of age in feed, (10 <sup>7</sup> -10 <sup>8</sup> cfu/g feed), oral	10 <sup>8</sup> cfu St# F98 Na1 <sup>r</sup> at 42 days of age, oral	very low reduction in shedding of challenge strain	
	chicken	St# F98 Spc <sup>r</sup> (inactivated)	two times 4 days of age (10 <sup>8</sup> cfu), im. 18 days of age (10 <sup>8</sup> cfu), im.	10 <sup>8</sup> cfu St# F98 Na1 <sup>r</sup> at 42 days of age, oral	low reduction in shedding of chal- lenge strain	

author, year	animal species	type of vaccine	dose/application	challenge	protection	additional information
	chicken	Stm F98 araA	two times 4 days of age ( $10^5$ cfu), im. 18 days of age ( $10^8$ cfu), im.	$10^8$ cfu Stm 798 Spc <sup>r</sup> at 32 days of age, oral	reduction in shedding of challenge strain, but longer persistence of the challenge strain in immunized than in control chicken	
	chicken	Stm F98 Na1 <sup>r</sup>	two times 4 days of age ( $10^5$ cfu), im. 18 days of age ( $10^8$ cfu), im.	$10^8$ cfu Stm 798 Spc <sup>r</sup> at 32 days of age, oral	reduction in shedding of challenge strain, but longer persistence of the challenge strain in immunized than in control chicken	

author, year	animal species	type of vaccine	dose/application	challenge	protection	additional information
Barrow et al. (1990c)	chicken	Stm F90 arcA	single 4 days of age ( $10^8$ cfu), oral	$10^8$ cfu Stm Spc <sup>r</sup> at 46 days of age, oral	not significant re- duction in shedding of challenge strain	
	chicken	Stm F98 Na1 <sup>r</sup>	single 4 days of age ( $10^8$ cfu), oral	$10^8$ cfu Stm Spc <sup>r</sup> at 67 days of age, oral	long excretion of the immunization strain, signifi- cant reduction in shedding of chal- lenge strain	
Hassan, J.O., Curtiss III, R. (1990)	chicken	$\Delta$ cya $\Delta$ crp Stm X3985	single 3 days of age ( $10^9$ cfu), oral	$10^6$ cfu Stm X3761 at 3 weeks of age, oral	significant re- duction in shed- ding of challenge strain, but not reduction in caecal colonization	

author, year	animal species	type of vaccine	dose/application	challenge	protection	additional information
	chicken	A cyaΔcrp St <sub>a</sub> X3985	two times 1 and 14 days of age (10 <sup>9</sup> cfu), oral	10 <sup>6</sup> cfu St <sub>a</sub> X3761 at 3 or 4 weeks of age, oral	high reduction in caecal coloni- zation when chal- lenge at 4 weeks of age, but not at 3 weeks of age	
Uyttebroek et al. (1991)	pigeon	5 different pre- parations of in- activated St <sub>a</sub>	single or two times, sc.	2x10 <sup>8</sup> cfu St <sub>a</sub> VC, oral	no protection	
Uyttebroek et al. (1991)	pigeon	St <sub>a</sub> gal E mutant	single or two times, oral	2x10 <sup>8</sup> cfu St <sub>a</sub> VC, oral	no protection	
Dorn et al. (1950)	breeder- flocks	St <sub>a</sub> (his <sup>-</sup> /pur <sup>-</sup> )	two times oral and or sc.	challenge of the chicken 10 <sup>3</sup> cfu St <sub>a</sub> TGD at 3 days of age, oral	significant re- duction in shed- ding of challenge strain at 7 days age, low or no protection from 14 until 35 days of age	

author, year	animal species	type of vaccine	dose/application	challenge	protection	additional information
	a) breeder- flocks b) their progeny	St <sub>8</sub> (his <sup>-</sup> /pur <sup>-</sup> )	a) two times oral and or sc. b) three times (7, 21, 28 days of age)	challenge of the chicken 10 <sup>3</sup> cfu St <sub>8</sub> TGD at 3 days of age, oral	significant re- duction in shed- ding of challenge strain until 42 days of age	
Times et al. (1990)	chicken	Se vaccine (whole-cell, oil-adjuvant)	single 3 weeks of age (10 <sup>11</sup> cfu), sc.	10 <sup>9</sup> cfu Se at 5 weeks of age, i.m.	good	
	chicken	Se vaccine (whole-cell, oil-adjuvant)	single 3 weeks of age (10 <sup>11</sup> cfu), sc.	10 <sup>8</sup> cfu Se at 8 weeks of age, iv.	good	
	chicken	Se vaccine (whole-cell, oil-adjuvant)	two times 3 and 6 weeks of age (10 <sup>11</sup> cfu), sc.	10 <sup>9</sup> cfu Se at 8 weeks of age, iv.	high	

author, year	animal species	type of vaccine	dose/application	challenge	protection	additional information
Bouzoubaa et al. (1987)	chicken	Sg (UMP with and without oil-adjutant)	two times 8 and 12 weeks of age (50-400 ug/100 g bw), i.v.	Sg at 15 weeks of age, oral	high protection with 400 ug/100 g bw	
	chicken	Sg (formalin- inactivated)	two times 8 and 12 weeks of age	Sg at 15 weeks of age, oral	low	

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ANNEX III

SALMONELLA EPIDEMIOLOGY \*

1. SURVEILLANCE OF SALMONELLOSIS

A major objective of salmonella surveillance programmes is the collection of data concerning salmonella infections and contaminations in man, animals and the environment, as well as their territorial and temporal distribution.

1.1 Using this surveillance programme, the following data have to be collected and evaluated:

- salmonella findings from the environment and preliminary stages of primary production, e.g. feedstuff and water;
- salmonella detections in animal production considering the draft of the EC guideline on zoonoses;
- salmonella isolations from diseased animals;
- data on salmonellae in food;
- salmonella findings in connection with infections of individuals and/or families, especially for salmonella outbreaks caused by food poisoning.

1.2 The informational value of salmonella data must be increased by using modern typing methods, such as phage typing, biotyping and plasmid profile analysis.

1.3 To further qualify the salmonella surveillance programme it is considered necessary:

- to record the total number of samples investigated, as well as the percentages of positive results in veterinary medicine and isolations from man. This must include the registration of salmonella findings in food in connection with disease in man;
- to present these results in territorial structures down to the community level (mapping) and in correlation with time;
- to intensify the interdisciplinary cooperation between medicine and veterinary medicine during the evaluation of the collected data.

\*Elaborated by a group during the meeting (Drs Blaha, Bögel, Koulikovskiy, Kühn and Schlüter).

1.4 This surveillance system must be further completed by including the following active measures in order to increase its efficiency:

- elaboration and imposition of a consequent surveillance scheme, as pilot projects, starting from the feedstuff industry, continued in animal production and ending in the food industry (slaughter houses) ultimately aiming at a salmonella-free production;
- realization of sentinel studies covering selected hospitals and practitioners in defined territories.

1.5 Creation of a national board for quality insurance with respect to salmonella-controlled production and products.

Under the auspices of this institution:

- the salmonella surveillance system will be elaborated, data processing will be carried out and resulting conclusions will be drawn;
- the pilot projects mentioned under paragraph 1.4 must be worked out and tested in practice;
- the collaboration between all authorities, institutions and confederations (e.g. feedstuff producers, breeding companies, industrial groups) participating in these surveillance and pilot programmes must be initiated;
- the international cooperation must be furthered including supranational producers and organizations.

## 2. EPIDEMIOLOGICAL RESEARCH

In recent years the efficacy and feasibility of immunoprophylactic measures have been proven both in experiments and in field trials. In the former GDR (during the 80's) it was possible to carry out successful vaccinations of calves and pigs (in one egg production farm) that led to a remarkable decrease of the salmonella occurrence in the livestock. The question, however, whether the vaccination can result in a real elimination of salmonella serovars which are not adapted to one host species from herds or flocks is not completely answered yet. So far, there is still little knowledge on herd (flock) immunity and herd (flock) susceptibility.

To get further information on this problem it is necessary to carry out pilot projects in the poultry industry under the auspices of WHO. At least 3 kinds of pilot projects are to be carried out:

Pilot project 1: Protection of salmonella-free herds (flocks)  
against the introduction of salmonellae

Pilot project 2: Elimination of salmonellae from a herd (flock)  
with a low infection pressure and protection of  
the freedom from salmonellae

Pilot project 3: Elimination of salmonella from a herd (flock)  
with a high infection pressure and protection of  
the freedom from salmonellae

The following complex measures are necessary for all 3 pilot projects:

- supply of feed absolutely free from salmonellae (heated or pelleted or irradiated or whatsoever);
- storage, transport and handling of the feedstuffs which guarantee the prevention of any salmonella contamination;
- highest hygiene level in the herd (flock);
- elimination of salmonella carriers from the herd (flock);
- introduction only of animals which have the same salmonella status as the herd (flock) of the pilot project (salmonella free or vaccinated);
- transport and slaughter of all animals under utmost hygienic conditions to prevent any salmonella contamination after the animals leave the herd (flock);
- processing of meat and eggs according to the guidelines on prevention and control of salmonellosis (WHO, Geneva, 1983).
- it may be necessary to vaccinate all animals in the herds (flocks) of pilot projects 2 and 3.

One epidemiological approach to answer the question of the contribution of immunoprophylaxis to the elimination of salmonella from infected herds could be to stop vaccinating the herd (flock) within pilot 2 or 3, when no salmonellae are found any longer. The following period with the observation of all measures necessary for the pilot projects, but without vaccination, has to be carefully monitored. The result of such a trial within the pilot projects will answer the question whether salmonellae can really be eliminated by means of vaccination and hygiene or whether they linger on in the herd (flock) under the "protection of immunoprophylaxis".

Considering the fact that in some countries serious regulatory measures have to be taken in case of salmonella infections, WHO should discuss with O.I.E. and FAO the necessity of modifying legal requirements to facilitate the voluntary cooperation of farms with pilot projects and longer field studies of salmonella surveillance and control in feed, poultry and livestock, and slaughter processes.

### 3. APPROACHES

Salmonellosis surveillance and control activities as well as the strategy under discussion for EC countries require the mobilization of resources in various subsectors of the food chain. Poultry and/or red meat product boards are being established in a number of countries in order to achieve essential cooperation of such subsectors. For poultry this would mean the "integration" of feed and parent stock production, broiler and egg production including hatcheries, slaughter technology industries and slaughter houses. Retailers and consumers may be represented in such an "integration".

All reasonable and feasible surveillance and control operations should be considered a pilot programme which could serve as an operational research project. National authorities should take an initiative by organizing planning meetings of such a "resources and coordination group" henceforth called product board, although this cooperation should largely be based on responsibility and self-control by the private sector.

Attempts to ensure salmonella-free breeding stocks, feed, farming, transportation and slaughter processes should eventually lead to a system of quality assurance and control.

Presently there are three procedures in use for quality assurance:

- (1) Good Manufacturing Practice (GMP), described in detail, prescribed, endorsed and controlled.
- (2) ISO 9000 or EN 29000 Management procedure for quality assurance, describing and controlling production and testing procedures but not product properties and criteria for interventions.
- (3) HACCP-systems based on monitoring of defined parameters at critical points and specifying criteria for intervention.

The HACCP-system has mainly been developed for on-line control of single product processing and is therefore used in slaughter processes and food processing industries, whereas the GMP is the basis for the operation of most of the existing "integrations" including farming. In general the effective integrations combine GMP and monitoring (though not called HACCP) in a strict manner.

Existing "integrations" differ greatly in the extent to which they entrust or share inspection and quality management with the government and with completely independent bodies. The ISO 9000 or EN 29000 procedure is principally based on verification and certification by private sector organisations independent of both the government and the producer.

Pilot schemes of salmonellosis control product boards may proceed with the following activities in mind:

- (a) Requirement setting for basic materials and products, step by step.
- (b) Assurance of accuracy of tests and monitoring services.
- (c) Description of GMP according to (1) and (2) above.
- (d) Surveillance and evaluation mechanisms.

It should be noted that the first pilot projects may apply all possible procedures to eliminate or greatly reduce risks of infection and contamination. Preference may be given to poultry and invasive salmonella in line with earlier WHO recommendations.

This may include salmonella-free feed production and storage, salmonella-free animal breeding and farming including the use of competitive flora, probiotics etc., special precautions in transport and slaughtering avoiding cross contamination, and, as far as possible, the application of terminal disinfections.

WHO may assist governments in setting up national boards and plans for the purpose of salmonellosis control. This board should also organize surveillance systems and information services towards food chain transmission. Moreover the board should assist in the application of research findings and help in recommending areas for additional research.

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