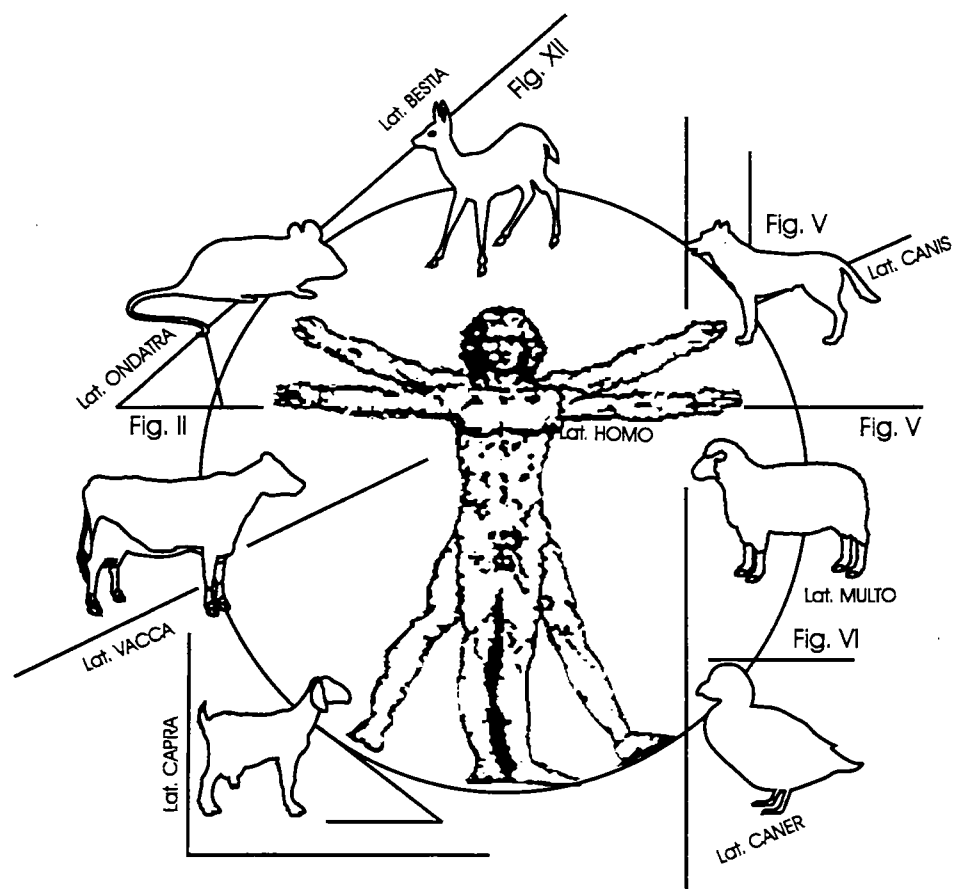


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WHO-FEDESA-FEP WORKSHOP ON COMPETITIVE EXCLUSION, VACCINATION AND ANTIMICROBIALS IN SALMONELLA CONTROL IN POULTRY

OBERNKIRCHEN, GERMANY
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WORLD HEALTH ORGANIZATION
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OF ANIMAL HEALTH

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1. INTRODUCTION

Over the last decade WHO has increased its involvement in research on animal reservoirs of human salmonellosis. Following the epidemic spread of *S. enteritidis* in poultry, various WHO projects have dealt with the monitoring and control of this infection which accounts for the overwhelming majority of human cases in many countries. Other invasive salmonellae, e.g. *S. typhimurium* have also been considered in the development of guidelines for Salmonella control.

The meetings in Ploufragan (1992)¹ and Jena (1993)² specified the need to provide detailed guidelines for day-to-day practice in (a) cleaning, disinfection and rodent control³, (b) microbiological monitoring⁴ and (c) proper application of biological and chemotherapeutic substances. The first two aspects have been dealt with by WHO, while the third area of interest has been entrusted to the Workshop and is the subject of this report.

The participants (Annex I) were welcomed by the First Deputy Mayor of Obernkirchen and representatives of the three sponsoring organizations: Dr Stöhr, WHO; Dr Vanhemelrijck, FEDESA; and Dr Bögel, FEP.

Following these addresses, the group unanimously supported the nomination of Dr Dr K. Bögel as Chairman. Dr Bögel accepted the position and the participants then approved unanimously the nomination of Dr Vanhemelrijck as Vice-chairman. Dr Bögel asked Dr G. Mead to act as Rapporteur for the Workshop.

The Chairman sought approval of the agenda. On the recommendation of Dr Geilhausen and with the agreement of the participants, the term "antibiotic" was replaced by "antimicrobial" in further discussions. The agenda was approved.

The Chairman reminded the participants that the objective of the Workshop was to produce guidelines for use in the field. Following discussion on

the principles of *S. enteritidis* control, four working groups dealt with specific elements of the proposed guidelines:

- (a) use of vaccines in combination with antimicrobials (Drs Vielitz, Selbitz, Blaha, Sisak)
- (b) exclusion flora alone or in combination with antimicrobials (Drs Hafez, Schneitz, Goren, Ehinger, Froyman, Mead)
- (c) combination of vaccines and exclusion flora (Drs Meyer, Humbert, Houghton, Kovarik, Käsbohrer)
- (d) Safety, legal limitations and codes of practice (Drs Vanhemelrijck, Vanhoorde (European Union observer), Ducatelle, Geilhausen)

The proposals of these sub-groups were adopted at the plenary session and incorporated in the following report.

This report contains recommendations and suggestions for the use of antimicrobials, competitive exclusion (CE) products and vaccines as management tools to aid in reducing the incidence of Salmonella in poultry flocks, and in particular *S. enteritidis* and *S. typhimurium* (invasive serotypes).

Any guidance described here should be read in conjunction with the manufacturer's recommendations. Claims that are made on data sheets of licensed products are supported by efficacy and safety data. Any use of products without the manufacturer's recommendations may or may not compromise the effectiveness of these products. Manufacturers may well not accept any liability for problems encountered when their recommendations are not followed.

The guidance provided does not indicate that any one product or procedure is necessarily more or less effective than any other.

Dr Vanhoorde reminded participants that he was

¹ WHO Consultation on National and Local Schemes of Salmonella Control in Poultry. Ploufragan, France, 18-19 September 1992. Document WHO/CDS/VPH/92.110, WHO, Geneva, 1992.

² WHO Consultation on Control of Salmonella Infections in Animals: Prevention of Foodborne Salmonella Infections in Humans. Jena, Germany, 21-26 November 1993. Document WHO/CDS/VPH/93.129, Geneva, 1993.

³ WHO Guidelines on Detection and Monitoring of Salmonella Infected Poultry Flocks with particular reference to Salmonella enteritidis, Graz, Austria, 1994 (Document WHO/ZOON./94.173).

⁴ WHO Guidelines on Cleaning, Disinfection and Vector Control in Salmonella Infected Poultry Flocks with particular reference to *S. enteritidis*. Report of a Workshop on Preparation of Guidelines on Cleaning and Disinfection/Sanitation in Farms with *S. enteritidis*, Bakum/Vechta, Germany, 7-11 June 1993. Document WHO/ZOON. 94.172, Geneva, 1994.

Dr Vanhoorde reminded participants that he was attending this Workshop as an EU observer. He indicated that the WHO guidelines may not be in line with the EU schemes and efforts should be made to avoid confusion, so that trade barriers do not result.

He also stressed that it was impossible to include unlicensed therapeutic products in a legal framework and that legal and trade issues could result from the use of such products.

Dr Vanhoorde summarised the current EU situation concerning the control of zoonoses, including *Salmonella* (working document no.19). He deplored the lack of response from Member States, particularly as far as notification of measures for preventing the introduction of *Salmonella* on the farm was concerned.

Dr Stöhr pointed out that the WHO schemes are more detailed than those proposed by the EU. This reminded the Workshop that, as a result of the latest developments, it was difficult to maintain continuous harmonization with other organizations like the OIE or the EU.

The Chairman thanked Dr Vanhoorde for attending the Workshop as an EU observer. He congratulated the EU for having initiated a most useful development in the form of the Zoonoses Directive.

2. STRATEGIES FOR SALMONELLA CONTROL IN POULTRY

Dr Meyer presented his report (working document no. 6) on the advantages and disadvantages of the three methods for controlling *Salmonella* and his conclusions regarding the possibility of combining two or all of them.

The Chairman commented that, in practice, veterinary surgeons faced three situations : known *Salmonella* -free birds, known infected birds, unknown microbiological status.

Dr Ducatelle reported on Belgian experience (working document no.11) and suggested that it was important to distinguish between invasive and non-invasive serotypes, since non-invasive

serotypes would not be transmitted vertically. He recommended concentrating on invasive salmonellae, except in the case of grandparent flocks. He also mentioned that monitoring may give misleading results once the animals were treated. In this connection, Dr Stöhr referred to the WHO Guidelines on Detection and Monitoring of *Salmonella* Infected Poultry Flocks with particular reference to *Salmonella enteritidis*.

The group went on to discuss the need for separate consideration of broilers and layers from the technical point of view, although available data did not necessarily support such a distinction. However, the Chairman suggested a separate approach in each case to help users of the recommendations.

Dr Selbitz's report (working document no.8) referred only to vaccination.

Dr Stöhr reminded the participants that WHO was essentially concerned with methods of eliminating or reducing the risk of *Salmonella* infection in poultry and the purpose of the Workshop was to provide advice on control strategies, especially in relation to *Salmonella enteritidis*. This would be based on scientific knowledge and would be in accordance with the recommendations of product manufacturers. Such recommendations were required worldwide.

Dr Vanhemelrijck drew the attention of participants to the fact that a number of animal health products would disappear from the market as a result of the revalidation process in the EU for product licences. Furthermore, EU legislation prevented manufacturers from recommending combined treatment programmes, and only an independent group of experts could do this.

Where required, the Workshop could formulate recommendations for further research in relevant areas.

The Chairman asked Drs Goren and Houghton to draft an introductory paragraph for the guidelines to indicate that the recommendations are offered for guidance and should not imply any deviation from the manufacturer's instructions.

The Chairman asked Dr Ducatelle to consider whether the requirement to monitor flocks for *Salmonella* infections affected the treatment strategy.

3. RESULTS OF FIELD STUDIES WITH COMPETITIVE EXCLUSION FLORA IN EGG AND BROILER PRODUCTION

3.1 Czech Republic (working documents nos.12 & 13)

Dr Sisak presented the results of field studies in the Czech Republic and concluded that vaccination was a successful method for preventing flock infection. At the request of the Chairman he clarified the point that the term "probiotic", included the exclusion flora. However, the Chairman requested that probiotics be separated from the exclusion flora in future discussions.

3.2 Finland (working document no.14)

Dr Schneitz reported that all grandparent breeders and over 90% of broilers were treated with competitive exclusion preparations in Finland. In 1993 only 0.4% of all broiler flocks were infected with *Salmonella*. The tendency in Finland was for *Salmonella* infected flocks not to be medicated. *S. enteritidis* had been isolated in very few cases.

3.3 France (working document no.20)

Dr Humbert presented the results of trials conducted under controlled conditions with naturally infected birds.

3.4 Germany

Dr Vielitz reported on the success of vaccination (working document no. 10) leading to a considerable decrease in the prevalence of *Salmonella typhimurium* and *Salmonella enteritidis* in vaccinated flocks. Furthermore, there had been some experience showing the benefit of combining antimicrobial treatment and subsequent vaccination.

3.5 The Netherlands

In preventive treatment of newly hatched broilers (field experiment with 9 million broilers on 44 farms over an 18-month period), the incidence of *Salmonella* positive flocks was reduced to 50% in comparison with untreated flocks. Within *Salmonella* positive flocks, the incidence of infected birds was three times higher than in treated flocks.

Treatment of *S. enteritidis* infected breeder flocks with enrofloxacin (10 days) and then CE preparations (on days 11 and 13) was successful in 74% of cases (no isolation from the flock or its prog-

eny and a change from serologically positive to negative within a 2-month period).

Dr Goren reported on the situation in the Netherlands where a combination of egg dipping in a gentamycin sulphate solution was combined with inoculation of the eggs with enrofloxacin. This was considered an attractive alternative to the financial losses resulting from destruction of infected eggs.

3.6 United Kingdom (working document no.3)

Dr Mead reported recent evidence of a decline in *Salmonella enteritidis* infection in humans and in poultry. He also referred to the difficulty of obtaining information on exclusion flora field trials, which mostly were carried out privately. Nevertheless, a large proportion of breeder chicks has been given exclusion flora treatment.

4. STRATEGY FOR SALMONELLA CONTROL IN POULTRY: USE OF ANTIMICROBIALS AND COMPETITIVE EXCLUSION FLORA (NORMAL GUT FLORA)

4.1 Non-infected flocks

Progeny not infected with salmonella at day-old; free from salmonella organisms after hatching ($p \leq 0.05$).

4.1.1 Breeder flocks

Include turkey, chicken, layer type and meat type. The following recommendations apply particularly to *S. enteritidis* and *S. typhimurium* (invasive serotypes).

Recommendations:

Application of CE flora at day-old as soon as possible after hatching, in order of preference, in the hatchers, at the hatchery, on the farm. Application by coarse spraying is preferred over administration via the first drinking water.

Remarks:

- (i) Further research on "in-ovo" inoculation is needed.

- (ii) Evidence suggests there is no incompatibility between spray inoculation of day-old chicks and use of Infectious Bursitis, Newcastle Disease or Mareks Disease vaccines. There is no information in relation to other agents, e.g. mycoplasmas, and research is needed on this topic.
- (iii) Spraying of CE products on the feed : further research is needed.
- (iv) Generally present-day growth promoters and anti-coccidials do not appear to interfere with the protective function of the normal gut flora. Otherwise it is not advisable to use antimicrobials in feed or water simultaneously with a CE product, unless this is unavoidable as a veterinary intervention, in association with GMP.

During the rearing and production period any use of antimicrobial treatment (e.g. for respiratory colibacillosis) may require the barrier flora to be repaired with a CE flora.

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Remark:

The interrelationship between antimicrobials and CE products is an area for further research.

4.1.2 Layer flocks

This strategy applies particularly to *S. enteritidis* and *S. typhimurium* (invasive serotypes) which may directly contaminate the surface and contents of some table eggs.

Recommendations:
(See 4.1.1 - Breeders)

4.1.3 Broiler flocks

This strategy applies to all serotypes, since poultry meat is frequently contaminated with a variety of food-poisoning salmonellae as a result of flock infection on the farm and cross-contamination in the processing plant. The recommended treatment can be expected to control any serotypes present.

Recommendations:

Administration of CE flora at day-old as soon

as possible after hatching (see 4.1.1 - Breeders).

Remarks:

- (i) On broiler farms all birds should have received a CE preparation as newly hatched chicks.
- (ii) Recolonisation of the intestinal tract with a CE preparation is advisable after antimicrobial medication.

4.1.4 Broiler turkeys

The control strategy applies to all serotypes (See 4.1.3 - Broiler flocks). In relation to competitive exclusion treatment, there is evidence that normal gut flora preparations from chickens are protective for turkeys and vice versa.

Recommendation:

The safety aspects of using chicken preparations to treat turkeys (or vice versa) need to be further investigated.

4.2 Infected flocks

Infection of poultry in the context of this report implies the presence of host-non-specific salmonellae, including both invasive and non-invasive serotypes, and its detection may depend upon the method used.

4.2.1 Breeder flocks

Include turkey, chicken, layer type and meat type. The strategy applies particularly to *S. enteritidis* and *S. typhimurium* (invasive serotypes).

- A. Infection in day-old birds, shown by hatchery debris (including fluff) or meconium, box liners, dead on arrivals, second quality birds.¹

Recommendations:

Option 1. Slaughter is preferable particularly in the case of invasive serotypes (e.g. *S. enteritidis*, *S. typhimurium*).

Option 2. Treatment consists of three consecutive steps :

Removal of debilitated or sick birds

Addition of an appropriate antimicrobial to water or feed (dose and duration to be specified according to the product)

Application of a CE product according to the manufacturer's recommendations. Oral administration of a CE product within 48 hours of completion of antimicrobial medication. A second dose 24 hours later can be beneficial.

Remarks:

- (i) Intensive monitoring of these flocks is essential, and should follow the recommendations in Section 7.3.
- (ii) Additional hygiene measures are necessary in the hatchery.
- (iii) Specific hygiene measures are necessary on the farm before the introduction of the next flock.

B. Flocks infected during rearing

Recommendations:

Option 1. Slaughter is preferable particularly in the case of invasive serotypes (e.g. *S. enteritidis*, *S. typhimurium*).

Option 2. See infection acquired by day-old chicks (A).

Remarks:

- (i) Treatment should be introduced as soon as possible after diagnosis to minimize environmental spread.
- (ii) Whenever possible movement of the flock to a clean site is recommended. The flock must be transferred before the antimicrobial medication has been completed.
- (iii) If intensified monitoring, as recommended in Section 7.3, shows that re-infection or a relapse has occurred, the flock should be slaughtered.

C. Flocks infected during egg production

Recommendations:

Option 1. Slaughter is preferable particularly in the case of invasive serotypes (e.g. *S. enteritidis* and *S. typhimurium*).

Option 2. Treatment (See A above)

Remarks:

- (i) Treatment should be introduced as soon as possible after diagnosis to minimise environmental spread.
- (ii) Movement of the flock to a clean site would be desirable but is normally impossible.
- (iii) Hatching eggs produced up to 3 weeks prior to recognition of flock infection should be traced and should not be incubated or sold for direct consumption.
- (iv) Hatching eggs from birds being treated should be dipped in an antimicrobial solution according to the recommendations given in Section 7.4.5 and Annex 3.
- (v) Hatching eggs produced during the treatment and during the legal withdrawal period must not be used for human consumption.
- (vi) If breakdown occurs, the flock should be slaughtered.

4.2.2 Commercial layers

A. Infection at day-old and during rearing

See A. - Breeders

Remark:

Withdrawal times for antimicrobials must be satisfied.

B. Infection during egg production

Recommendations:

Option 1. Slaughter is preferable particularly in the case of invasive serotypes (e.g. *S. enteritidis* and *S. typhimurium*). This would

be followed by cleaning of the house and intensive monitoring of the subsequent flock.

Option 2. Table eggs should be sent for effective treatment.

4.2.3 Chicken and turkey broilers

The strategy applies to all serotypes.

Recommendations:

Option 1. Treatment can be considered. Chicken CE flora is protective in turkeys and vice versa.

Remark:

Withdrawal times for antimicrobials must be satisfied

Option 2. Flocks should be slaughtered at an appropriate time to avoid cross-contamination in the processing plant.

Option 3. This group favours consideration of alternative carcass decontamination procedures, e.g. lactic acid, trisodium phosphate or ionising radiation.

5.2 Competitive exclusion (CE)

The use of normal gut flora for prophylactic purposes is only applicable at day 1 in the hatchery. CE should be administered by spraying the eggs at hatching. Ideally, CE should be given on day 1 and day 3 in the hatchery machines (days 19 and 21 of incubation). If treatment within the hatchery is not allowed, then CE may be sprayed in the containers used for transport. CE is most effective in hatcheries where the parent flocks are free from salmonellae, but it may be partially effective in the presence of a low level of infection.

5.3 Vaccination

Only licensed products should be used.

5.3.1 First vaccination

Live vaccines should be given orally at day 1 only if CE is not applied at this time. If CE is given at day 1, then the first live vaccine dose should be delayed until at least day 7, but this needs further investigation to determine the efficacy of such a schedule.

Inactivated vaccines may be given at day 1 whether CE is given or not. Killed vaccines may also be given later in the rearing period, for example at 12 weeks.

5.3.2 Booster vaccinations

For both live and killed vaccines a minimum of two doses is required during the rearing period. Examples of vaccination schedules known to be effective are given in Table 1. It is recommended that the minimum interval between final vaccination and the onset of laying is 3-4 weeks for live vaccines and 2 weeks for killed vaccines. This is to ensure peak immunity at the onset of laying and also in the case of live vaccines to minimise the chances of the vaccine strain being transmitted in or on the eggs.

5.3.3 Special considerations

During the laying period it is possible to vaccinate with live vaccines in the case of breeders, but not commercial layers, because of the risk of egg transmission of the vaccine strain.

5. COMPETITIVE EXCLUSION AND VACCINES, AND VACCINES AND ANTIMICROBIALS

5.1 Introduction

The successful control of Salmonella in poultry is fundamentally based on the use of good hygiene and husbandry on the farm. The application of competitive exclusion products (normal gut flora) and the use of vaccines with and without antimicrobials are additional management tools which may be of benefit in the control of Salmonella, with particular reference to *S. enteritidis* and *S. typhimurium*. It should be noted that the primary aim of Salmonella control is to prevent these organisms from entering the food chain.

Killed vaccines may be given during the laying period, but it should be noted that this may have an adverse effect on production.

Antimicrobial treatment immediately before, during or immediately after vaccination with live vaccines should be avoided. If emergency treatments are necessary at these times, then the vaccination must be repeated. Present day growth promoters and anticoccidials do not interfere with live *Salmonella* vaccines. Antimicrobial treatment and the simultaneous application of inactivated vaccines are completely compatible.

It is strongly recommended that the *Salmonella* prophylaxis is carried out only by the use of vaccines and/or CE. The use of antimicrobials should be restricted to epidemiologically justified veterinary intervention and be guided by GMP which are covered in the following guidelines.

5.3.4 Use of vaccines and antimicrobials

For infected flocks, the following schedule may be applied:

- Antimicrobial treatment with a suitable drug for 5 to 10 days. This should be started in breeders and commercial layers as early as possible after confirmation of the infection.

- If live vaccines are used, a treatment-free interval (1 to 3 days according to the properties of the drug) must be observed. Under these circumstances there may be an adverse effect of the drug on the efficacy of the vaccine. Therefore, it is advisable to vaccinate the birds twice on 2 consecutive days and to revaccinate at an interval of 6 to 8 weeks.
- Inactivated vaccines can be applied simultaneously with antimicrobials. At least one revaccination at an interval of 4 to 6 weeks is recommended.

5.3.5 Recommended research

- The impact of vaccination without accompanying measures on the prevalence of salmonellae in infected flocks.
- The effect of transferring birds to clean premises on the effectiveness of vaccination and/or antimicrobial treatment.
- Necessary duration of antimicrobial treatment prior to use of normal gut flora or vaccination and the optimum interval between antibiotic treatment and each of the other measures.



Table 1 – Examples of vaccination schedules in current use

	1d (*)	1w (*)	2w	3w	4w	7w	12w	15w	18w	22w (**)	60w
Killed	✓				✓						
Killed	✓				✓				✓		
Killed							✓	✓			
Live	✓		✓	✓							
Live	✓		✓	✓				✓			
Live	✓				✓	✓		✓			
Live	✓				✓				✓		

(*) d = day; w = week

(**) point of lay

6. QUALITY ASSURANCE, SAFETY AND LEGAL ASPECTS

6.1 Vaccines and antimicrobials

Vaccines and antimicrobials are medicines. Consequently, only licensed antimicrobials and vaccines should be used for the control of *Salmonella* infections. Licensing is based on quality, safety and efficacy criteria.

6.2 Competitive exclusion flora

Since exclusion flora preparations cannot be defined in the same manner as either a vaccine or a veterinary medicinal product, WHO should recommend that the authorities create a special product category called "NORMAL GUT FLORA".

In relation to the avian intestinal tract, "normal gut flora" is an undefined preparation of live obligate and facultatively anaerobic bacteria originating from normal, healthy, adult individuals of an avian species, which is free from specific pathogenic micro-organisms and is quality controlled. The purpose of such a preparation is to compensate for any deficiencies in the composition of the normal intestinal microbiota that relate to the natural control of undesirable micro-organisms and arise from modern systems of poultry production.

"Normal gut flora", as defined above, should be distinguished from live "probiotics" which are preparations of only one or a few strains of micro-organisms, the primary purpose of which is to improve animal performance.

It is recommended that WHO should collaborate with FAO and OIE in elaborating the criteria for a licensing procedure for this type of product. Companies producing "normal gut flora" for commercial purposes should license their preparations according to the new guideline and apply good laboratory and manufacturing practices in order to guarantee the safety of the product and the desired response in recipients.

6.3 Legal aspects

The combination of treatments proposed within these guidelines should not overrule any local legal provisions.

6.4 Education

As neither eradication nor treatment can give an absolute guarantee of freedom from *Salmonella* in the final product the workshop participants recommend that WHO urges Member States to initiate further education of retailers and consumers on the proper hygienic handling and treatment of foodstuffs.

7. INTERACTION BETWEEN MONITORING AND TREATMENT

7.1 Objective

To evaluate the response to treatment;

To evaluate the effectiveness of control measures in order to allow reintroduction of the treated flock in a quality assurance scheme.

7.2 General comments

Microbiological testing is generally necessary whether or not the serological test is positive (see Table 2), whereas serology is a screening method and, depending on the antigen preparations used, there may be differences in specificity and sensitivity.

Antibacterial medication alone is likely to have only a temporary effect.

A number of treatments proposed during this workshop will or could interfere with the detection and monitoring of *Salmonella* infected poultry flocks.

The following proposals should be considered as a supplement to the WHO guidelines on detection and monitoring of *Salmonella* infected poultry flocks with particular reference to *Salmonella* enteritidis.

Therefore, for treated flocks, the guideline should be adapted to take account of the possible interaction between vaccination, the use of antimicrobials and competitive exclusion on the one hand, and the monitoring scheme on the other hand.

Table 2

	Serology	Microbiology
a. Recent infection	Negative	Positive
b. Established infection or high rate of excretion	Positive	Positive
c. Chronic infection with little excretion	Positive	Negative (or variably positive)

Major interactions are expected to occur if animals are treated with antimicrobials or with vaccines. No major interaction with the monitoring scheme is expected if only competitive exclusion preparations ("normal gut flora") are used.

All *Salmonella* vaccines will interfere with serological monitoring. In addition, live vaccines may interfere with bacteriological testing. Thus, the vaccine strain should be "marked" to distinguish it from wild strains. WHO should coordinate research and development towards a vaccine that allows a clear distinction between infection and seroconversion due to vaccination. Meanwhile, serological tests following vaccination should be serotype specific and serotypes other than the vaccine serotype should be included in the test.

Antimicrobials are expected to interfere with bacteriological monitoring. In addition to the bacteriological testing described in the guideline it is recommended that serological monitoring is also carried out, the aim being to find out more about the effectiveness of the antimicrobial used and any eventual recurrence of infection.

In the case of treatment by either vaccination or use of antimicrobials, intensified monitoring should reveal an absence of *Salmonella* infection. Only under these conditions can flocks be declared successfully treated and receive a certificate indicating such a status (see Annex 2).

7.3 Intensified monitoring

Following infection and treatment, samples of fresh caecal droppings should be taken at 2-weekly intervals up to the point of transferring the birds. These will be used for bacteriological examination as in the guideline (Footnote 3). The

minimum requirement for broilers is one negative sampling. A certificate indicating successful treatment can only be issued if at least the last three samplings are negative.

7.4 *Salmonella* monitoring of breeder flocks

7.4.1 Day-old delivery from grandparent flocks Day-old chicks

- a) vaccinated with inactivated vaccine : follow Section 2.2 in the relevant WHO guideline (Footnote 3).
- b) vaccinated with live modified vaccine : the relevant WHO guideline (Footnote 3) can be followed providing the vaccine is "marked". Adapt culture media to the marker used for the vaccine strain. As part of a quality assurance scheme, it is recommended that serological testing of day-old chicks is carried out. A positive serological result from chicks derived from a grandparent flock which is not supported by a certificate of successful treatment should be considered as an unacceptable risk.
- c) Antimicrobial treatment : where antimicrobial treatment is used either at the hatchery (including egg dipping or egg injection) or on the farm for day-old chicks, the bacteriological testing may show false negative results. Consequently, serological testing at day-old is strongly recommended.

7.4.2 Seven-day-old birds

- a) vaccinated with inactivated vaccine during the first 7 days : follow Section 2.2 of the relevant WHO guideline (Footnote 3).

- b) vaccinated with live modified vaccine during the first 7 days: follow the relevant WHO guideline (Footnote 3). Adapt culture media to the "marker" used for the vaccine strain.
- c) antimicrobial treatment : where antimicrobial treatment is used either at the hatchery (including egg dipping or egg injection) or on the farm for day-old chicks over the first 7 days, the bacteriological testing may show false negative results. Consequently, serological testing is strongly recommended in addition to the bacteriological monitoring described in the guideline.

7.4.3 Four-week old birds

- a) vaccinated with inactivated vaccines : follow the relevant WHO guideline (Footnote 3).
- b) vaccinated with live modified vaccines : follow the relevant WHO guideline (Footnote 3) and adapt culture media to the "marker" used for the vaccine strain.
- c) antimicrobial treatment : sampling should be limited to fresh caecal droppings (option (a) of the guideline (Footnote 3)). Where antimicrobial treatment is used either in the hatchery (including egg dipping or egg injection) or over the first 4 weeks on the farm, the bacteriological testing may show false negative results. Consequently, in addition to the bacteriological monitoring in the guideline (Footnote 3), serological testing is strongly recommended.

7.4.4 Sixteen and 22-week old birds

The recommendations are the same as those for 4-week old birds.

7.4.5 After 22 weeks/during production

If an infected flock is treated during production, it is essential to sample the eggs present in the hatchery as well. This should be done in addition to the intensified monitoring scheme for the flock, in order to take all possible precautions. In addition to the treatment of hens, it is recommended that systematic egg dipping or egg injection is begun alongside recognised methods, as described in

Annex 3.

Hatching eggs produced 3 weeks prior to the diagnosis of infection must be identified and sampled according to the method described in Section 2.4 of the relevant WHO guideline (Footnote 3).

Chicks hatched from these eggs should be monitored serologically and bacteriologically.

Following infection and treatment, fresh caecal droppings should be sampled at 2-weekly intervals for bacteriological examination. A serological surveillance scheme should be implemented for the hen flock and a statistically relevant group of hens should be followed serologically on a 2-weekly basis (see relevant WHO guideline, Section 2.2 (Footnote 3)).

7.5 *Salmonella* monitoring of grandparent and elite flocks

Taking into account the well recognised risk of spreading infection from elite and grandparent flocks to the rest of the breeding pyramid, many countries prohibit treatment of grandparent and elite flocks, and insist upon slaughter.

If it is decided to treat infected flocks, all monitoring schemes and precautionary measures should aim at reducing the risk of spreading the infection. In that context, samples should be doubled in numbers and not pooled. Should there be no statutory control of *Salmonella* in grandparent and elite flocks, and treatment is implemented, then modifications in monitoring are recommended as for parent flocks, taking into account the doubling of the number of samples required in the WHO guideline (Footnote 3, Section 2.3).

7.6 *Salmonella* monitoring of the birds

7.6.1 Broilers

A. Day-old chicks

- a) vaccinated with inactivated vaccine : follow Section 2.2 of the relevant WHO guideline (Footnote 3) as for breeder flocks.
- b) vaccinated with live modified vaccine : guideline (Footnote 3) can be followed providing the vaccine is "marked". Vaccination with

live vaccine against Salmonella at day 1 requires modification of monitoring as described for parent flock day-old chicks. As part of a quality assurance scheme, it is recommended that serological testing of day-old chicks is carried out. A positive serological result for chicks from a parent flock which is not supported by a certificate of successful treatment should be considered an unacceptable risk.

- c) Antimicrobial treatment : Where antimicrobial treatment is used either at the hatchery (including egg dipping or egg injection) or on the farm for day-old chicks, the bacteriological testing may show false negative results. Consequently, serological testing at day-old is strongly recommended.

B. *Monitoring after treatment*

Treatment with antimicrobials should take into account the manufacturer's withdrawal time. Sampling can only start 5 days after antimicrobial treatment, but should continue as close as possible to slaughter (recommendation: slaughter date less 10 days).

At least one statistical sampling of caecal droppings (or bird ceca, gall bladder, liver) should be negative.

Following negative results for Salmonella, a certificate of successful treatment can be issued.

Notification of diagnosis, eventually accompanied by a certificate of successful treatment should accompany the birds to the slaughterhouse.

7.6.2 Layers

A. *During entire rearing period*

The modification of the monitoring scheme associated with treatment of layers is the same as that for the parents.

B. *At start of, or during production*

Replacement layers treated around the period of movement or during production : eggs should only be used for human consumption after the withdrawal period recommended by the manufacturer of the antimicrobial. Eggs produced after the withdrawal period and before the certification of successful treatment should not be marketed as fresh eggs for direct human consumption except after an appropriate treatment which gives a sufficient guarantee that Salmonella has been inactivated. A certificate of successful treatment requires the same conditions as those stated in the guideline for parent stock.

In addition, following certification of successful treatment, fresh caecal droppings should be sampled at 2-weekly intervals for bacteriological examination. A serological surveillance scheme should be implemented for the hen flock, and a statistically relevant group of hens followed serologically on a 2-weekly basis, throughout the further production period.

For a flock found to be infected with Salmonella, eggs can only be delivered for direct human consumption after a certificate of successful treatment has been obtained and provided that the intensified monitoring plan has been implemented and controlled.

For sampling of caged birds, follow Section 2.5 of the relevant WHO guideline (Footnote 3).

Annex I

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Annex 2 Certificate of successful salmonella treatment

Parent Stock / Laying Flock

FLOCK IDENTIFICATION:

Name of owner/stockman

Address

Breed and/or brand name

Source

Breeder code

Date of placement

NUMBER OF BIRDS: Hens :

Cockerels:

18

HOUSING:

Type of building

Litter

Disinfection prior to placement

FEED SOURCE AND CONTROL

Identification

Date of delivery

Quantity

SALMONELLA TREATMENT AND POST-TREATMENT MONITORING

Treatment

Confirmation of diagnosis (date)

Type of treatment

Lot no.

Mode of application

Treatment started (date):

Treatment ended (date):

Annex 3

Egg Dipping

Equipment and materials

Dipping tank	Commercially available
Antimicrobial	Gentamycin sulphate

Technical data

Pressure used	500 mbar
Time under pressure	10 minutes
Time under atmospheric pressure	10 minutes
Antimicrobial dose	1.5g Gentamycin sulphate/l water (about 1000 ppm Gentamycin)

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Procedure

Clean or washed, uncracked hatching eggs must be totally immersed in antimicrobial solution under 500 mbar pressure for 10 minutes, then left in the same solution under atmospheric pressure for a further 10 minutes before being removed.

After treatment broken eggs must be discarded before moving the treated eggs to an incubator.

Precaution

The dipping solution must be monitored every two weeks for gentamycin concentration and microbial contamination.