National Poultry Salmonellosis Control Plan

Sri Lanka - 2026

— Department of —

Animal Production and Health

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1. Introduction

Salmonellosis is one of the most significant foodborne zoonotic diseases and remains as a major disease of concern due to its considerable impact on human health, animal health, and national economies. The World Organization for Animal Health (WOAH) identifies pullorum and fowl typhoid, caused by serovar Gallinarum, as notifiable diseases due to their severe economic consequences and potential for rapid spread within poultry populations. The WOAH as well as the World Health Organization (WHO) recognize non-host-specific Salmonella serovars as major zoonotic agents responsible for a substantial proportion of foodborne gastroenteritis in humans worldwide, frequently linked to contaminated poultry, eggs, and poultry products.

The economic impact of salmonellosis includes production losses, increased mortality, reduced feed efficiency, and trade restrictions. In addition, the public health risk associated with foodborne salmonellosis highlights the importance of effective control measures in animal populations.

The Department of Animal Production and Health (DAPH) conducts surveillance and control programs for poultry salmonella, mainly focusing on poultry breeder farms, export-oriented poultry processing establishments and pet bird establishments and layer farms. However, there are gaps in current surveillance, control and monitoring programs indicating the need for a comprehensive and systematic national control plan. This national poultry salmonellosis control plan includes national surveillance, prevention, control, and monitoring programs in grandparent farms, parent farms, commercial layer farms, poultry processing establishments, backyard poultry, and pet bird establishments.

1.1 Objectives

The main objective is to reduce and to minimize the prevalence of salmonella in poultry farms, poultry meat, meat products, and eggs, to minimize the public health risk of salmonellosis in Sri Lanka and facilitate the exportation of birds, meat, meat products, eggs.

Specific objectives

To establish systematic poultry salmonella surveillance

To update poultry salmonella preventive and control programs in farms

To ensure Salmonella-free safety of poultry meat, meat products and eggs.

2. Overview of Salmonellosis

The genus Salmonella belongs to the family Enterobacteriaceae and consists of two main species: Salmonella enterica and Salmonella bongori. Out of these, Salmonella enterica is of the greatest

importance in poultry and public health. More than 2,600 serovars have been identified and around 1500 serovars account for over 99% of all Salmonella infections in warm-blooded animals and humans. Salmonella infections in poultry, primarily caused by Salmonella enterica subspecies enterica, are of particular concern because poultry meat and eggs are among the most common sources of human salmonellosis.

Fowl Typhoid and Pullorum Disease, are host-adapted to chickens and turkeys and often result in high mortality, especially in young birds (S. Gallinarum/S. Pullorum). In contrast, non-host-adapted serovars such as S. Enteritidis and S. Typhimurium usually cause subclinical infections in poultry but pose major zoonotic risks. Emerging serovars of global concern include Salmonella Infantis, Salmonella Kentucky, Salmonella Heidelberg, Salmonella Newport, and monophasic Salmonella Typhimurium, many of which are associated with poultry and human foodborne disease.

Transmission occurs through both vertical and horizontal routes. Vertical transmission from infected breeders to chicks via eggs is a key driver of persistence in production systems, while horizontal spread occurs through contaminated feed, water, litter, equipment, rodents, insects, and personnel. Poor biosecurity, inadequate farm hygiene, and improper handling at hatcheries and processing plants increase the risk of contamination and spread. Live bird markets and backyard farms represent additional high-risk environments due to mixed sourcing of birds, overcrowding, and suboptimal sanitary conditions.

Clinical signs of poultry salmonellosis vary with bird age, immune status, infecting serovar, and environmental conditions. High mortality may occur in flocks affected by biovar Pullorum (pullorum disease) or biovar Gallinarum (fowl typhoid). Biovar Pullorum mainly affects young chicks in the first 2–3 weeks of life, causing depression, white diarrhea, poor growth, and high mortality, whereas biovar Gallinarum primarily affects growers and adult birds, leading to septicemia, reduced egg production, and high mortality. Typical post-mortem lesions include hepatomegaly, splenomegaly, pericarditis, peritonitis, and white necrotic foci in the liver or spleen. Non–host-adapted Salmonella often circulates sub-clinically, with many birds acting as asymptomatic carriers; therefore, flock-level surveillance, bacteriological testing, and serology are essential for early detection and control.

Human salmonellosis typically results from consuming contaminated poultry meat, eggs, or egg products. Children, the elderly, and immunocompromised individuals are particularly vulnerable.

Therefore, controlling Salmonella in poultry is not only vital for animal health and production, but is also a key public health priority.

3. Situation Analysis

3.1 Global situation of Salmonella

Salmonella infections are reported worldwide and are considered endemic in most poultry-producing regions. In many countries, pullorum and fowl typhoid have been successfully eradicated from commercial operations through stringent testing, culling, and biosecurity protocols, yet they persist in backyard and small-scale farming systems. Non-host-adapted serovars such as S. Enteritidis and S. Typhimurium remain widespread globally and account for the majority of human foodborne salmonellosis outbreaks linked to poultry products.

Regions with intensive poultry production, including Europe, North America, and parts of Asia, have implemented comprehensive control strategies involving surveillance, breeder flock testing, vaccination, and stringent hygiene standards. These integrated approaches have substantially reduced Salmonella prevalence in commercial poultry. However, the pathogen remains a major challenge in low-and middle-income countries, where resource constraints, fragmented production systems, and limited regulatory oversight contribute to persistent contamination and recurring outbreaks.

3.2 Salmonella Situation in Sri Lanka

Sri Lanka has a growing poultry industry consisting of grandparent farms, parent, commercial layers, broilers, small scale producers and backyard flocks. The poultry population is as follows: broiler grandparent mil. 0.05, broiler parent mil.1.80, layer parent mil. 0.15, poultry commercial broiler mil.15.42, commercial layer mil.15.40 and backyard poultry mil'. 2.70 (DAPH, statistical bulletin, 2024). A substantial proportion of the poultry population is concentrated in the Western and North Western Provinces, with together account for approximately 37% of the poultry population in Sri Lanka.

Pullorum and fowl typhoid are notifiable diseases under the Animal Diseases Act No. 59 of 1992 Sri Lanka. The history of poultry salmonella in the country, Pullorum disease and fowl typhoid are recorded among major poultry disease conditions between 1975–1979 in Sri Lanka (wickramasuriya U. G. J. S, 1985). In the 1990s**,** there was evidence that non-host-specific salmonellosis was increasing in poultry in Sri Lanka as the industry expanded (Priyantha, M.A.R., 2009). In breeder farms and hatcheries, S. Enteritidis has detected in previously identified

Salmonella carrier farms and hatcheries in 2008 (Wijemanne et al., 2008). During 2011–2012 S. Gallinarum, S. Enteritidis, and Salmonella Typhimurium in breeder flocks were identified, while S. Enteritidis, S. Gallinarum, and S. Pullorum were detected in hatchery samples. Breeder farms have shown evidence of carrier status from 2008 to 2016, while Salmonella organisms were isolated from some hatcheries from 2011 to 2014 (DAPH Annual report, 2008; 2011; 2014; 2016). Motile Salmonella serotypes such as S. Typhimurium, S. Enteritidis, S. Kentucky, Salmonella Chester, Salmonella Muenster and S. Infantis have been identified in poultry farms, broiler meat, and processing environments in Sri Lanka, illustrating that foodborne salmonellosis is an established concern in the poultry sector (Kottawatta et al. (2014) and Jayaweera et. Al. , 2020).

3.2.1. History of salmonella control in Sri Lanka

The Department of Animal Production and Health (DAPH) has conduct a salmonella control program in poultry for more than 20years mainly targeting poultry breeder farms. A structured and intensive control program was officially launched in 2001, involving serological screening of all breeder flocks for Salmonella Gallinarum. Initially, the program was implemented on a voluntary basis with support from DAPH, but by 2003, it was made mandatory for all registered poultry breeder farms. (Veterinary epidemiological bulleting, Sri Lanka, volume 3. No. 1, 2009)

The control program includes periodic screening of breeder flocks by whole blood agglutination test and culling of serologically positive birds to prevent vertical transmission. In addition, regular monitoring of commercial hatcheries is carried out, including isolation and identification of Salmonella organisms using standard bacteriological methods, in accordance with the national procedures for prevention, control, and eradication of pullorum disease and fowl typhoid.

Although Sri Lanka has implemented a Salmonella control program (Pullorum and Fowl Typhoid control program in poultry breeder farms) for many years, the scope of this program was limited to grandparent and parent breeder operations. While it contributed significantly to reducing vertical transmission and improving flock health within the breeding sector, coverage of other critical sectors of the poultry value chain is very important. Biosecurity compliance varies significantly across different production systems, and the absence of a national monitoring system covering all production systems limits the ability to track trends and respond effectively to emerging risks. However, in 2022, Salmonella surveillance and control activities were initiated in an export-oriented poultry processing plant.

4. National surveillance program

The National Salmonella surveillance program for poultry includes surveillance in poultry grandparent, parent, commercial layer, commercial broiler, backyard poultry, export-oriented pet bird establishments, and poultry processing plants (broilers, poultry meat and meat products) and poultry imports. Both active and passive surveillance are conducted. In passive surveillance, clinical cases of suspected salmonellosis or other diseased birds are presented to the VIC or Government Veterinary Office (GVO). The active surveillance is mainly conducted by relevant VIOs.

The active surveillance programs are as follows:

a. Salmonella in poultry Grand Parent (GP) and parent farms
    - Screening of birds by Whole Blood Agglutination Test (WBAT)
    - Isolation and identification of Salmonella from the reactors of WBAT
    - GP and parent hatchery testing for salmonella
b. Commercial layer and back yard poultry
c. Surveillance in poultry Imports and export
    - Importation of DOC, meat products and pet birds
    - Export-oriented pet bird establishments, broilers, poultry processing establishments and commercial layer farms.

4.1 Salmonella in poultry Grand Parent (GP) and parent farms

The surveillance is conducted in all poultry GP and parent farms registered in the country. Poultry GP farms/parent farms follow the procedure adapted for the surveillance and control measures by the Department of Animal Production and Health (DAPH) with the objective of minimizing Salmonella and achieving Salmonella-free status. Salmonella surveillance in GP and parent farms includes mainly flock screening using the whole blood agglutination test (WBAT), isolation and identification of Salmonella from the reactors of WBAT, hatchery testing for salmonella and environmental sampling of the farm (rearing facilities).

4.1.1 Screening of Grand-Parent (GP) and parent birds by Whole Blood Agglutination Test (WBAT)

The Whole Blood Agglutination Test (WBAT) is conducted only in salmonella non-vaccinated parent farms.

- The owner or the farm management test all the birds in the flocks at 45 to 60-day intervals, starting from 16 weeks of age, prior to the 5% production using the WBAT with stained standard Salmonella antigen (refer Appendix A). The stained Salmonella antigen for WBAT used for this test is a product of Veterinary Research Institute.
- The number of samples is determined based on a standard statistical table. The sample size is calculated to detect 1% prevalence at a 95% confidence interval.
- The WBAT is performed by the Veterinary Research Officer (VRO) at the bacteriology division for grandparent farms and by the Veterinary Investigation Officer (VIO)of the relevant area for parent farm
- It is a responsibility of the farm authority to test the whole flock at 16wks age and inform the relevant VRO or VIO for testing. The farm authority may use commercially available or VRI produced stained Salmonella antigen for WBAT.
- In determination of the number of birds testing

  - If a single batch of birds is housed in an enclosure without partitions, and the enclosure is divided by wire mesh partitions, the total number of birds is considered the population size for determining the sample size.

  - If the enclosure is divided by solid walls, the birds in each partitioned area is regarded as separate flocks, and the sample size is determined accordingly.

  - In cases where one batch of birds is distributed across multiple houses, each house is treated as a separate population unit.

  - If the birds are raised in different locations, each location should be managed in the same manner and treated as a separate population unit.

- If the flock is negative, re-test of birds at age 56 weeks (after the peak production)
- If farm is positive follow the guidelines for corrective action taken for salmonella positive farm/establishment (refer annex 5-Corrective action taken for salmonella positive farm/establishment).

4.1.2 Isolation and identification of Salmonella

Laboratory testing of samples for salmonella is performed by the VRO bacteriology division or the relevant VIO. The samples are collected from the reactors of the WBAT and environmental samples.

a. The reactors of WBAT grand-parent and parent flocks

It is recommended for the isolation and identification of Salmonella from the reactors. The reactors detected in WBAT are sacrificed and tissue samples (liver, spleen, cecum/cecal tonsils, ovaries, reproductive tract) are collected. The laboratory isolation and identification of salmonella is conducted either in the VRI bacteriology division (GP farms) or VIC (parent farm). Serotyping is performed in the VRI bacteriology division.

b. Environmental sampling of the farm (rearing facilities)

The surveillance of flock environment includes litter sample (boot swabs), feed, and water. The isolation and identification of salmonella in the flock environment is conducted with the targeting all serovars of poultry Salmonella. The VIO collect boot samples, feed and water samples bi-annually during breeder farm visits every six months. In addition, the farm authority also sends samples to VRI or VIC for laboratory testing in order to test all the batches in the farm (refer Appendix B-Protocol for isolation and identification of Salmonella in boot swabs). The age of sampling is preferably 32 and 48 weeks. The representative feed samples and water samples are collected and tested (refer No 4.5 and No 4.6 - feed and water sampling) (refer Appendix C-Protocol for isolation and dentification of Salmonella in feed and water)

Boot swabs: A pair of commercial boots or autoclaved cotton socks is considered as a sample. The sample collector should wear the polyethene cover on his legs and then wear socks in boot sampling to minimize cross-contamination.

- In a GP farm, a total of five samples shall be taken to represent all four lines of birds: A, B, C, and D, In a closed house. At least 100 feet at the perimeter need to be walked with a pair of socks.
- In a parent farm, 2 samples are collected from each flock with single cage housing. If it is multiple cages, one sample is collected from one cage and at least 3 cages are included for the sampling. Further, at least 100 feet along the perimeter must be walked while wearing a pair of socks (refer annex 1- Sample collection and laboratory testing of samples for Salmonella).

4.1.3 GP and parent hatchery testing for salmonella

The surveillance of the hatchery is performed to take precautions entering Salmonella infected/contaminated chicks to poultry commercial layer and broiler farms. The hatcheries are tested for Salmonella. The samples are collected quarterly from each hatchery by a VRO/VIO

under the salmonella control program conducted by the Animal Health Division /DAPH. The laboratory testing is performed in either VRI or VIC and serotyping is performed at the bacteriology division at VRI. In addition, laboratory testing of samples is conducted by the farm authority either from farm own laboratory or external laboratory (government or accredited laboratory) at 32, 40, and 48 weeks of age. The results must be documented at the hatchery for auditors during their visits, as necessary.

The hatcher is considered as the sample unit.

Types of samples; (refer annex 1- Sample collection and laboratory testing of samples for Salmonella).

- Fluff - Fluff from the relevant hatcher (refer Appendix D-Protocol for isolation and identification of Salmonella in fluff)
- Unhatched eggs (Egg surface washings) or crushed eggshells -10 samples (eggs/ eggshell) per hatcher (Refer Appendix E-Protocol for isolation and identification of Salmonella in unhatched eggs (egg surface washing) or egg shell)
- Dead in Shell (DIS) – 10 dead in shell. (refer Appendix F-Protocol for isolation and identification of Salmonella in Dead in Shell (DIS
- Meconium- from 50 chicks per flock (refer Appendix G - Protocol for isolation and identification of Salmonella in meconium sample)
- Internal organs (yolk, liver, spleen) from dead chicks – Maximum 5 chicks per sample (refer Appendix H- Protocol for isolation and identification of Salmonella in internal organ (yolk, liver, spleen) from dead chicks)

4.2 Commercial layer

The samples are collected from commercial layer farms in the country. The farms are selected randomly and cloacal swab samples are collected from 300 farms, 3 pool samples per farm 5 cloacal swabs are pooled into 1 sample (300×3×5 = 4500) (refer annex 1- Sample collection and laboratory testing of samples for Salmonella). The sample collection and laboratory testing are performed by relevant VIO. The number of samples for each district is calculated to the ratio of the commercial layer population. The isolated Salmonella is sent to bacteriology division/VRI for serotyping (refer Appendix I-Protocol for isolation and identification of Salmonella in cloacal swabs).

4.3 Backyard

The samples are collected from backyard poultry farms in the country. The farms are selected randomly and cloacal swab samples are collected from 300 farms, 3 pool samples per farm 5 cloacal swabs are pooled into 1 sample. However, the number of pooled samples vary with the number of birds in the farm (refer annex 1- Sample collection and laboratory testing of samples for Salmonella). The sample collection and laboratory testing are performed by relevant VIO (refer Appendix I- Protocol for isolation and identification of Salmonella in cloacal swabs). The number of samples for each district is calculated to the ratio of the backyard population. The isolated Salmonella is sent to bacteriology division/VRI for serotyping.

4.4 Surveillance in poultry imports and export

4.4.1 Importation of Day-Old Chicks (DOC), meat products and pet birds

a. Day Old Chicks (DOC) for breeder farms (parent or grand-parent)

Each consignment of imported day-old chicks is tested for Salmonella at arrival as part of the import health protocol. The Animal Quarantine Officer (AQO) collects and submits to the Veterinary Investigation Centre (VIC) of the area or VRI. The samples include average of 1 to 3 pooled meconium samples (pool meconium of 30 chicks). Swabs from meconium-contaminated papers/material in the chick boxes (1- 3 pooled samples, 5 swabs pool in 1 pooled sample) and samples from 3 to 5 dead chicks (if available). During the quarantine period of 30 days 'on farm quarantine' period, at day 28, 6 pooled samples, 5 swabs pool in 1 pooled sample (6×5=30) of cloacal swabs are collected for laboratory testing for Salmonella (refer annex 1- Sample collection and laboratory testing of samples for Salmonella). The number of samples may adjust depend on the size of the consignment. The isolated Salmonella is sent to bacteriology division/VRI for serotyping

b. Meat and meat products

Imported meat and meat products are tested by the Medical Research Institute (MRI). The samples are collected by the quarantine officers of Dept. of Animal Production and Health and dispatch to MRI for laboratory testing. The laboratory report is issued to quarantine office and Veterinary Regulatory Affairs Division and Animal Health Division/ DAPH.

c. Pet birds

The cloacal swabs samples are collected from imported birds at arrival and at the end of quarantine period (28th day of quarantine). The swabs of fresh wet droppings samples are collected (5 swabs

pool depending on the number of birds 10 % of birds) from the cages or cloacal swabs from at least 10% birds (refer annex 1- Sample collection and laboratory testing of samples for Salmonella). The laboratory testing of sample is performed VIC and the isolated Salmonella is sent to bacteriology division/VRI for serotyping (refer Appendix I-Protocol for isolation and identification of Salmonella in cloacal swabs).

4.4.2 Export-oriented pet birds, poultry processing establishment and commercial layer

The surveillance program is conducted in export- oriented processing plants, broiler farms, layer farms and pet bird farms. The samples are tested in VRI or VIC (Refer. annex 1Sample collection and laboratory testing of samples for Salmonella).

a. Export oriented broiler farms (broiler bird supplier farm to export-oriented poultry processing plant)

The broiler birds received to the processing plant are tested for salmonella. The broiler supplier farms registered in relevant processing plant are under the broiler surveillance program. The birds are selected randomly and cloacal swab samples are collected from 30 birds, 6 pooled samples (5 cloacal swabs are pooled into 1 sample) bi-annually and transport samples at 2-8^{0}C (refer annex 1- Sample collection and laboratory testing of samples for Salmonella). The sample collection and laboratory testing are performed by relevant VIO. The isolated Salmonella is sent to bacteriology division/VRI for serotyping (refer Appendix I-Protocol for isolation and identification of Salmonella in cloacal swabs).

b. Export-oriented poultry processing establishment

The samples are collected by relevant VIO biannually according to the sampling plan. The laboratory isolation and identification of samples is performed at Central Veterinary Investigation Center (CVIC)/VRI and serotyping at bacteriology division VRI.

The samples are collected from processing area, evisceration room, packing section, cold room, equipment, packing material, water, workers hand ect. (refer annex 2- Export-oriented poultry processing plant sample plan).The sample testing performed described by the SLS guideline in Sri Lanka (Source: SLS 1161:2003).Further, workers are tested for salmonellosis by the Medical Officer of Health (MOH) or registered medical laboratory twice a year with the request of processing establishment authority.

export-oriented poultry layer farms

In export -oriented poultry layer farms, eggs and birds' samples are tested for Salmonella.

- Testing of eggs

One egg surface washing sample is collected from the eggs of one flock. (refer annex 1- Sample collection and laboratory testing of samples for Salmonella). The number of samples depends on the no. of flocks in the farm. The sample collection and laboratory testing are performed by the relevant VIO (refer Appendix J-Protocol for isolate from table eggs).

The isolated Salmonella is sent to bacteriology division/VRI for serotyping.

- Cloacal swabs

  The birds in farms are randomly selected and cloacal swab samples are collected from each flock of the farm. The number of 6 pooled samples per farm 5 cloacal swabs are pooled into 1 sample (6×5 = 30) (refer annex 1- Sample collection and laboratory testing of samples for Salmonella). The sample collection and laboratory testing are performed by relevant VIO. The isolated Salmonella is sent to bacteriology division/VRI for serotyping (refer Appendix I- Protocol for isolation and identification of Salmonella in cloacal swabs).

- Boot swabs

  A pair of commercial boot or autoclaved cotton socks is considered as one sample. Sample collector should wear the polyethene cover on his legs and then wear socks in boot sampling to minimize the cross-contamination

  In a farm, 2 samples are collected from one flock with single cage housed. If it is multiple cages one sample from one cage an at least 3 cages are included for the sampling. Further, at least 100 feet of the perimeter must be walked while wearing a pair of socks (refer annex 1- Sample collection and laboratory testing of samples for Salmonella).

- Fan belt samples

  In battery cages, 10 swab samples are collected from each fan belt and pooled in to one sample. The samples are collected from each house of the farm (refer annex 1- Sample collection and laboratory testing of samples for Salmonella).

4.5. Feed:

Feed samples from poultry breeder farms, export-oriented farms, are tested for Salmonella. The samples are collected from both feed storage and feeders. The feed samples may originate from starter, grower, breeder, layer etc... At least three feed samples (25 g each) need to be collected to sterile bags (refer annex 1- Sample collection and laboratory testing of samples for Salmonella). The bi-annual sample collection and testing is performed in VICs. The isolated Salmonella is sent

to bacteriology division/VRI for serotyping (refer Appendix C-Protocol for isolation and dentification of Salmonella in feed and water). Testing of feed samples is recommended annually by farm authority in commercial layer and broiler. In export-oriented establishment all these reports are to be inspected/audited by the biosecurity inspection team and auditing team.

4.6 Water

Water samples from poultry breeder farms, export-oriented farms, are tested for Salmonella. If water samples are collected from farms, the samples are supposed to originated from well, tube well, water tank, tap tine and waterers from cages. At least three water (25ml) samples need to be collected from waterers for a clean container and used for the isolation and identification process (refer annex 1- Sample collection and laboratory testing of samples for Salmonella). When multiple cages are involved, a single sample from a single cage is collected. In addition, at least two samples from the source/well, and storage tank are collected. The sample collection is done by relevant VIO bi- annually from export-oriented establishment. It is responsibility of poultry breeder farm and hatchery authority to test water samples by their own or provide samples to the biosecurity inspection team or out sourcing. The laboratory isolation and identification of Salmonella is performed either in VRI bacteriology division or VIC. Serotyping is performed in VRI bacteriology division (refer Appendix C- Protocol for isolation and dentification of Salmonella in feed and water). In export-oriented establishment all these reports are to be inspected/audited by the biosecurity inspection team and auditing team

5. Salmonella control program

The salmonella control program consists of salmonella control, prevention and monitoring in breeder farms, hatcheries, imports and exports, poultry processing establishments, live bird markets, and commercial layer and broiler as mentioned below.

- Grandparent and parent farms
- Poultry hatcheries
- Import of day-old chicks, pet birds, and poultry meat and meat products
- Export-oriented broiler farms, layers and poultry processing plants (meat and meat products)
- Export-oriented pet bird establishments
- Live bird market/wet market
- Commercial layer
- Commercial broiler

- Backyard poultry

All the measures are taken to control and prevent the disease in the farms/establishments and spreading. The control program targets food security for availability of healthy birds for human consumption and food safety for public health. In the development of the salmonella control program, the following areas are addressed

a. Biosecurity
b. Vaccination
c. Awareness program
d. Public health

5.1. Biosecurity

Biosecurity is considered as one of the major concerns in the prevention and control of salmonella in poultry establishment. The Department of Animal Production has produced biosecurity guidelines to prevent and control of pathogen entering and spreading to poultry premises (refer. DAPH website- Biosecurity guidelines for poultry and pet birds). These biosecurity guidelines mainly include the following instructions:

- Selection of the site /location
- Physical and functional separation from the outside environment
- Management in production area
- Health requirements; vaccination, laboratory testing etc..
- Waste disposal
- Record keeping

The biosecurity guidelines provided by the DAPH are monitored bi- annually by an appointed team with the responsibility of Veterinary Investigation Officers of the area.

5.2 Vaccination

If the salmonella is isolated from breeder farms and commercial layer farms, vaccination of the birds is recommended. The vaccination schedule is based on the manufactures instructions. Inactivated vaccine is recommended for breeder and commercial layer farm. Live vaccines are allowed under user permit. If non-motile Salmonella (Gallinarum /Pullorum) is isolated with severe mortality, SG9R is permitted in a special situation identified by DAPH. It is recommended to vaccinate healthy flock while implementing adequate biosecurity measures. Vaccination

monitoring is recommended for vaccinated flocks. The whole blood agglutination test is not performed in vaccinated poultry breeder farm.

5.3. Awareness Programs

The awareness programs are conducted for farmers and processing plant authority. Training and updating knowledge on Salmonella including salmonellosis in poultry, biosecurity, salmonella surveillance and control program conducting in DAPH are programmed for government veterinary surgeon, Veterinary investigation officers and technical staff.

	Trainee	Topic	Time schedule	program
	Veterinary Investigation Officer/Veterinary Surgeon	-Poultry salmonellosis, diagnosis, control and prevention -Biosecurity monitoring/inspection	One day physical program/ twice a year	Institute of continuing education(ICE) /DAPH and online 1 hr program
	Veterinary Investigation Officer	Laboratory testing of samples (hatchery/birds/environmental sample etc)- Hands on experience	4 days laboratory training	Bacteriology division, Veterinary Research Institute
	Poultry farmers Breeder farmers	Control and prevention of salmonella and biosecurity	1 day physical/year	ICE/DAPH
	Poultry Processors	Review of laboratory findings of poultry processing plant and biosecurity	1day/year	ICE/DAPH

5.4. Public health

Salmonella in poultry is an important public health concern, causing food-borne illness in human. With the occurrence of food-borne Salmonella outbreak, the Medical Officer of Health (MOH) or regional or national level one health approach trace back the origin of the chicken meat or meat products or eggs. The MOH communicates with the Veterinary Surgeon of the area who is

responsible to contact with the relevant VIO and support to investigate the case. Based on joint investigation and laboratory findings, the origin of Salmonella is identified. If salmonella originates from the farm Veterinary Surgeon take action to control the disease in the farm and prevent the organism entering the food chain.

6. Salmonella monitoring

Salmonella monitoring is mainly through the registration of poultry farms/establishments in the DAPH. Salmonella monitoring program is as follows:

- Registration of farms/establishment in DAPH and renewal of registration annually
  - VRA division/DAPH registers poultry breeder farms, export-oriented poultry processing establishment, pet bird establishment and layer farms.
  - The Government Veterinary Office (GVO) registers commercial layer farm, commercial broiler farm, backyard poultry
- Registration is based on the biosecurity inspection and monitoring of poultry breeder farm, export-oriented poultry processing establishment, pet bird establishment and layer farms bi- annually. The biosecurity monitoring/inspection team is provided a checklist which is prepared based on the biosecurity guidelines (refer DAPH website- Biosecurity guidelines for poultry and pet birds). An inspection team visits these places with the biosecurity monitoring checklist and provides their recommendation with any corrections or non-recommendation. The inspection report is sent to the Director Animal Health (D/AH). Based on inspection team recommendation, the D/AH recommend the registration of the farm/establishment to D/VRA, and D/VRA registers them in D/APH. The renewal of registration annually in VRA division, D/APH after the inspection by the team.
- Monitoring of records and reports in farms/establishment – The biosecurity inspection team monitors the records on laboratory testing records available in the farms/establishment. The records are on laboratory testing of salmonella in birds, poultry meat and meat products feed, water, and litter and vaccine sero-monitoring records. In addition, the farms/establishments are monitored for disease situation, disease diagnosis, disease investigation which are performed by VRO bacteriology or relevant VIO.
- Monitoring of broiler supplier farm records -In processing establishment, in addition to the records in the place, the records on broiler supplier farms are monitored by the inspection team. Poultry processing plan authority should register all broiler supplier farms who

provide broiler to them and keep the records on health, laboratory test reports, and biosecurity with them to provide to the inspection team.

- Auditing of the export-oriented farms/establishment – Auditing of farms /establishment those are under biosecurity bi-annual monitoring program once in 3 yrs, in addition to biannual inspection. The auditing teams are appointed by DAPH for each audit.

7 Disease Reporting, Investigation, Diagnosis, Outbreak Management and post outbreak management

The disease investigation in poultry farms mainly based on the mortality and the complaint by the farmer in Salmonellosis. However, in a situation of paratyphoid infection where the public health significance Salmonellosis in poultry, occurs as a concurrent disease and the organism is found as incidental finding. Therefore, disease investigation performed in case of Fowl Typhoid /Pulorum disease situation and the situation of paratyphoid salmonellosis outbreaks occurs among human which is presented to the medical doctor.

7.1 Case definition of Salmonella in poultry

A suspected Salmonella case is defined based on the clinical presentation and confirmation is defined with the isolation and identification of the organism.

Suspected case definition:

Host adapted Salmonella (Pullorum disease (*S. Pullorum*) and Fowl typhoid (*S. Gallinarum*)

It is primarily based on the identification of the specific biovar through laboratory confirmation, as clinical signs are often non-specific

- Pullorum Disease **-** Characterized by acute septicemia, high mortality, anorexia, and white diarrhea specifically in chicks of 2-3 weeks of age. In adults, it often presents as a subclinical carrier state with reduced egg production.
- Fowl Typhoid **-**Typically affects mature or growing birds. The clinical signs include acute septicemia, depression, dehydration, and a characteristic surface metallic green liver observed during post-mortem.

  The samples are liver, spleen, yolk sac, ovaries or cloacal swab.

However, currently, host adapted Salmonella is not detected in the country.

Non-host-adapted Salmonella (*S.* Enteritidis and *S.* Typhimurium)

An infected flock is where *Salmonella* is detected through microbiological testing of samples. The samples include cloacal swabs, droppings, drag swabs, boot swabs, dust, or environmental samples from the poultry house or hatchery and tissue samples (liver, spleen, yolk sac)
Clinical signs are often sub-clinical, in acute salmonellosis may show clinical signs of anorexia, diarrhea, dehydration, and high mortality, particularly in young chicks.

- A definitive case is confirmed when the specific pathogen is isolated and identified through microbiological or molecular methods.

7.2 Disease Investigation and reporting

If any veterinary surgeon suspect salmonellosis based on the case definition, should perform primary investigation of the case. If salmonellosis is suspected, VS contact the relevant VIO for investigation for the confirmation of the disease. The VIO investigate the suspected case, collect samples and VIO made a tentative clinical diagnosis as salmonellosis based on the investigation findings, including post-mortem lesions. The samples are collected at the post -mortem and if salmonella is isolated, inform to veterinary surgeon of the area and farmer. At the same time, VIO submits a disease investigation report to D/AH, and weekly report (refer annex 3 - Disease Investigation Report). Once the diagnosis is made as salmonellosis, the treatment and the management of the diseased farm is done by the relevant VS

7.3 Disease diagnosis

The clinical diagnosis of salmonellosis is based on the case definition by VS. Further, case history and clinical picture including clinical signs, mortality, morbidity pattern and the post-mortem findings are also supportive in making a tentative diagnosis. The confirmatory diagnosis is made after the laboratory isolation and identification of salmonella either by VRI or VIC.

7.4 Laboratory Diagnosis

The post-mortem samples are dispatched to the VIC and perform laboratory isolation and identification of the organism. Isolated Salmonella is sent to the bacteriology division of Veterinary Research Institute for serotyping and molecular techniques such as conventional PCR. The bacteriology division send the laboratory report to VIO with a copy to D/AH.

7.5 Outbreak management and post outbreak management

7.5.1Outbreak management

Outbreak management of the disease includes mainly proper disposal of carcasses during the outbreak and improving the biosecurity measures and vaccination. The farmers are aware of

biosecurity measures to prevent the disease spreading inside the farm and out of the farm by the Veterinary Surgeon.

Carcass disposal

The carcasses are immediately removed and disposed of starting from the beginning of the outbreak. All dead birds in an infected farm are disposed of properly as per the guidelines (refer annex 4- Guidelines for disposal of dead birds).

Vaccination

The vaccination of the birds is based on the salmonella serotypes. After the infected flocks are removed from the farm, rest of the flocks are vaccinated. A vaccination program is initiated in the infected farm after the outbreak and other farms at risk. The killed vaccines are allowed for salmonella vaccination in the country and available in the market. However, currently, live vaccines are permitted under user permit by DAPH.

7.5.2 Post-outbreak management

In the post-outbreak of Salmonella in a farm, cleaning/disinfection, restocking and strict monitoring are recommended. After the disposal of dead birds and litter, the houses and the farm environment are cleaned properly and disinfected. A minimum of 3-month resting period is recommended in a situation where the farm is affected by poultry salmonellae such as S. Galinrum before restocking.

7.6 Corrective action taken for salmonella positive farm/establishment

It is necessary to follow the guidelines if the salmonella is isolated from a farm/ any poultry establishment or rectors for the WBAT (refer annex 5- Guidelines for corrective action taken for salmonella positive farm/establishment**)**.

08. Data analysis, reporting and information sharing

Analysis of surveillance data (passive and active surveillance) is conducted by the Veterinary Public Health (VPH) Unit of the Animal Health Division. Reports on public health-significant Salmonella are submitted to the Director General and shared with the Ministry of Health (MOH). The findings are also shared with Provincial Directors, Subject Matter Specialists, and Veterinary Investigation Officers (VIOs) by the VPH Unit. Additionally, the results are presented quarterly at disease review meetings and published in the epidemiological bulletins of the DAPH.

References

De Lima, M.S., Isolan, L.W., Hessel, C.T., Pessoa, J.P., Tondo, E.C., 2018. Prevalence of Salmonella spp. in poultry carcasses samples collected in slaughterhouses of Southern Brazil from 2006 to 2015. J Infect Dev Ctries 12, 1034–1038. doi:10.3855/jidc.10290

Donado-Godoy, P., Gardner, I., Byrne, B.A., Leon, M., Perez-Gutierrez, E., Ovalle, M.V., Tafur, M.A., Miller, W., 2012. Prevalence, Risk Factors, and Antimicrobial Resistance Profiles of Salmonella from Commercial Broiler Farms in Two Important Poultry-Producing Regions of Colombia. Journal of Food Protection 75, 874–883. doi:10.4315/0362-028X.JFP-11-458

Ferrari, R.G., Rosario, D.K.A., Cunha-Neto, A., Mano, S.B., Figueiredo, E.E.S., Conte-Junior, C.A., 2019. Worldwide Epidemiology of *Salmonella* Serovars in Animal-Based Foods: a Meta-analysis. Appl Environ Microbiol 85, e00591-19. doi:10.1128/AEM.00591-19

Hardie, K.M., Guerin, M.T., Ellis, A., Leclair, D., 2019. Associations of processing level variables with Salmonella prevalence and concentration on broiler chicken carcasses and parts in Canada. Prev Vet Med 168, 39–51. doi:10.1016/j.prevetmed.2019.03.027

Newton, K., Withenshaw, S.M., Cawthraw, S.A., Davies, R., 2021. In-depth farm investigations and an exploratory risk factor analysis for the presence of Salmonella on broiler farms in Great Britain. Prev Vet Med 197, 105498. doi:10.1016/j.prevetmed.2021.105498

Parry, C.M., Thuy, C.T., Dongol, S., Karkey, A., Vinh, H., Chinh, N.T., Duy, P.T., Thieu Nga, T.V., Campbell, J.I., Van Minh Hoang, N., Arjyal, A., Bhutta, Z.A., Bhattacharya, S.K., Agtini, M.D., Dong, B., Canh, D.G., Naheed, A., Wain, J., Tinh Hien, T., Basnyat, B., Ochiai, L., Clemens, J., Farrar, J.J., Dolecek, C., Baker, S., 2010. Suitable Disk Antimicrobial Susceptibility Breakpoints Defining *Salmonella enterica* Serovar Typhi Isolates with Reduced Susceptibility to Fluoroquinolones. Antimicrob Agents Chemother 54, 5201–5208. doi:10.1128/AAC.00963-10

Rivera-Pérez, W., Barquero-Calvo, E., Zamora-Sanabria, R., 2014. Salmonella contamination risk points in broiler carcasses during slaughter line processing. J Food Prot 77, 2031–2034. doi:10.4315/0362-028X.JFP-14-052

Salvat, G., Guyot, M., Protino, J., 2017. Monitoring *Salmonella* , *Campylobacter* , *Escherichia coli* and *Staphylococcus aureus* in traditional free-range 'Label Rouge' broiler production: a 23-year survey programme. J Appl Microbiol 122, 248–256. doi:10.1111/jam.13313

Talorico, A.A., Bailey, M.A., Munoz, L.R., Chasteen, K.S., Pal, A., Krehling, J.T., Bourassa, D.V., Buhr, R.J., Macklin, K.S., 2021. The use of roller swabs for Salmonella detection in poultry litter. Journal of Applied Poultry Research 30, 100163. doi:10.1016/j.japr.2021.100163

Tzani, M., Mandilara, G., Dias, J.G., Sideroglou, T., Chrysostomou, A., Mellou, K., 2021. Impact of Salmonella Control Programmes in Poultry on Human Salmonellosis Burden in Greece. Antibiotics 10, 121. doi:10.3390/antibiotics10020121

Wegener, H.C., Hald, T., Wong, D.L.F., Madsen, M., Korsgaard, H., Bager, F., Gerner-Smidt, P., Mølbak, K., 2003. *Salmonella* Control Programs in Denmark. Emerg. Infect. Dis. 9, 774–780. doi:10.3201/eid0907.030024

Whiley, H., Ross, K., 2015. Salmonella and Eggs: From Production to Plate. IJERPH 12, 2543–2556. doi:10.3390/ijerph120302543

Withenshaw, S.M., Cawthraw, S., Gosling, B., Newton, K., Oastler, C.E., Smith, R.P., Davies, R.H., 2021. Risk factor analysis for Salmonella contamination of broiler chicken (Gallus gallus) hatcheries in Great Britain. Preventive Veterinary Medicine 196, 105492. doi:10.1016/j.prevetmed.2021.105492

Witkowska, D., Kuncewicz, M., Żebrowska, J.P., Sobczak, J., Sowińska, J., 2018. Prevalence of *Salmonella* spp. in broiler chicken flocks in northern Poland in 2014-2016. Ann Agric Environ Med 25, 693–697. doi:10.26444/aaem/99528

Procedure for prevention/Control/Eradication of Pullorum and Fowl typhoid from Poultry breeder farms and Hatcheries, Department of Animal Production and Health

Appendix

A. Whole blood agglutination test: The blood is collected from wing vein of the bird. The loopful of WAT antigen is placed the glass or tile. Then prick the wing vein and get loop full of blood and place on the top of the antigen. The combination is mixed gently by rotating the testing tile and wire loop simultaneously. The agglutination is shown 60-90 seconds after the mixing. If salmonella antibodies are present agglutination appears. The suspicious bird is kept aside and retest later. The agglutination indicates test positive and they are considered as reactors (serologically positive). If agglutination appears more than 120 Seconds are not considered for the test. The positive controls are required at the beginning of the test.

B. Protocol for isolation and identification of Salmonella in boot swabs (Wegener et al., 2003) Boot sampling is performed in each pen using socks. The socks are removed after walking and it is put into a sterile zip lock bag with and transported in 4^0 C to the laboratory. The socked are enriched by adding 150-225 ml of buffered peptone water and massaged by hand. Incubate the sample at 34–38°C for **18** ± 2 hours. Add 1ml of the incubated sample in buffered peptone water to 10ml of Tetrathionate broth (TT broth with 2% Iodine-Iodide solution) incubating at 37°C for 24hs. for selective enrichment. After incubation, streaked onto XLD plates, incubated at 37°C for 24hs. The cultured typical colonies of Salmonella are carried out for biochemical tests such as TSI, SIM, , Simmons citrate, Urease and LIM test. The positive culture is inoculated into Nutrient agar and serotyping is done for Polyvalent Salmonella O antigens and H antigens. The positive cultures are stored in TSB broth with 40% glycerol at -80^0C.

C. Protocol for isolation and dentification of Salmonella in feed and water

Weight of 25g of feed (collected100g and 25g use for testing) and 25 ml (collected100ml and 25ml use for testing) of water 225 ml buffered peptone water (1:10 ratio) and mix thoroughly incubated at 37°C for 18 ± 2 h. The 1ml of incubated sample is added into 10 ml tetrathionate or RVST (Rapport Vassidalis) broth and incubated at 41.5°C for 24hrs. The broth is steaked on XLD and BGA agar plates and incubated 37^0C for 24 hours. The cultured typical colonies of Salmonella are carried out for biochemical tests such as TSI, SIM, Simmons citrate, Urease and Indole test. The positive culture is inoculated into Nutrient agar and serotyping is done for Polyvalent Salmonella

O antigens and H antigens. The positive cultures are stored in TSB broth with 40% glycerol at -80^0C.

D. Protocol for isolation and identification of Salmonella in fluff

The fluff is collected from the hatcher and dispatch to the laboratory at 4^0 C. Twenty-five grams of fluff is added to 225 ml buffered peptone water (1:10 ratio), mix thoroughly and incubated at 37°C for 18 ± 2 hours. The 1ml of incubated sample is added into 10 ml tetrathionate and RVST (Rapport Vassidalis) broth and incubated at 41.5°C for 24hrs. The broth is steaked on XLD and BGA agar plates and incubated 37^0C for 24 hours. The cultured typical colonies of Salmonella are carried out for biochemical tests such as TSI, SIM, Simmons citrate, Urease and Indole test. The positive culture is inoculated into Nutrient agar and serotyping is done for Polyvalent Salmonella O antigens and H antigens. The positive cultures are stored in TSB broth with 40% glycerol at -80^0C.

E. Protocol for isolation and identification of Salmonella in unhatched eggs (egg surface washing) or egg shell

Ten unhatched eggs or egg shells crushed and washed in 100ml buffered peptone water. The sample is incubated at 37°C for 18 ± 2 hours. The 1ml of incubated sample is added into 10 ml tetrathionate broth and incubated at 37°C for 24hrs. The broth is steaked on XLD and BGA agar plates and incubated 37^0C for 24 hours. The cultured typical colonies of Salmonella are carried out for biochemical tests such as TSI, SIM, Simmons citrate, Urease and Indole test. The positive culture is inoculated into Nutrient agar and serotyping is done for Polyvalent Salmonella O antigens and H antigens. The positive cultures are stored in TSB broth with 40% glycerol at -80^0C.

F. Protocol for isolation and identification of Salmonella in Dead in Shell (DIS)

Sterile egg surface, open eggs and swab samples are collected by inserting sterile swabs inti the DIS eggs. One swab from one egg. All 10 swabs are pooled in 10ml buffered peptone water and incubated at 37°C for 18 ± 2 hours . The 1ml of incubated sample is added into 10 ml tetrathionate broth and incubated at 37°C for 24hrs. The broth is steaked on XLD and BGA agar plates and incubated 37^0C for 24 hours. The cultured typical colonies of Salmonella are carried out for biochemical tests such as TSI, SIM, Simmons citrate, Urease and Indole test. The positive culture

is inoculated into Nutrient agar and serotyping is done for Polyvalent Salmonella O antigens and H antigens. The positive cultures are stored in TSB broth with 40% glycerol at -80^{0}C

G. Protocol for isolation and identification of Salmonella in meconium sample

Meconium is collected from 50 DOC. The volume of meconium is added to buffered peptone water at a ratio of 1:10 and mix. The sample is incubated at 37°C for 18 ± 2 h. The 1ml of incubated sample is added into 10 ml tetrathionate broth and incubated at 37°C for 24hrs. The broth is steaked on XLD and BGA agar plates and incubated 37^{0}C for 24 hours. The cultured typical colonies of Salmonella are carried out for biochemical tests such as TSI, SIM, Simmons citrate, Urease and Indole test. The positive culture is inoculated into Nutrient agar and serotyping is done for Polyvalent Salmonella O antigens and H antigens. The positive cultures are stored in TSB broth with 40% glycerol at -80^{0}C

H. Protocol for isolation and identification of Salmonella in internal organ (yolk, liver, spleen) from dead chicks

The internal organs of dead chicks in the hatcher are added into 100ml of buffered peptone water at a ratio of 1:10. The maximum samples is 5 chicks per sample. The organs are crushed and mix well to make a proper solution by hand. The sample is incubated at 37°C for 48 ± 2 h. The 1ml of incubated sample is added into 10 ml tetrathionate broth and incubated at 37°C for 24hrs. The broth is steaked on XLD and BGA agar plates and incubated 37^{0}C for 24 hours. The cultured typical colonies of Salmonella are carried out for biochemical tests such as TSI, SIM, Simmons citrate, Urease and Indole test. The positive culture is inoculated into Nutrient agar and serotyping is done for Polyvalent Salmonella O antigens and H antigens. The positive cultures are stored in TSB broth with 40% glycerol at -80^{0}C

I. Protocol for isolation and identification of Salmonella in cloacal swabs

A sterile swab is inserted into the cloaca of the bird and then turned slowly to take the sample. The samples are pooled in buffered peptone water (5 swabs each) and transported at 2-8^{0}C to the laboratory. The sample is incubated at 37°C for 18 ± 2 h. The 1ml of incubated sample is added into 10 ml tetrathionate broth and incubated at 37°C for 24hrs. The broth is steaked on XLD and BGA agar plates and incubated 37^{0}C for 24 hours. The cultured typical colonies of Salmonella are carried out for biochemical tests such as TSI, SIM, Simmons citrate, Urease and Indole test. The positive culture is inoculated into Nutrient agar and serotyping is done for Polyvalent Salmonella

O antigens and H antigens. The positive cultures are stored in TSB broth with 40% glycerol at -80^0C

J. protocol to isolate Salmonella from table eggs:

20 eggs are washed in 225 ml buffered peptone water, mix well and incubated at 37°C for 18 ± 2 h. The 1ml of incubated sample is added into 10 ml tetrathionate broth and incubated at 37°C for 24hrs. The broth is steaked on XLD and BGA agar plates and incubated 37^0C for 24 hours. The cultured typical colonies of Salmonella are carried out for biochemical tests such as TSI, SIM, Simmons citrate, Urease and Indole test. The positive culture is inoculated into Nutrient agar and serotyping is done for Polyvalent Salmonella O antigens and H antigens. The positive cultures are stored in TSB broth with 40% glycerol at -80^0C

Annexure 1

Sample collection and laboratory testing of samples for Salmonella

	Type of farm	Sample type	Sample collection method	No. of samples
1	Grandparent Parent farms (environmental samples)	Boot sample	Bi-annually	Single cage - 2 samples/flock (pair of socks per sample) in single cage Multi cages- 1 sample/cage min of 3 cage/farm
2	Commercial layer farms	Cloacal swabs from laying birds	Annually	300 farms, 3 pooled per farm, 5 pool in each sample Total = (300×3×5)= 4500
3	Backyard	Cloacal swabs	Annually	300 farms, 3 pooled per farm, 5 pool in each sample (minimum of 1 pooled sample /farm and maximum 3 pooled sample /farm) Total = (400×1×5) = 2000 (minimum)
4	Day old chicks' imports	1.Meconum 2. Meconium contaminated papers 3. Cloacal swabs 4.Dead chicks	At arrival Day 28 Day 1-Day30	1-3 pooled samples (30 chicks meconium to 1 pooled sample) 1-3 pooled samples (5 swabs pooled in 1 sample) 6 pooled samples 5 pool in each sample. Total = (6×5) =30 1-3 chicks (if available
5	Hatchery samples	1.Fluff 2.Unhatched eggs 3.Dead in shell 4.Meconium 5.Dead chick (internal organs)	One hatcher	25g 10 eggs (surface washing) 10 eggs 50 chicks (meconium) Max 5 chicks
6	Pet bird imports	Wet droppings /cloacal swabs	At arrival and Day 28	10% of the birds (5swabs pooled in 1 sample)

7	Poultry processing plant (export oriented)	Meat and meat products	Bi- annually	Processing plant sample plan (muscle/workers hand/equipment/water etc) (refer annex 2- Export-oriented Poultry processing plant sample plan)
8	Broiler farm (export-oriented Poultry processing plant suppliers)	Cloacal swabs from broiler birds at receiving	Bi-annually	6 pooled samples 5 pool in each sample. Total = (6×5) =30
9	Export-oriented layer farm	1.Cloacal swabs from layer birds 2. Egg surface washing 3 Boot sample 4. fan belt sample	Bi-annually	6 pooled samples/per flock, 5 pool in each sample. Total = (6×5) =30 1 sample (washing of 20 eggs)/eggs from one flock Single cage - 2 samples/flock (pair of socks per sample) Multi cages- 1 sample/cage min of 3 cage/farm 1 pooled sample from 1 flock (5 swabs from the fan belt pool into 1 sample)
10	Export oriented farms (pet bird/layer), poultry breeder farms	Feed	Bi-annually	Min of 3 feed sample (100g each) Use for testing 25g
11	Export oriented farms (pet bird/layer),poultry breeder farms	Water	Bi-annually	Min of 3 water sample (100ml each from well/tank/cage) Use for testing 25ml Multiple cages- 1 sample per cage

Annexure 2

Poultry processing plant sample plan

Export-oriented Poultry Sample collection plan for poultry processing/further processing establishment monitoring and sampling program

	Sample	No of sample
1	Water samples – 100 ml of water into sterile bottles	
a	Water source (Waterline/ tap line in the plant)	01
b	Chilling tank	02
2	Final product before packing and storing - 200g of meat (Samples should be collected to represent multiple batches as much as possible - batch No/name of the farm to trace back)	
a	Meat from whole carcass - 200g x 5carcasses	5 carcasses
b	Swabs from skin surface of the neck ($2.5cm^2$) -10 pooled swabs in 1bottle of buffered peptone/distilled water x 10 bottles (10x1x10 =100 necks of birds)	10 bottles
c	a. Further processed product (200g) - Raw materials (MSM/Breast meat etc…) - Each final product	N sample
3	Final processed/further processed products to be exported (either before packing and storing or products in stores)	N sample
4	Meat from cold stores processed or further processed product - (longest period stored)- 200g x 1	1 sample
5	Packing material	
a	Swabs from packing material 5 areas (approximately $50cm^2$ surface inside and outside of the pack for 1 swabs)- 5 swabs x 1bottle of buffered peptone/distilled water x 2 (5x1x2 =10 packing material), random selection of packing material = 2 bottles	2 bottles

6	Surface swabs from equipment, tables and knives - By rubbing 5 areas (approximately 50cm^2 surface for 1 swab) -5 swabs x 1bottle of buffered peptone/distilled water x 1 from each section	
a	Evisceration	1
b	Cut up/deboning, etc..	1
c	Further processing (each product area separately)	N areas
7	Workers hand swabs - pooled hand swabs of 5 workers x 1bottle of buffered peptone/distilled water x 1	
a	Cutting/deboning/maceration area (if physically separated areas more than one, collect from each area)	N areas
b	Packing area	N areas
c	Further processing areas (swabs from each area of further processed product) 1 x No. of product(N1) x no of areas (N2) (if physically separated areas more than one, collect from each area) = 1xN1xN2	1x N1x N2
8	Environmental samples -Air sampling with open plate of Nutrient agar	
	a. Packing area	1
	b. Cut up/deboning/maceration	1
	c. Further processing area (if physically separated areas more than one, collect from each area) N	N areas

Annexure 3

Disease Investigation Report

Epidemiological Investigation Report

1. Administrative Information

a. Investigation Reference No -

b. Date and Time of Investigation -

c. Reporting Officer -

d. Veterinary Investigation Centre -

e. Veterinary Range -

2. Event Notification Details

a. Source of Notification -

b. Date Notified -

c. Complaint -

d. Reason for Investigation -

3. Investigation Team (name /designation/Institution)

4. Geographic and Epidemiological Location

a. Province and district -

b. Divisional Secretariat Division -

c. Grame Niladari Division -

d. Village -

e. Farms GPS Coordinates:

Latitude -

Longitude -

f. Farm Boundaries / Nearby Landmarks: (Surrounding GN and Vs rangers):

g. Map sketch attached: Yes / No

5. Farm Identification and Ownership

Name of the Farmer/s name/ address/ Contact No	Farm registration No	Farm type (Cattle/Buffalo /Poultry/Swine/ Goat/Backyard	Purpose of rearing

6. Animal Population Details:

Species	breed	Total population	Age	Sex	
				Male	Female

7.Investigation findings

a History (Past and Present**)**

(include clinical history/clinical course No affected/index case/onset of signs/No at risk)

b.Farm management assessment

- Management (housing/feed/ water)
- Biosecurity status
- Waste disposal method

c. Animal movement and risk factors

- Recent animal introduction (Date / Number / Source / Health status)
- Contact with neighboring animals
- Exposure to wildlife

d. Vaccination History/vaccination record:

e Findings at date of investigation (describe investigation findings in detail including morbidity rate/mortality rate case fatality rate etc..)

8. Postmortem examination findings

- Postmortem conducted: Yes / No
- Macroscopic finding (gross) :
- Photographs sent: Yes / No

09. Sample collection

Animal ID	Sample type	Sample no	No. of samples	Test requested

a. Any field tests conducted:

10 Samples Submitted to Laboratory

a. Test performed in VIC

b. Samples dispatch to VRI

11. Differential diagnosis

12. Tentative diagnosis

13. Immediate action taken with tentative diagnosis

14. Laboratory Results

15. Confirmatory diagnosis

16. Conclusion and Recommendations

17. Follow-up Plan /visit

……..

Veterinary Investigation Officer:

Date:

Annexure 4

Guidelines for disposal of dead birds

Safe disposal of carcasses is crucial in preventing the spread of the disease in an outbreak situation.

- Carcasses must be immediately removed from the infected pen and disposed. (perform post-mortem immediately if necessary and dispose)
- The person who handles carcasses should wear gloves, masks, and boots and disinfect after the process.
- Do not throw carcasses into open fields, water bodies, or garbage pits.
- Do not use dead birds as feed for other animals.
- Selling, giving away, or moving dead birds off the farm is strictly prohibited.

Recommended method

1.Disposal of carcass to the pit in the farm

- The carcass disposal pit should be 50 meters away from water sources, public access and should be located within the farm boundary.
- The pit should be at least 1.5–2 meters deep and 1 meter wide.
- The top of the pit should be sloped and sealed with an openable coverage size of 24 x 24 inches for the disposal of carcasses to the pit. Lime is added to the bottom of the pit.
- Put carcasses to the pit and add another layer of lime.

2.In deep burial a new disposal pit is made for disposal of large no of carcasses.

- 50 meters away from water sources with the size 1.5–2 meters deep and 1 meter wide.
- The bottom of the pit is lined with lime.
- Place carcasses in the pit and add another layer of lime.
- Cover with soil and compact to seal the pit to prevent scavenger access.
- Mark burial sites clearly with date and number of carcasses.
- If the same disposal is used again, make the time gap as follows (200 birds – at least 6 months and > 200 birds – at least 1 year)

3. Incineration

- Should reach >800°C for proper destruction.
- Ashes should be buried after the incineration process.

Annexure 5

Guidelines for corrective action taken for salmonella positive farm/establishment

Procedure adapted for the reactors for the WBAT at screening of GP and parent flocks

- The flocks with more than 1% reactors, the flock must be retested by the owner after a 45 to 60-day interval until the reactor rate is reduced to less than 1% or no reactors are detected.
- The flocks with less than 1% reactors for two consecutive tests, or 100% of negative birds, will be tested and verified within 2 to 4 weeks of notification.
- Salmonella verification is conducted only after the farm management has confirmed two consecutive negative test results, in accordance with the stipulated protocol.
- Further, all birds in the flock are to be screened using WBAT at 56 weeks by the farm authority and the records available with them. The records are to be available for theVRI verification team or audit teams.
- All positive reactors identified during each testing should be culled & removed from the farm immediately.
- The relevant VIO should provide guidelines to the farm authority based on the farm management, structure and the national control plan of the country.

Procedure adapted for salmonella positive poultry farms/ establishment

If salmonella is isolated from the following areas during surveillance or disease investigation, the control and preventive measures are taken into action based on the place it is isolated.

a. Grandparent and parent

If breeder farm is positive for whole blood agglutination test, sacrifices a few birds to isolation and identification of salmonella in the laboratory (VIC or VRI). The positive birds are re-tested every 45 days until test negative results are achieved. The eggs from are not recommended for hatching. Once the flock become negative, the eggs should incubate separately in a hatcher to prevent any possibility of contamination the hatchery.

If Salmonella is isolated from a breeder farm, either cull the whole batch and initiate vaccination for other batches or initiate vaccination for all batches depending on the severity and the serotype of Salmonella. It is recommended to improve biosecurity to prevent the

disease spreading inside the farm and to the hatchery contamination as well as infected DOC introduced to the poultry farms.

b. Poultry hatcheries

If the whole hatchery is contaminated, a special cleaning and disinfection should be conducted in the hatchery. Identify the origin of the contaminated eggs (eggs from an infected flock or contaminated eggs from the hatchery) and incubate using a separate incubator/hatcher for eggs from suspected flocks. Special biosecurity measures should be adapted in the hatchery to prevent introduction of infected DOC to the farms.

c. Import of live birds, poultry meat and meat product.

Infected live birds are identified during quarantine, they are not allowed to be sent to farms. All Salmonella positive meat and meat products are destroyed.

d. Export oriented broiler farms and processing plant

The contaminated meat and meat products are not allowed for export. The processing plant should be subjected to a special cleaning and disinfectant procedure. Trace back infected the farm and adapted control measures for the broiler farm. Strictly adhere for the testing of birds (surveillance) in the birds at receiving.

e. Export oriented pet bird establishment

The birds are separately reared and not recommended to introduce salmonella positive to the farm.

f. Commercial layer

If it is poultry salmonella (non-motile) causing high mortality in birds, treat the flock to minimize mortality or cull the whole flock and vaccinate the rest of the birds. If it is food-borne motile salmonella, initiate vaccination of flock. It is recommended to improve the biosecurity measures to prevent the disease spreading inside the farm. In chicks of <10days positive for Salmonella, the chick origin breeder farm/hatchery is informed to take action for the prevention of disease spreading.

g. Backyard poultry

Improvement of biosecurity measures to prevent the disease inside the farm and spread to outside the farm. The birds in surrounding farms are vaccinated and type of vaccine is based on the serotype (public health significance or mortality)

h. Export -oriented poultry processing establishments (refer annex 6)

Annexure 6

Guideline for corrective measures for production of safe poultry meat and products in poultry processing/further processing establishments which do not satisfy recommended microbiological standards

This guideline is provided for processing/further processing establishments which are found to be positive for Salmonella and/or exceed the standard microbiological (*E.coli* O157:H7, Coliforms, *Staph aureus,* APC) limits undergoing DAPH inspection and surveillance (bi- annual monitoring and sampling) program with the objective of producing safe quality poultry meat and product for export or local market.

Guideline for the control measures to be adapted in Salmonella-positive poultry processing/further processing establishments

- Under the DAPH surveillance in poultry processing/further processing establishments, the samples are collected biannually for microbiological examination. The laboratory report is expected to be negative for Salmonella (including other parameters) in a processing/further processing establishment.
- If the samples tested are positive for Salmonella, the processing plant is considered contaminated with Salmonella, and the following guideline is recommended

1. Determination of the origin of Salmonella detected in processing plant
    - Depending on the isolation of Salmonella in the processing/further processing establishment, it is required to identify the exact meat item or commodity or source of contamination (dressed meat/final product, water, processing plant environment, and source grower farm, etc..)
        - List out Salmonella isolated places/items (whole carcass, chicken parts, chicken products, water, etc.) in the processing plant
        - Identify the contaminated area/section of the processing/further processing plant
        - Identify the batch/es and the suspicious source grower farm/s

- Identify the batch/es (and source grower farm/s) slaughtered in the previous day (to check whether the contamination is due to previous day samples and improper cleaning).

- Examination of suspicious broiler grower farms (suppliers)
  - Detail testing of the areas/sections/meat/water in the processing plant (for Salmonella for further information if necessary).
  - Testing of animals in out-grower farm (mention below)
  - Testing of animals in own grower farm (mention below)

2. If the Salmonella originated from out-grower farms, proceed as follows
   - Ensure the introduction of Salmonella free day-old chicks to the broiler farm
     - Testing of the source breeder farm and hatchery for Salmonella (if an owned breeder farm or hatchery)
     - Test Day Old Chick (DOC) for Salmonella.
     - If DOC is positive for Salmonella improve the biosecurity measures and other measures to control Salmonella
   - Screening of the farm for Salmonella
     - Routine testing litter, water, feed and workers for the presence of Salmonella
     - All the batches (birds) in the farm should be tested at least once in the cycle for Salmonella
   - Improve biosecurity measures in the broiler farm
     - Improve biosecurity measures to prevent Salmonella entering into the farm and spreading among the batches in the farm
     - Poultry litter/feces and other potentially contaminated farm waste should be disposed in a safe manner
     - Special care should be taken in cleaning and disinfection of poultry houses and equipment
     - Adequate downtime (recommend at least for two weeks) should be allowed between batches if the previous batch/flock was positive for Salmonella
     - Buildings, surfaces, and equipment should be cleaned and disinfected properly.
   - Adapt other measures to control Salmonella such as use of probiotics, acidifiers, etc
   - Adopt rodent control program in the farm

3. If the Salmonella is originated from the processing/further processing establishment, proceed as follows
    - Testing of fecal samples/cloacal swabs prior to delivery for slaughter (pooled sample)
    - Salmonella-free farms/batches should be slaughtered initially.
    - Strict biosecurity measures should be adapted in the process of the processing/further processing establishment
        - Cleaning and disinfection of bird transport vehicles, crates, and hanging area
        - Improve the arrangement of the evisceration section to prevent cross-contamination
        - Maintain chiller tank temperature 4-8 °C
        - Make arrangements to change the gloves of the workers regularly at a reasonable frequency according to the nature of the activity
        - Keep hand washing/disinfection facilities in every section
        - Cutting, deboning, packing, and Mechanically Separated Meat (MSM) meat production areas should be separated physically
        - Each type of chicken parts/product items handling and packing should be done by separate workers
    - Testing of the processing establishment for Salmonella
        - Chilling tank water testing 2 times /week
        - The final product (whole carcass/chicken parts/MSM) should be tested regularly (samples weekly)
    - Record keeping, monitoring, and analysis of Salmonella status along the value chain from broiler farms to final products (testing records - farm records and processing plant records)
    - Health monitoring of the food handlers and providing training for food handlers on food safety measures
    - Review and take measures for correcting if any in the cleaning and disinfection procedures, temperature control points including CCPs

In addition, follow the currently using checklist as the guideline to improve the hygienic and biosecurity measures to be adapted in a poultry processing/further processing establishment.