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# Risk to public and/or animal health of the treatment of dead-in-shell chicks (Category 2 material) to be used as raw material for the production of biogas or compost with Category 3 approved method

## EFSA Panel on Biological Hazards (BIOHAZ)

### Abstract

In 2011, EFSA assessed the risk posed by the possible use of dead-in-shell chicks for the production of processed pet food. In 2014, the European Commission asked for an update on the risk posed by the same material but as raw material for the production of biogas and compost according to standard transformation parameters normally used for Category 3 material. According to current European Union legislation, the following processing method was considered: maximum particle size before entering the processing unit: 12 mm; minimum temperature in all material in the unit: 70°C; and minimum time in the unit without interruption: 60 minutes. A list of pathogens potentially present in the material to be treated was compiled and available literature data were used to assess the ability of the processing methods to inactivate the most resistant pathogens identified. Spores of *Clostridium botulinum* were identified as the most resistant hazard potentially present in the material to be processed. Circovirus and parvovirus and *Enterococcus faecium* were considered the most heat-resistant viruses and non-sporulating bacterium, respectively. Moreover, depending on storage conditions, the formation of bacterial toxins is possible. The processing methods considered were therefore assessed for their ability to inactivate those hazards. The probability of survival of pathogens related to the use of dead-in-shell chicks subjected to the treatment process was considered to be extremely low for the heat-sensitive pathogens that could be present in the material, which include bacteria such as *Salmonella* and avian influenza virus. However, this treatment is unable to sufficiently inactivate other relevant hazards (e.g. bacterial spores, thermoresistant viruses and certain bacterial toxins).

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**Keywords:** animal by-products, hatchery, dead-in-shell chicks, biogas, compost, hazards, thermal inactivation

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## Summary

Following a request from the European Commission (EC), the Panels on Biological Hazards (BIOHAZ) and on Animal Health and Welfare (AHAW) were asked to deliver a scientific opinion on hatchery waste as animal by-products, in order to update an opinion produced in 2011, considering a different process and end use.

Standard transformation parameters for biogas and composting plants are set out in Chapter III of Annex V to Regulation (EU) No 142/2011. In accordance with those standard transformation parameters, Category 3 material (lowest risk material) which is used as raw material in a biogas plant equipped with a pasteurisation/hygenisation unit or in a composting plant must be submitted to the following minimum requirements:

- maximum particle size before entering the unit: 12 mm;
- minimum temperature in all material in the unit: 70°C; and
- minimum time in the unit without interruption: 60 minutes.

According to Article 13(e) of Regulation (EC) No 1069/2009, animal by-products of Category 2 material (medium-risk material), including dead-in-shell chicks, may be transformed into biogas or compost following processing by pressure sterilisation and subsequent permanent marking. However, certain Category 2 materials, including eggs and egg products, may be transformed into biogas or compost without prior processing if the Competent Authority does not consider them to present a risk of spreading any serious transmissible disease.

After discussion with the EC, it was agreed that the final Terms of Reference of the mandate should be to assess the risk to public and/or animal health during the treatment of dead-in-shell chicks (Category 2 material), to be used as raw material for the production of biogas or compost-by applying the standard transformation parameters for Category 3 material.

Considering the mandate received, it was agreed that its scope was to assess the ability of the above-mentioned processing methods to inactivate the biological hazards that may be potentially present in the material to be treated. In this context, the AHAW Panel compiled a list of the possible pathogens that could be present in the material via vertical or horizontal transmission and the BIOHAZ Panel had responsibility for evaluation of the occurrence of pathogens, including zoonotic agents, and also used the available scientific literature to assess the ability of the processing methods to inactivate the most heat-resistant pathogens identified.

Spores of *Clostridium botulinum* were identified as the most resistant hazard potentially present in the material to be processed. Avian circovirus and parvovirus and *Enterococcus faecium* were considered to be the most resistant viruses and non-sporulating bacterium, respectively. Depending on the storage conditions of the material to be processed, the generation of heat-resistant bacterial toxins was also considered to be possible. Consequently, the currently approved standards for compost and biogas production using Category 3 material were assessed for their ability to inactivate those hazards. Furthermore, considering that the material is subjected to heat as part of the biogas production or composting process, and the tissues of dead-in-shell chicks may have a relatively high moisture content, the assessment was based on the assumption that the heat treatment would be performed in a moist environment. There was uncertainty associated with the assessment since data were not available for the specific matrices and processing conditions considered.

The origin of the material and both intrinsic and extrinsic factors can play a major role in determining the occurrence, growth and survival of the target microorganisms and, consequently, the final risk they may pose. Moreover, the methods and specific equipment used and the conditions of heat treatment are known to affect microbial inactivation, as well as the potential for inefficiencies in the treatment processes, such as reduced dwell time due to rapid transit of a proportion of the material in some continuous flow systems.

In conclusion, the potential for survival of pathogens in dead-in-shell chicks submitted to the compost or biogas process was considered to be very low for the heat-sensitive pathogens. This includes bacteria such as *Salmonella*, which can be associated with the hatching process as a result of vertical and horizontal transmission and can multiply outside the host, and avian influenza or Newcastle disease viruses, which are major statutory and disease control priorities when they occur in poultry

breeding flocks. However, this treatment is not able to sufficiently inactivate other relevant hazards such as bacterial spores, thermoresistant viruses and some bacterial toxins, and this conclusion was not compromised by uncertainties resulting from lack of data relating to the specific matrix to be treated.

The BIOHAZ Panel concluded that there is no scientific basis for supporting a treatment of dead-in-shell chicks according to the biogas and composting standards for Category 3 material because of the inability of the process to sufficiently reduce the concentration of thermoresistant pathogens.

More realistic studies, particularly under field conditions and using the actual matrices, were recommended in order to provide data to better quantify the reduction of pathogenic agents. Validation of the efficacy of biogas plants using a representative indicator organism and development of a protocol to verify the efficacy of the treatment processes in terms of their ability to achieve the required level of inactivation of pathogenic agents would also be desirable.

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## 1. Introduction

### 1.1. Background and Terms of Reference as provided by the European Commission

According to Article 13(e) of Regulation (EC) No 1069/2009,<sup>1</sup> animal by-products of Category 2 material may be transformed into biogas or compost following processing by pressure sterilisation and subsequent permanent marking. However, certain Category 2 materials, including eggs and egg products, may be transformed into biogas or compost without prior processing if the Competent Authority does not consider them to present a risk of spreading any serious transmissible disease.

Standard transformation parameters for biogas and composting plants are set out in Chapter III of Annex V to Regulation (EU) No 142/2011.<sup>2</sup> In accordance with those standard transformation parameters, Category 3 material which is used as raw material in a biogas plant equipped with a pasteurisation/hygienisation unit or in a composting plant must be submitted to the following minimum requirements:

- (a) maximum particle size before entering the unit: 12 mm;
- (b) minimum temperature in all material in the unit: 70°C; and
- (c) minimum time in the unit without interruption: 60 minutes.

The minimum requirements referred to in points (b) and (c) above must be applied in case of eggs and egg products of Category 2 material that may be transformed into biogas without prior processing by pressure sterilisation.

The application of standard transformation parameters for Category 3 material in transformation of dead-in-shell chicks into biogas or compost should be limited only to daily mortality under normal production condition. They cannot be applied in case of health restriction referred to in Article 6 of Regulation (EC) No 1069/2009.

#### 1.1.1. Terms of Reference as initially provided by the European Commission

In view of the above, and in accordance with Article 29 of Regulation (EC) No 178/2002,<sup>3</sup> the Commission asks EFSA:

1. to assess the risk to animal and public health arising from the transmission of the most important infectious agents in hatchery by-products, such as *Salmonella* spp., avian influenza virus and Newcastle disease virus, through the transformation of dead-in-shell chicks into biogas or compost according to standard parameters for Category 3 material;
2. to assess the risk to animal health arising from the transmission of infectious avian diseases, such as avian leukosis, chicken infectious anaemia, infection with avian adenoviruses (egg drop syndrome), reticuloendotheliosis, avian encephalomyelitis, *Mycoplasma* infection and avian psittacosis, through the transformation of dead-in-shell chicks into biogas or compost according to standard parameters for Category 3 material;
3. to assess the risk to animal and public health arising from the transmission of other biological hazards such as *Campylobacter*, *Enterobacteriaceae*, Erysipelas, botulism toxins and toxoplasmosis, through the transformation of dead-in-shell chicks into biogas or compost according to standard parameters for Category 3 material;

<sup>1</sup> Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation). OJ L 300, 14.11.2009, p. 1–33.

<sup>2</sup> Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive. OJ L 54, 26.2.2011, p. 1–254.

<sup>3</sup> Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, OJ L 31, 1.2.2002, p. 1–24, as last amended.

4. to indicate, in the case of identified risks mentioned in points 1 to 3, the most important factors which should be monitored in the transformation into biogas or compost.

### 1.1.2. Clarifications on the Terms of Reference

After discussion with the requestor it was agreed to replace the above Terms of Reference of the mandate with the following:

- To assess the risk to public and/or animal health during the treatment of dead-in-shell chicks (Category 2 material) – to be used as raw material for the production of biogas or compost – by applying the standard transformation parameters for Category 3 material.

It was clarified that the hazards considered during the risk assessment will cover at least those mentioned in the original Terms of Reference.

### 1.1.3. Approach taken to answer the Terms of Reference

After having received this request from the European Commission (EC), EFSA assigned the mandate to the Panel on Biological Hazards (BIOHAZ) and the Panel on Animal Health and Welfare (AHAW). The AHAW Panel drafted and endorsed aspects related to the hazard identification (as presented within Section 3.2), whereas the BIOHAZ Panel had responsibility for the evaluation of the efficacy of standard transformation parameters for Category 3 material to inactivate pathogens including zoonotic agents and adoption of the entire opinion.

## 1.2. Interpretation of the Terms of Reference

It was also agreed with the requestor that the biological hazards considered in the assessment should only include those present in the European Union (EU), since fertilised eggs are not imported into the EU. The assessment should focus on the risks associated with the treatment processes: from dead-in-shell chicks to raw material for biogas or compost. No exposure assessment was carried out to assess the risks associated with survival of pathogens or toxins after the treatment in the biogas or compost plant.

In the current opinion, 'risk' is defined as the probability of survival of pathogens in dead-in-shell chicks submitted to the biogas and compost process.

## 1.3. Additional information

The inactivation of pathogens and contaminants by heat is influenced by several factors related to the physical and chemical properties of the treated material, the type of technical equipment used for treatment and the control of the biotechnological process involved as described in Section 3.3.1. None of these factors were considered in the establishment of the parameters contained in the legislation and may compromise the effectiveness of the process.

## 2. Data and Methodology

### 2.1. Data

For the hazard identification section (3.2), available data were compiled from literature searches on (a) the biohazards that may be found in the embryos of several poultry species, (b) evidence of presence in eggs and initial level of contamination as well as (c) the levels of thermal resistance of specific or similar agents, preferably in the substrate under assessment (dead-in-shell chicks) and, if not existent, in comparable substrates.

Annex I (Infectious Agents present in Eggs) of a reference textbook (Pattison et al., 2008) was used to compile a list of agents potentially present in dead-in-shell chickens. This list of hazards was completed with the most up-to-date and comprehensive textbook of avian diseases (Swayne et al., 2013).

In addition, internet-based searches (PubMed) on thermostability of the relevant microorganisms included searches using the keywords 'thermal stability' and 'thermal inactivation'.

For the risk reduction section (3.3), internet-based searches were carried out in PubMed and Scopus using the keywords 'D-value', 'z-value', 'heat resistance' in combination with the name of target microorganisms.

## 2.2. Methodology

Considering the mandate received, it was agreed that the scope of this assessment was to assess the ability of the biogas and compost methods to inactivate the biological hazards that may be potentially present in the material currently produced in the EU.

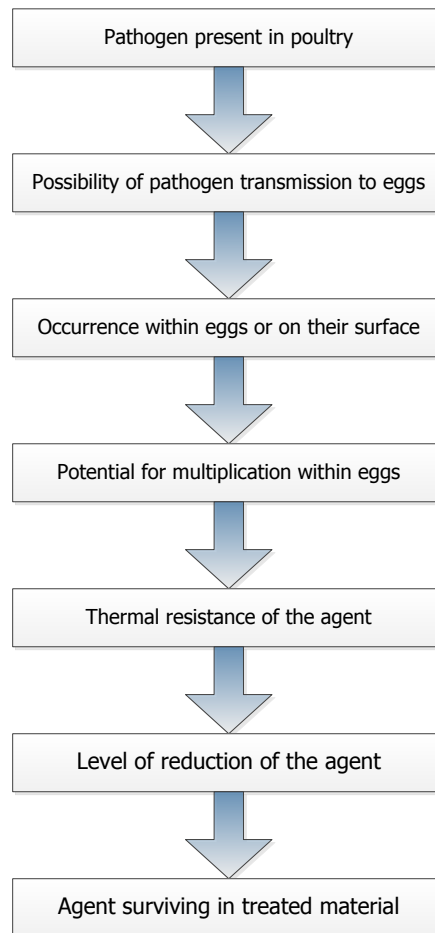
In this context it was further agreed that:

- The EFSA Scientific Panel on Animal Health and Welfare (AHAW) would conduct an update of the evidence available and compile a list of the possible pathogens potentially present in the material.
- The EFSA Scientific Panel on Biological Hazards (BIOHAZ) would have responsibility for the evaluation of the efficacy of standard transformation parameters for Category 3 material to inactivate pathogens, including zoonotic agents. The assessment would make use of the available literature as described in the previous point to evaluate the ability of the processing method to inactivate the most resistant pathogens identified.

A search for biohazards that may be found in the embryos of several poultry species, either transmitted vertically or as a result of horizontal transmission or contamination by infectious agents that may be found in eggs, was conducted by accessing standard textbooks on avian diseases as described in Section 2.1. The list of agents considered follows an aetiological classification (i.e. viruses, bacteria, fungi and toxins). Poultry species affected by these agents were indicated. Information related to the likelihood of finding the different agents in eggs from several poultry species and the initial level of contamination was also sought and, whenever found, included. Spoilage bacteria, which are environmental contaminants and can be found on eggs, were also considered. Basic information was collected on the agent, as well as on relevant physicochemical properties, such as thermal resistance, when available. Experimental data on the presence and viability of agents in the substrate under assessment, i.e. dead-in-shell chicks, have been presented. This, together with the information collected on the heat resistance parameters (D- and z-values: see Section 3.3.1 for definitions), was considered to evaluate the ability of the combined time and temperature parameters of the pasteurisation method under assessment to inactivate them. Target biological hazards, characterised by their inherent high thermal resistance, were identified and the efficacy of the proposed treatment method to inactivate them (in terms of number of log reductions) was estimated (see Section 3.3).

A flow diagram of the evaluation process is presented in Figure 1.





**Figure 1:** Flow diagram of the evaluation process

The level of uncertainty associated with the extrapolations made from experimental data produced by studies using different substrates, agents and experimental conditions is considered in Section 4. The majority of the studies conducted to determine the thermal resistance of the different pathogens were conducted in liquid eggs. In the opinion of the working group experts, it is easier to inactivate those microorganisms in that medium than in the actual matrix (dead-in-shell chicks, which consists of solid material as well as fatty yolk within the yolk sac) (EFSA BIOHAZ Panel and EFSA AHAW Panel, 2011). Infertile eggs may be present amongst those containing dead-in-shell embryos and, when mixed with free water that may be released during maceration of hatchery waste, coagulation of egg albumin may occur during heat treatment. The resultant solidification of material could interfere with inactivation of microorganisms (Lewith, 1890; Shimada and Matsushita, 1980; Bruzual et al., 2000).

The assessment was conducted with the data available at the time of preparing this scientific opinion. If new hazards are identified in the future, review of the risk assessment should be considered.

### 3. Assessment

#### 3.1. Introduction

##### 3.1.1. Hatching of poultry eggs

Eggs collected from breeding flocks are subject to a multitude of factors that may influence embryonic survival and hatchability (Tullett, 1990; Christensen 2001; King'ori 2011). The nutrition, health and environmental management of breeding birds, as well as the species and age of the birds (Tona et al., 2004), have an impact, and breeding companies manage these factors carefully, as well as controlling the hygiene of nest boxes/avoiding hatching of floor eggs to optimise fertility and hatchability (Heier and Jarp, 2001). Commercial-scale chicken eggs are typically collected twice a day using an auto-nest

system, whereas turkey and duck eggs are normally collected by hand from floor-level nest boxes several times a day. Eggs are then stored on the farm in cool conditions awaiting collection on a daily or less frequent basis. Eggs may be sanitised by washing, dipping or being fogged with disinfectant at the farm to minimise surface contamination from faecal organisms.

Eggs from several flocks and farms are usually hatched in a central hatchery owned by the company. The eggs may be sanitised on entry to the building using methods similar to those used on the breeding farms or, less commonly now, fumigated with formaldehyde vapour. This sanitation may be deferred until eggs are removed from cold storage at the hatchery and placed in the first-stage incubators (setters), where they are regularly turned by means of mechanised tray-tilting trolleys. The time and conditions of storage and exposure of birds or eggs to disinfectants or pesticides can influence embryonic mortality and the impact of this varies with the age of the parent flock (Fry, 1995; Tona et al., 2004). During first-stage incubation, eggs may be candled by viewing against a bright light, which allows infertile, damaged or grossly abnormal eggs to be identified and removed. Infertile eggs may be sold for human consumption in some countries. Eggs within the setters are often fogged with disinfectant to suppress bacterial contamination, especially in multi-stage setters in which eggs of different ages from different flocks are mixed. Such setters are only occasionally fully emptied for complete cleaning. The temperature and humidity conditions used during incubation of eggs are ideal for bacterial multiplication and if surface spoilage bacteria have gained access to eggs via shell defects or moisture/osmotic gradients the eggs may crack or burst, releasing more contamination. Premature hatching of a small proportion of eggs may also occur.

After an 18-day incubation (chicken eggs), the eggs are transferred to static hatcher baskets. They may also be candled at this stage. The transfer to hatcher baskets is largely automatic in modern commercial hatcheries by means of batteries of suction cups. The transfer process can facilitate cross-contamination between individual eggs and batches of eggs from different sources. Eggs are transferred to hatcher cabinets for 3 days, during which time chicks start to hatch, so a proportion of chicks may be outside the shell for up to 3 days. Carry-over of hatcher cabinet contamination between hatches is common as there is typically little time for deep cleaning and thorough disinfection. Organisms such as *Salmonella* may therefore be found residing in ducting and inaccessible places within hatchers for decades, giving rise to a form of horizontal transmission that is referred to as pseudo-vertical transmission. Failure to decontaminate hatcher baskets or contamination of the wash machines or storage areas used for the baskets can also contribute to ongoing infection of chicks (Davies and Wray, 1994). Management of conditions such as turning frequency, careful handling, temperature, humidity, levels of ventilation; oxygen and other atmospheric/exhaust gases, and bacterial or fungal contamination is important for maximising hatchability (Pattison et al., 2008; Kalita et al., 2013). Bacteria such as *Escherichia coli*, *Pseudomonas*, *Staphylococcus*, *Proteus*, *Streptococcus* and *Salmonella* are most likely to be found in association with embryonic mortality caused by bacterial contamination (Al-Sadi et al., 2000).

The incidence of bacterial contamination in turkey eggs which were rejected at candling or were 'dead-in-shell', was about 4% in one study, which was lower than that previously found in chicken eggs, although a single study cannot provide representative data for a whole poultry sector. Analysis of the bacterial flora of turkey eggs indicated that the proportion of Enterobacteriaceae was higher and the proportion of *Micrococcus* spp. was lower (Bruce and Drysdale, 1983).

Embryonic death directly attributable to bacterial contamination is, however, uncommon, since the egg possesses a battery of protective mechanisms designed to prevent this. The occasional transmission of a pathogen, such as certain *Salmonella* serovars (e.g. *S. Pullorum*, *S. Gallinarum*, *S. Enteritidis*, O18 'arizonae'), or mycoplasma can become a major pathway as secondary transmission amongst infected chicks can subsequently occur (Tullett, 1990; Berchieri et al., 2001a, b), and certain avian viruses can also be vertically transmitted at a very low within-batch prevalence (Grgic et al., 2006). This means that the risk associated with infectious agents in hatchery waste is likely to be low, except in the case of organisms that can multiply outside the host or within fertile eggs, e.g. *Salmonella*, or which may be highly infectious at low exposure, e.g. certain avian viruses.

After hatching, the hatcher baskets may be emptied manually or automatically, with viable chicks being sorted for grade, sex, vaccination and despatch and non-hatched eggs, eggshells (including egg membranes) and dead or abnormal embryos or chicks being separated from the baskets and their liners and either macerated or compressed for dispatch as hatchery waste (Das et al., 2002). The

proportion of eggs that fail to hatch is around 15%, or less, in a well-run chicken or turkey hatchery, but may be slightly higher for duck eggs.

### 3.1.2. Legal framework

This opinion focuses on the possible use of dead-in-shell chicks sourced from the poultry hatchery industry for the production of biogas and compost under the provisions currently applicable for these products. The document considers all the relevant poultry species produced in hatcheries in the EU.

Standard transformation parameters for biogas and composting plants are set out in Chapter III of Annex V to Regulation (EU) No 142/2011. In accordance with those standard transformation parameters, Category 3 material (lowest risk material) which is used as raw material in a biogas plant equipped with a pasteurisation/hygenisation unit or in a composting plant must be submitted to the following minimum requirements:

- (a) maximum particle size before entering the unit: 12 mm;
- (b) minimum temperature in all material in the unit: 70°C; and
- (c) minimum time in the unit without interruption: 60 minutes.

According to Article 13(e) of Regulation (EC) No 1069/2009, animal by-products of Category 2 material (medium risk material), including dead-in-shell chicks, may be transformed into biogas or compost following processing by pressure sterilisation and subsequent permanent marking.

According to Regulation (EU) No 142/2011, Annex IV, Chapter III, point A-processing method 1 (pressure sterilisation) is described as follows:

#### *Reduction*

1. If the particle size of the animal by-products to be processed is more than 50 mm, the animal by-products must be reduced in size using appropriate equipment, so that the particle size after reduction is no greater than 50 mm. The effectiveness of the equipment must be checked daily and its condition recorded. If checks disclose the existence of particles larger than 50 mm, the process must be stopped and repairs made before the process is resumed.

#### *Time, temperature and pressure*

2. The animal by-products with the particle size of no greater than 50 mm must be heated to a core temperature of more than 133°C for at least 20 minutes without interruption at a pressure (absolute) of at least 3 bar. The pressure must be produced by the evacuation of all air in the sterilisation chamber and the replacement of the air by steam ('saturated steam'); the heat treatment may be applied as the sole process or as a pre- or post-process sterilisation phase.
3. The processing may be carried out in batch or continuous systems.

However, certain Category 2 materials, including eggs and egg products, may be transformed into biogas or compost without prior processing if the Competent Authority does not consider them to present a risk of spreading any serious transmissible disease. This assessment considers the ability of the processing standards currently approved for the manufacture of biogas or compost to inactivate the possible biological hazards present in dead-in-shell chicks, without the need for pressure sterilisation. Exposure assessment of any of the hazards identified is excluded from the current opinion as agreed with the EC.

It is important to translate the time and temperature parameters of the standard pasteurisation method into quantitative measures of the reduction of viability/infectivity of pathogens. In this regard, Section 2, Chapter III, Annex V, of Regulation (EU) No 142/2011 lays down the rules for the authorisation by the competent authorities of alternative transformation parameters for biogas and composting plants using Category 3 material. Point 1(d) states that the validation of the intended process referred to in point (c) must demonstrate that the process achieves the following overall risk reduction: for thermal and chemical processes a reduction of 5 log<sub>10</sub> in *Enterococcus faecalis* or *Salmonella* Senftenberg (775W, H<sub>2</sub>S negative) and a reduction in the infectivity titre of

thermoreistant viruses such as parvovirus of at least 3 log<sub>10</sub>, whenever they are identified as a relevant hazard.

## 3.2. Hazard identification

### 3.2.1. Background considerations on occurrence of pathogens in eggs and the effect of thermal treatment

There are many microorganisms that can be found in eggs (Pattison et al., 2008; Saif, 2013). There are several ways for microorganisms to infect or contaminate the chicken egg: through infection of the ovum, infection of the oviduct and subsequent transmission to the egg, contamination with droppings when the egg passes through the cloaca or after laying and contamination of the egg during storage with infectious agents found in droppings or in the environment in contact with the eggs.

The agents that may be found in the chicken egg, either transmitted vertically or as a result of horizontal transmission or contamination are discussed below, following a taxonomic order (viruses, bacteria, protozoa, fungi and bacterial toxins). Spoilage bacteria, which are environmental contaminants, are also considered. Some microorganisms are found only rarely in eggs and, in some other cases, evidence suggesting egg transmission is circumstantial.

A list of poultry pathogens and potentially zoonotic organisms, assessed according to their occurrence in poultry, evidence of zoonotic potential, transmission within eggs and thermal resistance characteristics has been prepared by the working group experts below. Thermal resistance values were obtained in small-scale experiments, usually carried out in liquid egg or liquid media. Inactivation of biological agents in such media is more easily achieved than in solid material such as macerated embryonated eggs, so the estimates of thermal resistance may underestimate values that would be obtained if actual biogas or compost conditions were used.

Determination of the log-linearity behaviour of the inactivation curve is a prerequisite for determination of the D-value by means of laboratory heat treatment studies. However, owing to the strong tailing of the inactivation kinetics of many viruses and lack of culturability, reported inactivation studies may not be suitable for calculation of D-values (Dimmioc, 1967). For many of the listed pathogens, little data could be found and it was therefore not possible to provide D- and z-values.

### 3.2.2. Viruses

#### ***Retroviridae and Oncovirinae***

##### *Leucosis/sarcoma (L/S) group of avian type C oncoviruses and reticuloendotheliosis viruses (REV)*

Viruses from the L/S group of avian type C oncoviruses may be found in almost any flock of commercial chickens (Fadly and Nair, 2008). Chickens are the natural hosts for all viruses of the L/S group, but they have been also isolated from pheasants, partridges and quail. Experimentally, however, some members of the L/S group have a wide host range and can be adapted to grow in unusual hosts by passage in very young animals. Unequivocal proof of a public health risk by these viruses has not been reported (Nair and Fadly, 2013). Reticuloendotheliosis viruses have a broad host range, which includes turkeys, chickens, ducks, geese, pheasants, quail and peafowl. Certain mammalian cells support at least limited viral replication, including dog sarcoma cells, rat kidney cells, mink lung cells and bovine cells. Uncertainty exists concerning the possibility of infection of human cells. Evidence concerning human infection by these viruses has been regarded as insufficient (Nair et al., 2013).

- *Presence in eggs:* embryo infection with L/S viruses is strongly related to infection in the oviduct and egg albumen. Depending on infection/immune status of the flock, very high numbers of eggs may be positive; up to 60% (Payne et al., 1982). REV is transmitted to the embryo with lower frequency, approximately 10% (Bagust et al., 1981).
- *Thermal resistance:* L/S viruses are inactivated rapidly at high temperatures; the half-life at 50°C is 8.5 minutes and at 60°C is 0.7 minutes (Dougherty, 1961). No information on REV

was found but, as it is the same class of viruses, the resistance could be similar, although one study suggests relatively higher thermal tolerance (Darlix et al., 1992).

## ***Picornaviridae***

### *Genus Tremorvirus, avian encephalomyelitis virus (AEV)*

AEV is classified as a unique member of the genus *Tremorvirus* (King et al., 2012; Suarez, 2013). It has a limited host range: chickens, pheasants, quail, pigeons and turkeys. AEV occurs worldwide (Tannock and Shafren, 1994). There is no evidence suggesting that it is transmissible from avian species to mammals or humans. AEV has been isolated from all over the world, including several EU Member States (UK, Italy, France, Belgium, the Netherlands and Germany) (Guy et al., 2008).

- *Presence in eggs:* when susceptible flocks are exposed to AEV after sexual maturity, the hens transmit the virus to a variable proportion of their eggs. Some researchers have reported that a high proportion of hatching eggs may not hatch due to embryo mortality; in one study, the drop was from 78.6% to 59.6% (Taylor et al., 1955). In addition, a high proportion of the hatched chicks are likely to be infected (up to 60%) if the breeders are fully susceptible (Calnek, 2008).
- *Thermal resistance:* thermal resistance of AEV has not been fully investigated. Infectivity<sup>4</sup> of AEV may be only slightly reduced following 56°C heating for 1 hour (Butterfield et al., 1969; Takase et al., 1989). As with other enterovirus-like viruses, the virus is protected against inactivation by various salts (e.g. MgCl), which dramatically increases thermal tolerance, and variations occur in the inactivation mechanisms at high and low temperatures and pH values (Dimmiok, 1967; Davis, 1987). Information on thermal resistance is well known for other related picornaviruses, like foot-and-mouth disease virus (FMDV). D-value for different FMDV strains at 70°C ranged from 6.06 to 10.87 seconds (Kamolsiripichaiporn et al., 2007). However, some strain differences in thermal sensitivity have been observed (Nettleton et al., 1982). As well as being relatively heat resistant, picornavirus may also be resistant to inactivation by high pressure (Kingsley et al., 2004).

## ***Reoviridae***

### *Avian reoviruses*

Avian reoviruses have been found in many avian hosts, including chickens, turkeys, ducks, pigeons, geese and psittacine birds (Menendez et al., 1975; Al-Muffarej et al., 1996). Attempts to establish active infection in the canary, pigeon, guinea pig, rat, mouse, hamster and rabbit have failed. This group of viruses is considered to be ubiquitous in commercial poultry (Jones, 2013). No public health significance has been reported.

- *Presence in eggs:* egg transmission rates in infected commercial flocks are considered to be low (in one experiment approximately 2%), and shell contamination is unlikely (Menendez et al., 1975). Higher vertical transmission rates for some strains of virus have been reported experimentally (Al-Muffarej et al., 1996), but under natural conditions the rate is still considered to be low.
- *Thermal resistance:* avian reoviruses are relatively heat resistant; it is claimed that infectivity remains following heat treatment at 50°C for 30 minutes (Estes et al., 1979). Others report that it is able to withstand 60°C for 8–10 hours (Matthews, 1982).

### *Avian rotaviruses*

Avian rotaviruses have been found in turkeys, chickens, pheasants, partridges, ducks, guinea fowl, pigeons and lovebirds (Theil and Saif, 1987). Rotaviruses have been isolated from poultry all over the world, including several EU Member States (the UK, France, Belgium and Germany) (McNulty and Reynolds, 2008).

<sup>4</sup> Reduction of infectivity of an agent, while describing its thermal resistance, in the current opinion, is the reduction of the capacity to infect chicks or embryos exposed to treated material in comparison with untreated material.



- *Presence in eggs:* egg transmission has not been demonstrated but, because of infection of very young birds, it has been speculated that shell contamination (the virus is excreted in large numbers in faeces) may occur at a low transmission rate (Theil and Saif, 1987).
- *Thermal resistance:* heating at 56°C for 30 minutes decreased infectivity by 100-fold (Kang et al., 1988), but some infectivity remained after this treatment (Estes et al., 1979). However, it has been recently shown (Chmielewski et al., 2011) that 82.2°C for 6 hours could totally inactivate avian rotavirus.

## **Adenoviridae**

### *Genus Aviadenovirus (previously known as group I avian adenoviruses)*

Chicken adenoviruses are ubiquitous in fowl populations, as demonstrated by the identification of antibodies in serological surveys (Fitzgerald, 2013). Common hosts include chickens, turkeys, pigeons, budgerigars, ducks and pheasants (and probably geese). In addition, particles, which were probably adenoviruses, have been observed in kestrels, herring gulls, lovebirds, parakeets, parrots, murre and cockatiels. Some adenoviruses have been associated with human infection (e.g. SMAM-1), which may play a role in human obesity, and other strains may have interest as possible gene transfer vehicles.

- *Presence in eggs:* no transmission rates have been reported but the transmission to eggs is considered to be common (McFerran and Adair, 1977).
- *Thermal resistance:* major variation concerning heat resistance seems to exist. Strains have been observed to be viable following 30 minutes' treatment at 70°C, but this seems to be an exception. Treatment at 80°C for 30 minutes has been shown to eliminate all infectivity (Clemmer, 1964). However, several reports have indicated that the stability of these viruses to heat is greater when they are suspended in monovalent cations compared with divalent cations, a property which adenoviruses have in common with other DNA viruses.

### *Genus Atadenovirus (duck adenovirus 1 or egg drop syndrome virus (EDSV))*

Chickens, ducks and geese are affected (Fitzgerald, 2013). Turkeys and pheasants may be infected experimentally. EDSV has been isolated from several EU Member States (Belgium, the United Kingdom, Italy and Denmark) (Adair and Smyth, 2008). The virus affects only avian species, and it is not relevant for public health.

- *Presence in eggs:* the prevalence of infection in embryos is considered 'low' but no exact figures have been obtained.
- *Thermal resistance:* EDSV is inactivated by heating for 30 minutes at 60°C (Yamaguchi et al., 1981).

## **Orthomyxoviridae**

### *Type A avian influenza virus (AIV)*

AIVs have been isolated from more than 90 species of free-living birds, representing 13 different orders (Cappucci et al., 1985). Several mammalian species, including humans, may be infected naturally or experimentally. The low-pathogenicity avian influenza viruses (LPAIVs) are widespread globally (including Europe), primarily in wild birds, but they are occasionally introduced into commercial production without major clinical signs necessarily appearing. In contrast, the highly pathogenic avian influenza viruses (HPAIVs) are not considered to be endemic in wild bird populations but are believed to develop from LPAIV of the H5 and H7 subtype following introduction into flocks of poultry (Swayne and Halvorson, 2008). However, following the avian influenza A (H5N1) virus crisis starting in 2003, this view on the epidemiology may need to be reconsidered. Now, avian influenza A (H5N1) virus is endemic in several countries, primarily in Asia, and it appears that the virus is circulating and is being maintained primarily in waterfowl (as well as migrating species of birds). Thus, the introduction of HPAIV into commercial poultry production globally has become more likely (Alexander, 2008). With the increase in global trade, egg products could present potential biosecurity problems and affect international trade in liquid and dried egg products (Chmielewski et al., 2011).

The EU has implemented an 'early warning system' for highly pathogenic avian influenza (HPAI), which ensures that outbreaks will be detected in a timely way.<sup>5</sup> Consequently, the risk of introducing the virus into hatcheries is extremely low, but could potentially occur during the early stages of infection of a poultry breeding flock (Cappucci et al., 1985; Promkuntod et al., 2006).

- *Presence in eggs:* both HPAIV and LPAIV can be present on the eggshell surface; however, only HPAIVs can be present in the internal contents of chicken eggs laid by infected hens (Cappucci et al., 1985). Definitive proof of true vertical transmission of the disease to eggs and progeny is lacking and has been demonstrated only experimentally (Malladi et al., 2015). However, AIV infection of hens has resulted in virus recovery from eggshell surfaces and the internal contents of the eggs (Cappucci et al., 1985). The internal contents of eggs from five of seven severely affected chicken flocks in the USA were found to be positive for the virus and more than 50% of the eggs sampled were positive in some cases, depending on the egg storage and time before sampling (Cappucci et al., 1985).
- *Thermal resistance:* thermal inactivation studies using A/chicken/Pennsylvania/1370/83 (H5N2) HPAIV in a fat-free liquid egg model resulted in  $D_{55^-}$ ,  $D_{56^-}$ ,  $D_{56.7^-}$ ,  $D_{57^-}$ ,  $D_{58^-}$  and  $D_{59^-}$ -values of 18.6, 8.5, 3.6, 2.5, 0.4 and 0.4 minutes, respectively. The z-value was 4.4°C. LPAIV A/chicken/New York/13142/94 (H7N2) had  $D_{55^-}$ ,  $D_{56.7^-}$ ,  $D_{57^-}$ ,  $D_{58^-}$ ,  $D_{59^-}$  and  $D_{60^-}$ -values of 2.9, 1.4, 0.8, 0.7, 0.7, and 0.5 minutes, respectively, and a z-value of 0.4°C. In a more realistic study, when considering the nature of dead-in-shell embryos, thermal inactivation of the H5N1 HPAIV strain A/chicken/Korea/ES/2003 (Korea/03) was quantitatively measured in thigh and breast meat harvested from infected chickens. The Korea/03 titres were recorded as the mean embryo infectious dose ( $EID_{50}$ ) and were  $10^{8.0}$   $EID_{50}/g$  in uncooked thigh samples and  $10^{7.5}$   $EID_{50}/g$  in uncooked breast samples. Survival curves were constructed for Korea/03 in chicken thigh and breast meat at 1°C intervals for temperatures ranging from 57 to 61°C. Although some curves had a slightly biphasic shape, a linear model provided a fair-to-good fit at all temperatures, with  $R^2$  values of 0.85 to 0.93. Stepwise linear regression revealed that meat type did not contribute significantly to the regression model and generated a single linear regression equation for z-value calculations and D-value predictions for Korea/03 in both meat types. The z-value and the upper limit of the 95% confidence interval for the z-value were 4.64 and 5.32°C, respectively. From the lowest temperature to the highest, the predicted D-values and the upper limits of their 95% prediction intervals (conservative D-values) for 57 to 61°C were 241.2 and 321.1 seconds, 146.8 and 195.4 seconds, 89.3 and 118.9 seconds, 54.4 and 72.4 seconds and 33.1 and 44.0 seconds. D-values and conservative D-values predicted for higher temperatures were 0.28 and 0.50 seconds for 70°C and 0.041 and 0.073 seconds for 73.9°C. The conditions applied to biogas production should therefore provide a large margin of safety (Thomas and Swayne, 2007). Standard industry pasteurisation protocols are effective for inactivation in homogenised whole egg, for example 60°C for 2 minutes (Swayne and Beck, 2004). The inactivation of AIV during composting has also been tested (Senne et al., 1994). The method of composting described by Murphy and Handwerker (1988) was used. Infected tissues from sick birds were used although the infectious titre was not determined. No virus was isolated from these tissues following the composting process (highest temperature recorded was 58.3°C on day 13). However, no conclusions could be drawn on the extent of viral titre reduction, as the initial viral concentration in tissues was not determined.

## ***Hepeviridae***

### *Avian hepevirus*

Under field conditions, chickens are the only known host for avian hepatitis E virus (HEV) (Meng and Shivaprasad, 2013). Turkeys have been infected experimentally. Attempts to infect monkeys and mice have failed. Avian HEV infection in humans has not been reported. Most reports on prevalence of the virus concern the USA and Australia but serological evidence has also been obtained from the United

<sup>5</sup> Council Directive 2005/94/EC of 20 December 2005 on Community measures for the control of avian influenza and repealing Directive 92/40/EEC, OJ L 10, 14.1.2006, p. 16–65.

Kingdom (Todd et al., 1993). However, it seems that few investigations have been performed in order to assess the occurrence of the virus.

- *Presence in eggs:* no exact figures have been reported, but large numbers of virus are present in faeces and genuine vertical transmission has been suggested. Following experimental infection of hens, avian HEV can be found in egg whites, but evidence of true vertical transmission (virus inside the egg transmitted to progeny) is lacking (Guo et al., 2007).
- *Thermal resistance:* infectivity of avian HEV has been reported to be lost after incubating at 56°C for 1 hour or 37°C for 6 hours (Meng and Shivaprasad, 2013).

## **Circoviridae**

### *Chicken anaemia virus (CAV)*

CAV is commonly found in commercially produced chickens and has a worldwide distribution. It is difficult to inactivate thermally or with common disinfectants, which limits the utility of normal sanitisation practices. The virus is important because of the disease it produces following transovarian transmission and because of its potential for inducing immunosuppression alone or in combination with other infectious agents (Rosenberger and Cloud, 1998).

Infection by CAV (unique species of genus *Gyrovirus*) has been recognised only in chickens, turkeys and perhaps Japanese quail and some European corvids but other circoviruses or circo-like viruses from the genus *Circovirus* have been found in other species of birds and mammals (Chettle et al., 1989; Engström, 1999). Serological data suggest that CAV is ubiquitous in all major chicken-producing countries of the world, and virus isolation has been carried out in all continents (Schat and van Santen, 2008).

- *Presence in eggs:* in one study with experimentally infected hens, 7.5% of the eggs were infected (Yuasa and Yoshida, 1983), whereas approximately 3% were positive in another study (Hoop, 1992). Duck circovirus has been suggested to be capable of natural vertical transmission (Li et al., 2014).
- *Thermal resistance:* circoviruses are among the most resistant viruses known. CAV is highly resistant to inactivation. It is only partially resistant to heating at 80°C for 30 minutes and is completely destroyed within 15 minutes at 100°C. However, inactivation of CAV in infected chicken by-products (viraemic carcasses minced in a meat grinder and heated within sealed tubes within a water bath) requires a core temperature of 95°C for 30 minutes or 100°C for 10 minutes (Urlings et al., 1993). Fermentation of CAV-positive minced meat by the addition of *Lactobacillus* and incubation at 20°C for 7 days did not inactivate the virus (Urlings et al., 1993). CAV has been shown to be almost completely resistant to dry-heat treatment up to 120°C for 30 minutes (mean log infectivity reduction of 0.6) (Welch et al., 2006). Circoviruses from other species (e.g. pigs) are also highly resistant to heat. Experiments have demonstrated that porcine circovirus 2 virus is very resistant to dry heat at up to 120°C for 30 minutes and to moist heat at 75°C for 15 minutes (O'Dea et al., 2008).

## **Parvoviridae**

### *Anseriform dependoparvovirus 1 (geese and duck parvoviruses)*

Parvoviruses have been reported from all the major goose and Muscovy duck farming countries of Europe (Gough, 2008).

- *Presence in eggs:* vertical transmission is well documented but no transmission rates have been reported.
- *Thermal resistance:* avian parvoviruses are highly resistant to inactivation. No reduction in titre was observed following heating to 65°C for 30 minutes (Gough et al., 1981). However, it has recently been found (Chmielewski et al., 2011) that 82.2°C for 6 hours can totally inactivate 6 log<sub>10</sub> infectious dose/ml of chicken parvovirus, as determined by in vivo tests. Bovine parvovirus has also been demonstrated to be highly heat resistant. The infectivity of this virus was not significantly influenced by exposure to 95°C for 2 hours (dry heat)



(Sauerbrei and Wutzler, 2009). Canine parvovirus remained infective after 7 hours at 80°C (McGaving, 2008).

### ***Paramyxoviridae: paramyxovirinae -Avulavirus***

#### *Newcastle disease virus (NDV)*

In Western Europe, sporadic epizootics of Newcastle disease (ND) occur on a fairly regular basis despite the widespread use of vaccination (Alexander and Senne, 2008). However, the true distribution of the NDV is difficult to assess because of vaccination. Surveillance programmes and several control measures have been set in place for many years by the EC. Outbreaks of ND in holdings need to be notified and control measures are put in place including destruction of the eggs (even hatching eggs). Consequently, the real risk of introducing and maintaining the virus within hatcheries is extremely low, even though a low rate of egg transmission may be possible.

- *Presence in eggs:* both the virulent Newcastle disease virus (vNDV) strains and low-virulence or lentogenic Newcastle disease virus (INDV) strains can be present on the eggshell surface, while the vNDV can be present in the internal contents of chicken eggs laid by infected hens (Chmielewski et al., 2011). The main reason that ND is not considered a truly vertically transmitted disease is that flocks infected with NDV stop laying eggs and that infected embryos will likely die during incubation. However, the virus may be present in dead-in-shell chicks (Alexander and Senne, 2008). In addition, virus-laden faeces may contaminate the outside of the eggs. Further, it has been documented that the virus may be present in eggs from vaccinated flocks (French et al., 1967).
- *Thermal resistance:* the thermal resistance of NDV is not high. In artificially infected chicken meat homogenate the following D-values were obtained: D<sub>65</sub> of 120 seconds, D<sub>70</sub> of 82 seconds and D<sub>80</sub> of 29 seconds (Alexander and Manvell, 2004). D-values for liquid whole egg have been deduced as 38 seconds at 64.4°C for one particular strain (Alexander and Chettle, 1998). According to other studies, the vNDV avian paramyxoviruses of serotype 1 (AMPV-1)/chicken/California/212676/2002 had D<sub>55</sub><sup>-</sup>, D<sub>56</sub><sup>-</sup>, D<sub>56.7</sub><sup>-</sup>, D<sub>57</sub><sup>-</sup>, D<sub>58</sub><sup>-</sup> and D<sub>59</sub><sup>-</sup>-values of 12.4, 9.3, 6.2, 5, 3.7 and 1.7 minutes, respectively. The z-value was 4.7°C. INDV AMPV-1/chicken/United States/B1/1948 had D<sub>55</sub><sup>-</sup>, D<sub>57</sub><sup>-</sup>, D<sub>58</sub><sup>-</sup>, D<sub>59</sub><sup>-</sup>, D<sub>61</sub><sup>-</sup> and D<sub>63</sub><sup>-</sup>-values of 5.3, 2.2, 1.1, 0.55, 0.19 and 0.17 minutes, respectively, and a z-value of 1.0°C (Chmielewski et al., 2011).

### ***Astroviridae***

#### *Avian nephritis virus (ANV)*

ANV is an astrovirus, classified in the genus *Avastrovirus* (Virus Taxonomy Reports, online). It has a wide host range, affecting at least chickens, turkeys, ducks, pigeons and guinea fowl, and the public health significance is unknown (Todd and Imada, 2013), but antibodies to turkey astrovirus have been recently found in humans (Meliopoulus et al., 2014).

- *Presence in eggs:* vertical transmission supported by field observations; ANV has been detected in dead embryos from chickens and ducks, but no transmission rates have been reported (Biđin et al., 2013).
- *Thermal resistance:* ANV is relatively heat labile. Between 3- and 4-log reduction in infectivity were observed in different strains after heating at 56°C for 30 minutes (Takase et al., 1989).

### **3.2.3. Bacteria**

#### ***Salmonella***

Serovar Gallinarum/Pullorum is host adapted to a wide range of avian species and is vertically transmitted (Berchieri et al., 2001a). *S. Enteritidis* (Gast and Beard, 1990; Keller et al., 1995) and *S. Typhimurium* (Cason et al., 1994; Liljebjelke et al., 2005) are considered to be the most important serovars in terms of zoonotic potential and can be vertically transmitted (EFSA, 2009; EFSA BIOHAZ Panel, 2014). Other serovars are normally transmitted through faecal contamination, which may lead to horizontal infection of embryonated eggs. The broad host range serovars have a very complex

epidemiology involving a wide variety of birds and mammals. In addition, they are often associated with wildlife and environment. Most European countries have a low incidence or absence of *S. Gallinarum/Pullorum* in commercial poultry production (Shivaprasad and Barrow, 2008). The paratyphoid serovars are widespread in Europe but with varying incidence in different countries (Gast, 2008).

- *Presence in eggs*: vertical transmission appears to be the major mechanism for dissemination of *S. Gallinarum/Pullorum* and up to 33% of eggs may be infected (Shivaprasad and Barrow, 2008). *S. Enteritidis* and, to a lesser extent, certain strains of *S. Typhimurium* may be vertically transmitted by internalisation within the egg, and the vertical transmission of *S. Enteritidis* was a major reason for the pandemic spread of this serovar starting at the end of the 1980s (Sobel et al., 2000), although only a very small percentage will be inside the egg: less than 1% of eggs from an infected flock are internally contaminated (Humphrey et al., 1989; Williams et al., 1998). *Salmonella* can multiply to achieve large numbers during the incubation process without affecting the embryo, which leads to extensive contamination and cross-infection of chicks when eggs hatch. Shell contamination may be very high in the case of progeny being hatched from *Salmonella*-positive flocks; contamination rates ranging from 17% to 75% have been reported depending on serovars involved, immune status of the flocks, etc. (Cox et al., 1990; Bailey et al., 1994). Although surface decontamination of eggs is often carried out before incubation, this is never totally effective (Russell, 2003).
- *Thermal resistance*: generally, *S. Gallinarum/Pullorum* is less resistant to heat than members of the paratyphoid groups and is killed within 10 minutes at 60°C (Snoeyenbos, 1991). Heating at 57°C for at least 70 minutes can eliminate *Salmonella* inside intact eggs (Brackett et al., 2001). Liquid whole egg can be pasteurised successfully at 60°C for 3.5 minutes (Baker, 1990).

### ***Escherichia coli***

Most, if not all, avian species are susceptible to colibacillosis. Clinical disease has been found most often in chickens, turkeys and ducks. Natural infection of quail, pheasants, pigeons, guinea fowl, waterfowl, ostriches and emus has been reported (Petersen et al., 2006; Nolan et al., 2013). *E. coli* is part of the normal intestinal flora of birds; however, little is known about the carriage of potentially virulent types. Most avian *E. coli* isolates are specific clonal types that are pathogenic for only birds and represent a low risk of disease for people or other animals. Although natural infection is rarely identified, chickens can be readily experimentally infected with Shiga toxin-producing *E. coli* O157 (STEC), which is an important pathogen for humans; however, natural infection in chicken flocks is extremely rare. Ducks, pigeons and turkeys can also occasionally carry toxigenic *E. coli* (Pilipcinec et al., 1999).

Serotypes, virulence factors and antimicrobial resistance are often shared between avian *E. coli* strains and strains from other sources. Thus, avian strains can potentially be a source of genes and plasmids that encode antimicrobial resistance and virulence factors (Dierikx et al., 2013). Avian *E. coli* strains may carry virulence factors that are identical to the ones found in human uropathogenic *E. coli* strains. Plasmids from avian strains can contribute to uropathogenicity of *E. coli* in a murine model of human disease (Maluta et al., 2014). Antimicrobial resistance may also be a relevant consideration for other bacterial pathogens considered in this opinion, but the diversity of strains, plasmids and resistance genes amongst intestinal commensal organisms such as *E. coli* is considered to be much greater than within batches of hatchery waste than for any specific clonal pathogens that are likely to be present in or on hatching eggs (Mathers and Guerrant, 2014).

- *Presence in eggs*: in one study, 2.7% of the eggs contained *E. coli* internally following colonisation of the oviduct (via oral infection) (Ardrey et al., 1968). Embryonated eggs may commonly contain *E. coli* (in the yolk sac), and a contamination rate of up to 6% has been reported. In addition, experimental infections of hens resulted in up to 26% of their eggs being contaminated with *E. coli* and vertical transmission of fluoroquinolone resistant *E. coli* in hatching eggs has been reported (Harry, 1957; Petersen et al., 2006). *E. coli*, like most environmental organisms, can readily multiply within eggs, often leading to embryonic death.
- *Thermal resistance*: inactivation of most strains will occur at temperatures ranging from 60°C for 30 minutes to 70°C for 2 minutes (Nolan et al., 2013).

## ***Staphylococcus***

All avian species are susceptible to staphylococcal infections, and all *Staphylococcus* spp. (Kizerwetter-Świda and Binek, 2008; Smyth and McNamee, 2008) are ubiquitous, normal inhabitants of skin and mucous membranes and are common environmental organisms where poultry are reared, hatched or processed (Andreasen, 2008).

Methicillin-resistant *Staphylococcus aureus* (MRSA), which has emerged as an important human pathogen, may also be of concern in poultry and poultry meat because of the possibility of contact infections (Kyeremateng-Amoah et al., 2014). MRSA has been reported in poultry sampled in the EU (Beninati et al., 2015).

- *Presence in eggs:* *S. aureus* is ubiquitous, and conditions during incubation of eggs are ideal for bacterial growth. Contamination of eggs with faeces containing *S. aureus* may cause embryo mortality and recently it has been shown that hatched and hatching chicks may easily be infected (Smyth and McNamee, 2008). Twenty per cent (5 out of 25) of 18-day-old chicken embryos were *Staphylococcus* positive in one study (Kizerwetter-Świda and Binek, 2008).
- *Thermal resistance:* some strains are moderately resistant to inactivation but significant strain variation occurs. A  $D_{72}$ -value of 4.1 seconds has been reported when measured in milk (stationary phase) (Adam and Moss, 1995e). In another study  $D_{58}$ -values ranging between 0.93 minutes to 0.17 minutes were described (Rodriguez-Calleja et al., 2006).

## ***Streptococcus***

Several species of *Streptococcus* have been isolated from a variety of bird species (Kizerwetter-Świda and Binek, 2008; Smyth and McNamee, 2008). Chickens, turkeys, ducks, pigeons and psittacines may all be susceptible to at least some *Streptococcus* species. In addition, rabbits and mice have been shown to be susceptible to some of these isolates in experimental studies. It has been suggested that certain streptococci should be regarded as zoonotic agents (Dumke et al., 2015). Streptococci are normal inhabitants of birds and mammals and can be recovered from poultry housing environments (Kizerwetter-Świda and Binek, 2008; Smyth and McNamee, 2008).

- *Presence in eggs:* as taxonomy is still quite confusing with many species being re-classified over the years it is difficult to assess egg-transmission rates, but it is recognised that streptococci can be isolated from hatcheries and that its spread may be direct through the egg (Smyth and McNamee, 2008).
- *Thermal resistance:* there is little available information on the thermal tolerance of streptococci that can be associated with avian hosts and there is considerable variability in heat resistance between strains, with some isolates being able to actively multiply at 45–50°C. Many strains can survive pasteurisation of milk at 62.8°C for 30 minutes (Sherman and Stark, 1931). Heat treatment of 55°C for 10 minutes each hour was sufficient to prevent the accumulation of biofilms of *Streptococcus thermophilus* in a cheese production plant, but not to eliminate it (Knight et al., 2004). In contrast, studies in which *S. lactis* or *S. pneumoniae* overnight broth cultures were adsorbed in filter paper and then subjected to heat treatment in saline solution, neither organism survived for more than 5 seconds at 70°C, although *S. thermophilus* survived for 5 minutes (Patzschke, 1916). There is substantial variation between strains.

## ***Enterococcus***

Enterococci can be recovered from the environment of any poultry flock (Kizerwetter-Świda and Binek, 2008; Smyth and McNamee, 2008).

*E. faecalis*, in particular, has been isolated from clinically affected poultry over the years and several bird species may be affected including canaries, chickens, turkeys and ducks. Other species of relevance include *E. hirae*, *E. durans* and *E. faecium*. Mammals, including humans, may also be infected by some of these organisms.

- *Presence in eggs:* enterococci are common in both embryos and on the surface of eggs (Kizerwetter-Świda and Binek, 2008). One investigation reported the presence of enterococci

in 20% of 18-day-old embryos. Eggs were from a flock with no particular disease problems identified (Kizerwetter-Świda and Binek, 2008).

- **Thermal resistance:** there is variation between strains. Many strains survive at 60°C for 30 minutes and *E. faecium* is considered relatively heat resistant. In one study, *E. faecium* isolates survived at 80°C for 3 minutes (Kearns et al., 1995), while  $D_{70}$ -values obtained for *E. faecium* (ATCC 49624) in another study varied from 0.33 to 1.73 minutes as a function of culture temperature and physiological state of cells (Martínez et al., 2003). However, z-values calculated were not significantly influenced by these factors. A mean z-value of  $4.50 \pm 0.39^\circ\text{C}$  was found. In another experiment, isolates of *E. faecium* and *E. faecalis* were inoculated into raw ham and subjected to heating conditions simulating the industrial processing of cured, pasteurised ham. Thermal resistance characteristics, including survival curves, D-values and z-values were determined. *E. faecium* P-1A was the most heat-resistant organism tested, showing a D-value of 29.04 minutes at 66°C. *E. faecium* E-20 showed a D-value of 13.69 minutes at this temperature and *E. faecalis* P-2A was the least heat-resistant organism with a D-value of 1.69 minutes at 66°C. It was demonstrated that these three organisms showed greater thermal resistance in the raw ham (a matrix that could be considered to be similar to macerated chick embryos) than in Sorenson's buffer or ham broth. The derived z-values for the raw ham suggest that the use of the z-value in determining pasteurisation process parameters for solid materials should be carefully reconsidered (Magnus et al., 1988).

### ***Mycobacterium avium***

Avian tuberculosis in poultry is caused by *Mycobacterium avium* subsp. *avium*, (referred to as *M. avium* henceforth) and is distributed worldwide. There are several reports of the isolation of *M. avium* from different European countries (Finland, Norway, Denmark, Germany and Great Britain) (Fulton and Sanchez, 2008). However, much of the information relies on old investigations and it is difficult to provide robust data on current incidence and distribution (Dale and Brown, 2013).

All species of birds may be infected with *M. avium*. These infections have been common in patients with acquired immune deficiency syndrome (Hafez and Hauck, 2015). *M. avium* isolates recovered from humans and animals have some similarities, but the human isolates are more closely related to pig isolates than those from birds (Pérez de Val et al., 2014). *M. avium* serovar 2, the organism most frequently isolated from chickens, is rarely isolated from humans (Meissner and Anz, 1977).

- **Presence in eggs:** *M. avium* has been isolated from eggs of naturally infected chickens although no transmission rates have been reported. However, it has been reported that the bacilli occur rarely (Barrow, 1994; Jordan and Hampson, 2008).
- **Thermal resistance:** *M. avium* does not survive in eggs after 6 minutes of boiling (Fulton and Sanchez, 2008). Isolates from swine and from humans representing serotypes 1, 2, 4, 8 and 10 of the *M. avium*-*M. intracellulare* complex were compared for heat tolerance in aqueous suspension. Inactivation rates were minimal at 60°C or below. Decimal reduction values were 4 minutes or less at 65°C and 1.5 minutes or less at 70°C. Inactivation rates were slightly higher at pH values of 6.5 and 7.0 than at 5.5 or 6.0 (Merkal and Crawford, 1979).

### ***Campylobacter* species**

Many prevalence studies have been conducted in Europe, which have reported *Campylobacter*-positive flocks ranging from 3% to 97% (Zhang, 2008). The majority of on-farm surveys have been conducted with broiler chickens but breeder flocks and laying hens are also commonly infected (Shane, 1992) and the organism is the most common bacterial zoonosis in Europe.

*Campylobacter* bacteria are found in a wide variety of warm-blooded animals but the preferred host for the thermophilic species, *C. jejuni*, appears to be poultry (Newell and Fearnley, 2003). They are common in chickens, turkeys and ducks and have also been found in various game birds, pigeons and several wild bird species.

- **Presence in eggs:** this is still quite a controversial subject. In early studies, *C. jejuni* was reported to be recovered from approximately 1% of eggs from colonised hens (Doyle, 1984). In addition, intact eggshells appear to be permeable to *C. jejuni*. Over 4% of eggs can be experimentally infected with *C. jejuni* by immersion in a suspension of organisms (Allen and

Griffiths, 2001). The sensitivity of *Campylobacter* to inhibitory substances in egg albumen, the lack of infection of broiler flocks in cold seasons even though broiler breeder flocks are consistently infected, and the differences in genotypes found in parent and progeny flocks suggest that vertical transmission involving survival or multiplication of the organism within eggs is unlikely and susceptibility to desiccation prevents survival on eggshells (Vidal et al., 2014).

- *Thermal resistance*: *Campylobacter* are delicate organisms and are easily destroyed by heating (e.g. for pasteurisation,  $D_{55}$  is approximately 1 minute) (Adam and Moss, 1995b).

### ***Ornithobacterium rhinotracheale***

*Ornithobacterium rhinotracheale* has been reported throughout the world and, although it is a serious disease of poultry, it is not considered to be zoonotic (El-Gohary, 1998; Chin et al., 2008). *O. rhinotracheale* has been isolated from numerous bird species, including chickens, chukar partridge, duck, goose, guinea fowl, gull, pheasant, pigeon, quail and turkey.

- *Presence in eggs*: *O. rhinotracheale* has been isolated from hatching eggs, infertile eggs, dead embryos and dead-in-shell chickens and turkeys (Chin et al., 2008). However, no transmission rates have been indicated.
- *Thermal resistance*: available data indicate that at 37°C, *O. rhinotracheale* does not survive on egg-shells for more than 24 hours (Varga et al., 2001).

### ***Riemerella anatipestifer***

The disease occurs worldwide and has been recognised in countries that have intensive duck production (Sandhu, 2008).

*Riemerella anatipestifer* infection is primarily a disease of domestic ducks and geese. Naturally occurring outbreaks have been reported in turkeys. The organism has also been isolated from pheasants, chickens, guinea fowl, quail, partridge and other waterfowl. Recently, it has also been isolated from gulls, budgerigars, guillemots and pigs. Guinea pigs may die following infection with large dosages of the organism.

- *Presence in eggs*: the pathogenesis is obscure. Some field observations indicate that this agent may occur in eggs but a definitive proof of transmission to eggs is lacking
- *Thermal resistance*: most strains do not survive on solid media for more than 3 to 4 days at 37°C or room temperature. Incubation at 55°C for 12–16 hours inactivates the organism (Bangun et al., 1981).

### ***Mycoplasma gallisepticum*, *M. meleagridis*, *M. iowae*, *M. synoviae***

*Mycoplasma gallisepticum* infections naturally occur primarily in gallinaceous birds, particularly chickens and turkeys in commercial production (Glisson and Kleven, 1985). However, it has also been isolated from natural infections in pheasants, chukar partridge, peafowl, quail, ducks, geese, Amazon parrot, greater flamingos and various finches. *M. gallisepticum* probably occurs in all countries where poultry are kept, although the primary breeding companies maintain *M. gallisepticum*-free stock. In countries with well-developed poultry industries, most commercial breeding flocks are also *M. gallisepticum*-free and 'breaks' in such flocks are generally sporadic (Bradbury and Morrow, 2008).

*M. meleagridis* is a specific pathogen of turkeys but has recently been isolated from normal birds of prey in Germany (Yamamoto et al., 1966). The organism is considered to be widespread (Chin et al., 2008).

The natural host of *M. iowae* is the turkey, but isolation of *M. iowae* from chickens is not uncommon, and it has also been isolated from geese (McClenaghan et al., 1981). In addition, *M. iowae* has been isolated from Amazon parrots and from wild and exotic birds. The organism is considered to occur worldwide (Jordan and Amin, 1980).



The natural hosts of *M. synoviae* are chicken and turkey. Natural infections have also been found in ducks, geese, guinea fowl, pigeons, quail, pheasants and partridges (Carnaghan, 1961). *M. synoviae* has also been isolated from house sparrows in Spain. *M. synoviae* occurrence is widespread but primary breeding companies are largely free of infection due to control programmes (Kleven and Ferguson Noel, 2008).

Avian mycoplasma is not considered to be zoonotic.

- *Presence in eggs:* for *M. gallisepticum*, more than 50% of eggs may be infected during the acute phase of the infection (Sasipreeyajan et al., 1987). For *M. meleagridis*, the egg transmission rate among individual hens may vary from 10–60% (Yamamoto et al., 1966). Few data are available for *M. iowae*. However, some individuals in a flock may lay 'many' infected eggs (McClenaghan et al., 1981). For *M. synoviae*, no data are available, but major variation is expected as observed with the other species.
- *Thermal resistance:* *Mycoplasma* is sensitive to heat. Most species and strains will not survive temperatures above 45–47°C. *M. gallisepticum* was inactivated in infected chicken hatching eggs that reached 45.6°C during a 12–14-hour heating procedure (Yoder, 1970).

### ***Chlamydia psittaci***

Antibodies to *Chlamydia psittaci* have been found in more than 400 wild avian species (Wittenbrink et al., 1993; Lublin et al., 1996). It is recognised that any species of bird may be infected, although the susceptibility to infection may vary considerably. Mice and guinea pigs can be naturally infected. Humans may get infected and develop severe pneumonia. Avian chlamydiosis occurs worldwide, with the incidence and distribution varying greatly with the species of bird and the serotype of the chlamydial organism. In Europe there have been a number of outbreaks in ducks (Lederer and Muller, 1999; Andersen and Van Rompay, 2008).

- *Presence in eggs:* its occurrence in the egg is considered to be low (Andersen and van Rompay, 2008).
- *Thermal resistance:* diluted suspensions (20%) of infectious tissue homogenates are inactivated by incubation for 5 minutes at 56°C (Page, 1959).

### ***Clostridium* spp.**

Several species, including *Clostridium perfringens* and *C. botulinum*, may be involved in disease development in poultry, and different species have been isolated from eggshell fragments, embryos and chick fluff from the hatchery (Craven et al., 2001a,b; Kizerwetter-Świda and Binek, 2008). In all likelihood, much of the contamination of eggshells is due to survival of spores during the fumigation process but little has been published concerning the issue of vegetative cells versus spores in contamination.

- *Presence in eggs:* the hatchery has been identified as a source of *C. perfringens* in a number of studies (Craven et al., 2001a, b). Organisms have been recovered from up to 20% of eggshells (Craven et al., 2001a). While no specific reports of eggs contaminated with *C. botulinum* have been found, as a ubiquitous pathogen, this possibility exists and biowaste, including poultry material, can therefore pose a risk of botulism (Böhnel and Lube, 2000; Neuhaus et al., 2015).
- *Thermal resistance:* spores of *C. perfringens*, which is a common cause of food poisoning and causes necrotic enteritis in chickens, show a wide interstrain variability concerning D-values, which may vary from 0.31 minutes to more than 38 minutes at 100°C (Adam and Moss, 1995c). *C. botulinum* can produce heat-resistant spores and D-values (see Section 3.3.1) of 46 to 100 minutes at 85°C have been reported, dependent on the strain (Peck et al., 2008). At 121°C D-values have ranged from 0.1 to 1.3 minutes (Gaze and Brown, 1988; Adam and Moss, 1995c; Betts and Gaze, 1995; Diao et al., 2014).

### ***Bacillus* spp.**

*Bacillus* species are present in the poultry house environment and have been reported as contaminants of table eggs (EFSA BIOHAZ Panel, 2014).

- *Presence in eggs*: *Bacillus* spp., including *B. cereus*, have occasionally been associated with embryonic mortality in chickens, turkeys and ducks (Barnes and Nolan, 2008).
- *Thermal resistance*: no information concerning heat resistance of spores of avian isolates of *B. cereus* has been found. However, spores from non-avian strains have demonstrated variable heat resistance; recorded D-values at 95°C in phosphate buffer range from around 1 minute up to 36 minutes. Resistance appears to vary with serovar (Adam and Moss, 1995a). There is no record of *B. anthracis* in poultry.

### ***Listeria monocytogenes***

Outbreaks of listeriosis occur sporadically in chickens, turkeys and waterfowl (occasionally other species) (EFSA BIOHAZ Panel, 2014).

- *Presence in eggs*: in one study, the organism was not shed in eggs of heavily inoculated laying hens (Mazette et al., 1991). However, the organism may be present in faeces and can colonise the hatchery environment, consequently a risk of surface contamination of the egg exists (Cox et al., 1997).
- *Thermal resistance*: despite some conflicting data in the literature, it appears that the heat resistance of *Listeria monocytogenes* is similar to that of other non-spore-forming Gram-positive organisms with a typical D<sub>60</sub> of a few minutes and a D<sub>70</sub> of a few seconds (Adam and Moss, 1995d).

### ***Erysipelothrix rhusiopathiae***

*Erysipelothrix rhusiopathiae* is a zoonotic pathogen that is worldwide in distribution (Reboli and Farrar, 1989). It is a Gram-positive rod-shaped bacterium. Different serotypes of the bacterium occur and infection has been reported in many species. Healthy and diseased pigs can carry *E. rhusiopathiae* in lymphoid tissues, and may therefore constitute a major reservoir. In turkey and chicken flocks, *E. rhusiopathiae* may cause severe disease outbreaks (Ericksson et al., 2009).

- *Presence in eggs*: there is no evidence of vertical transmission of *E. rhusiopathiae* (Mazaheri et al., 2006).
- *Thermal resistance*: *E. rhusiopathiae* is killed by heating at 55°C for 15 minutes (Grieco and Sheldon, 1970). Heating of sewage sludge by microwave suggested that the organism is more heat sensitive than *Salmonella* (Niederwohrmeier, 1985).

## **3.2.4. Fungi**

### ***Aspergillus* spp.**

*Aspergillus fumigatus* and other *Aspergillus* species may also be egg-transmitted (Eggert and Barnhart, 1953). Aspergillosis occurs in most domesticated and several wild animal species all over the world. Mycotic abortion is an important disease of dairy and beef cattle worldwide. Poultry, exotic and wild birds appear to be particularly susceptible to pulmonary aspergillosis. Aspergillosis in humans has increased over the years as a complication of therapeutic immune suppression.

- *Presence in eggs*: the fungi may penetrate the eggshell during incubation, and infected eggs that are opened (or explode in the incubator) during incubation release large number of spores that may infect hatchmates. Problems with infection of a large number of eggs may occur following, for example, *in ovo* vaccination as the puncture facilitates entry and dissemination of spores.
- *Thermal resistance*: the fungi are not considered highly resistant to heat. D-values for conidia from various strains at 60°C ranged from 8 to 59 seconds (Doyle and Marth, 1975).

### **3.2.5. Spoilage organisms**

Several organisms have been isolated from chicken and turkey eggs including Gram-positive cocci, *Proteus* spp., coliforms, *Pseudomonas*, *Klebsiella* spp., *Enterobacter* spp., *Bacillus* spp. and *Clostridium*

spp. (Jordan and Hampson, 2008; Kizerwetter-Świda and Binek, 2008). Most of these organisms have a broad host range but may be regarded as opportunistic pathogens occurring worldwide.

- *Presence in eggs*: spoilage organisms may be found in eggs as a result of shell penetration. They are likely to cause them to burst during the early stages of incubation, so they are not likely to be present within dead-in-shell chicks. Spoilage and environmental organisms may be found on the shells of eggs, but would not be expected to multiply in this location during the incubation process (Mayes and Takeballi, 1983).
- *Thermal resistance*: this group includes environmental microorganisms with a very variable thermoresistance. Among this group, the spores of *Bacillus* and *Clostridium* spp. have the highest resistance to heat (as described above).

### 3.2.6. Protozoa

#### *Toxoplasma gondii*

Naturally occurring infections have been diagnosed in chickens, turkeys, ducks and many wild birds from all over the world. It should be mentioned that commercial housed chickens have little exposure to *Toxoplasma gondii* oocysts, in contrast to free-range birds.

- *Presence in eggs*: it is unclear whether congenital infection occurs in chicks from naturally infected parents. One study showed that the organism could not be isolated from eggs from an infected flock of breeders (Jacobs and Melton, 1966) and another study showed a very low percentage of eggs carried the organism (Iannuzzi and Renieri, 1971). Only one study reports substantial embryonic mortality and malformation of surviving chicks (18%) following experimental infection of hens (Caballero-Servín, 1974).
- *Thermal resistance*: generally, it is recognised that cooking of, for example, pork to between 70 and 75°C provides a wide safety margin (Dixon, 1992). Twenty-gram samples of infected homogenised meat were sealed in plastic pouches, pressed to a uniform thickness of 2 mm, and subjected to water bath temperatures of 49, 52, 55, 58, 61, 64 or 67°C for 0.01, 3, 6, 9, 12, 24, 48 or 96 minutes. Treated samples were digested in HCl-pepsin solution and bioassayed in mice. *T. gondii* tissue cysts remained viable when heated at 52°C for 9.5 minutes but not at 58°C for 9.5 minutes, and tissue cysts from infected mouse brain, which were added to minced pork, were eliminated when heated at 61°C for 3.6 minutes (Dubey et al., 1990). *T. gondii* tachyzoites survived cooking for 3 minutes in artificially inoculated boiled or fried egg, especially in yolk (Dubey, 2010).

### 3.2.7. Bacterial toxins

Depending on the storage conditions of the material to be processed, the production of bacterial toxins could be possible. Some bacterial toxins have been shown to be thermostable. That is the case of cereulide, the emetic toxin produced by *B. cereus* strains, which has recently been shown to remain stable after more than 2 hours at 121°C (dry-heat treatment) (Rajkovic et al., 2008). In addition, *S. aureus* produces a highly resistant enterotoxin exhibiting activity after treatment at 100°C (Fung et al., 1973). In contrast, *Clostridium* toxins are more heat-labile. Losikoff (1978) reports an inactivation of *C. botulinum* toxin at 77°C after 6 minutes. A recent review of toxin plasmids of *C. perfringens* (Li et al., 2013) provides an overview of the range of toxins this microorganism produces. Although the *C. perfringens* enterotoxin is a heat-labile protein and is inactivated by heating for 5 minutes at 60°C, it is not generally produced in food (McDonel, 1980). The survival of bacterial spores in treated material could, however, lead to germination of spores and formation of toxins after treatment (Peck et al., 1995).



### 3.3. Ability of the currently approved standards to inactivate the possible biological hazards present in dead-in-shell chicks

#### 3.3.1. General considerations on thermal inactivation

Inactivation of microorganism and viruses can be achieved by chemical, physical and biotechnological treatments. While in chemical and biotechnological processes the inactivation kinetics cannot be easily described by mathematical terms, heat inactivation under comparable conditions follows mathematical laws which allow interpretation, characterisation and prediction of the effectiveness of such treatment processes. It is generally assumed that the inactivation of microbial populations exposed to lethal temperatures follows first-order kinetics. This principle is widely used in thermal process calculations although it does not describe all the experimental observations of microbial destruction. There is a mechanistic justification in this first-order kinetics as death is caused by inactivation of some essential enzymes or enzymatic complexes, and enzyme inactivation generally follows also first-order kinetics. Nevertheless, it should be pointed out that sometimes survival curves do not fit within first-order kinetics (Cerf, 1977). In such cases, shoulders and/or tails (downwards and/or upwards concavity phenomena) are observed. More sophisticated models have been proposed to fit survival data in these instances (Sapru et al., 1992; Peleg and Penchina, 2000). Some of these combine first-order kinetics for distinct microbial inactivation stages, while others use probability models. This last approach to microbial inactivation considers lethal events as probabilistic instead of deterministic and it is based on the fact that the survival curve of the population is considered to be the cumulative form of the survival curves of the individual units (cells, virus particles or units of infectivity) that show a biological variation in thermoresistance.

If the kinetics of first-order reactions are assumed, the time interval required to achieve one decimal reduction (i.e. a 90% reduction) in the number of survivors is constant; this means that the time to reduce the population from  $10^4$  to  $10^3$  is the same as the time required to reduce the population from  $10^3$  to  $10^2$ . Thermal calculations based on first-order kinetics are not only a common practice in the food industry but are also mandated by food safety regulations and codes of practice. The calculation of the efficacy of thermal treatments involves the need for knowledge of the initial concentration of microorganisms to be inactivated, the acceptable concentration of surviving microorganisms, the thermal resistance of the target microorganisms (the most heat-resistant) and the temperature–time relationship required for the destruction of the target microorganisms (Beney et al., 2000). These calculations are commonly based on the following parameters:

- D-value: the decimal reduction time, the time required at a certain constant temperature to inactivate 90% of the involved population of organisms.
- z-value: it can be defined as the temperature change (in °C) required for a 1-log reduction in the D-value. The D-value at a given temperature is specific to each type of microorganism, species, strain and particular physiological condition. Furthermore, microorganisms show a specific D-value at a given temperature and a specific z-value in a given food matrix. For instance, the D-value of *S. enterica* serovar Senftenberg in liquid whole eggs at 63°C ranges from 1.2 to 3.1 minutes, and its z-value is 5.2°C (Mañas et al., 2003).

These parameters are dependent on several factors, the most important are the physical properties of the material to be treated, e.g. the water activity ( $a_w$  value), the thermal conductivity and homogeneity of the matrix and the chemical properties of the material, e.g. pH value, inorganic ions and organic components, which can be protective or increase the effect of the thermal treatment. Moreover, the thermoresistance patterns within species are not identical and heat resistance is often strain-specific. This means that thermoresistance patterns have to be determined in the matrix to be treated; results from experiments with other treated materials can only be extrapolated within limitations.

Taking into account all these general considerations, in the current assessment the efficacy of processing of death-in-shell chicks of 12 mm particle size, for 60 minutes at 70°C (the minimum requirements established in the Regulation for treatment of Category 3 materials prior to biogas or compost production) to inactivate the main biological hazards identified in Section 3.2 was evaluated using the information available in the literature on heat resistance parameters (D-values and z-values)

of the identified hazards. It is important to note that there is a lack of available experimental data on thermal inactivation of microorganisms in the matrix under assessment ('dead-in-shell' chicks). The assessment has therefore been carried out using information on heat resistance parameters obtained in whole liquid egg or other egg products (when available) or in laboratory media or other food matrixes. It is well known that physicochemical properties of the treated matrix (e.g. pH, water activity) have a strong influence on microbial heat resistance parameters, therefore the level of uncertainty associated with assumptions and conclusions made in the assessment is high for some biological hazards.

### 3.3.2. Identification of the target biological hazards

Spores of *C. botulinum* have been identified as the most resistant bacterial hazard potentially present in the material to be processed. Other limiting spore-forming organisms contaminating the product, e.g. *C. perfringens*, may also be relevant.

As regards viruses, circovirus (chicken anaemia virus) and parvovirus (chicken parvovirus) are considered to be the most resistant.

Of the non-sporulating bacteria, *E. faecium* is regarded as the most resistant to heat treatments. The most heat-resistant serovar of *S. enterica* (*S. Senftenberg*, variant 775W) was also considered in the assessment. Nevertheless, these organisms are still relatively heat sensitive compared with spore formers and thermoresistant viruses.

### 3.3.3. Treatment of 12-mm particle size, at 70°C and for 60 minutes

The standard processing conditions for treatment of Category 3 materials prior to biogas or compost production (particle size 12 mm, 60 minutes, 70°C) (as explained in Section 3.1.2) are equivalent to a pasteurisation treatment, where the vegetative forms of pathogenic microorganisms are killed, while most spores remain viable.

Spores of *C. botulinum* have been identified as the most resistant of the pathogenic microorganisms potentially present in the material to be processed. *C. botulinum* spores may show D-values at 121°C as high as 0.2 minutes, ranging from 0.1 to 1.3 minutes, and would require a treatment at 121°C for approximately 3 minutes to achieve a 12-log reduction in their population (Diao et al., 2014). Despite the lack of studies assessing the thermal inactivation kinetics of *C. botulinum* spores in eggs, it can be concluded that a treatment at 70°C for 60 minutes will not be sufficient to inactivate them. Similarly, *C. perfringens* spores have D-values at 100°C of approximately 90 minutes (Raju et al., 2006), and will be also able to survive under the proposed treatment conditions.

Regarding vegetative bacteria, *E. faecium* is considered to be the most heat-tolerant non-spore-forming bacterium (Martinez et al., 2003; Fernández et al., 2009). Its high tolerance to adverse conditions and its ubiquitous nature makes *E. faecium* a reference microorganism appropriate to evaluate the efficacy of pasteurisation processes (Smith et al., 1990) and disinfection methods (Spicher et al., 2002). Sörqvist (2003) reviewed the thermal tolerance of *E. faecium* in several liquids, including liquid whole egg, and described D<sub>60</sub>-values ranging from 17.0 to 27.1 minutes and z-values ranging from 3.63 to 14.3°C. This would result in an 11.1-log reduction after treatment for 60 minutes at 70°C in the worst-case scenario. The thermal tolerance of other microorganisms belonging to the Enterobacteriaceae is well recognised to be lower than that of *E. faecium*. As an example, *S. enterica* serovar Enteritidis, one of the main biological hazards linked to hatchery waste, shows D<sub>60</sub>-values ranging from 0.15 to 0.5 minutes (Sörqvist, 2003; Jin et al., 2008). Taking into account that z-values described for *Salmonella* spp. range from 3.24 to 9.5°C (Sörqvist, 2003), a treatment for 60 minutes at 70°C will result in a > 1 000-log reduction. With regard to *S. Senftenberg* 775W, the *Salmonella* serovar with the greatest thermal tolerance (Alvarez-Ordóñez et al., 2009a,b), D<sub>63</sub>-values ranging from 1.2 to 3.1 minutes and z-values ranging from 4.5 to 9.1°C have been reported (Sörqvist, 2003). Thus, a treatment for 60 minutes at 70°C would assure a > 200-log inactivation. Therefore, it can be concluded that the proposed treatment method would be able to inactivate more than 5 log of *E. faecalis* or *S. Senftenberg* in the material to be treated, as required in Section 2, Chapter III, Annex V, of Regulation (EU) No 142/2011.

Among the viruses identified in Section 3.2 as potential hazards that may be present in the material to be thermally processed, circoviruses (e.g. chicken anaemia virus) and parvoviruses are the most

thermo-resistant. Although, to the best of our knowledge, there are no studies determining the thermal inactivation kinetics and describing D- and z-values for either family of avian viruses; various studies indicate that they may be able to survive under the recommended treatment conditions. Thus, Welch et al. (2006) showed that chicken anaemia virus and porcine circovirus 2 are almost completely resistant to dry-heat treatments of up to 120°C for 30 minutes (mean log infectivity reduction of 0.6) and Urlings et al. (1993) reported that inactivation of chicken anaemia virus in infected chicken by-products requires a core temperature of 95°C for 35 minutes, or 100°C for 10 minutes. Parvoviruses are also highly resistant to thermal inactivation. It has been shown that infectivity is not significantly reduced by exposure to 95°C for 2 hours (Sauerbrei and Wutzler, 2009). In laboratory conditions, a 10-fold reduction of the number of infectious porcine parvovirus was not obtained after 60 minutes at 60°C (Blümel et al., 2002). However, in manure held in experimental reactors for 11 to 54 hours at 55°C a 4 log<sub>10</sub> reduction of infectious porcine parvovirus resulted, whereas less than 1 log<sub>10</sub> reduction was obtained after 1 hour at 70°C (Lund et al., 1996). Lund et al. (1996) described a D<sub>70</sub>-value for porcine parvovirus of 42.8 to 100 minutes, which would result in a 0.6-log reduction after a treatment for 60 minutes at 70°C. Other relevant viral hazards linked to hatchery waste, AIV and NDV are less heat tolerant. Their thermal tolerance in homogenised whole egg has been assessed by Swayne and Beck (2004), who reported D<sub>59</sub>-values ranging from 0.36 to 0.37 minutes and 0.37 to 1.60 minutes for AIV and NDV, respectively. These authors also described z-values ranging from 3.2 to 3.6°C and 3.9 to 7.7°C for AIV and NDV, respectively. Thus, treatment at 70°C for 60 minutes would result in a > 180 000-log reduction for AIV and > 1 000-log reduction for NDV. Therefore, it can be concluded that the proposed treatment will be able to inactivate heat-sensitive viruses present in the material to be treated, while heat-resistant viruses, such as circoviruses and parvoviruses, could survive. It therefore cannot be concluded that a reduction of infectivity titre of thermo-resistant viruses, such as parvovirus, by at least 3 log can be achieved, as required in Section 2, Chapter III, Annex V, of Regulation (EU) No 142/2011.

A compilation of heat resistance parameters of selected relevant microorganisms are presented in Table 1. No suitable data were found for circoviruses.

**Table 1:** Thermal tolerance characteristics determined experimentally at the nearest temperature to 70°C and reduction in microorganisms under proposed thermal treatment conditions

Microorganism	Matrix	D <sub>t</sub> -value (minutes)	z-value (°C)	Log-reductions after treatment at 70°C for 60 minutes	Reference
<i>E. faecium</i>	Liquid egg	D <sub>60</sub> -value: 17.0–27.1	3.63–14.3	11.1–2 006.9	Sörqvist (2003)
<i>S. Enteritidis</i>	Liquid egg	D <sub>60</sub> -value: 0.15–0.5	3.24–9.5	1 354.6–488 067.3	Jin et al. (2008), Sörqvist (2003)
<i>S. Senftenberg 775W</i>	Liquid egg	D <sub>63</sub> -value: 1.2–3.1	4.5–9.1	113.8–1 796.9	Mañas et al. (2003), Sörqvist (2003)
<i>Porcine parvovirus</i>	Slurry and bleaching clay	D <sub>70</sub> -value: 42.8–100	No data	0.6–1.4	Lund et al. (1996)
<i>AIV</i>	Liquid egg	D <sub>59</sub> -value: 0.36–0.37	3.2–3.6	184 049.1–456 403.3	Swayne and Beck (2004)
<i>NDV</i>	Liquid egg	D <sub>59</sub> -value: 0.37–1.60	3.9–7.7	1 006.7–107 266.1	Swayne and Beck (2004)
<i>C. botulinum</i>	Various liquid matrixes	D <sub>121</sub> -value: 0.1–1.3	10.7–11.8	0.010–0.002	Diao et al. (2014)

### 3.4. Risk posed by the possible use of dead-in-shell chicks for the production of biogas or compost

The legal requirements for the different treatment of Category 2 and Category 3 material is based on the principle that Category 2 materials are considered more likely to contain pathogens and undesirable substances and as such can represent a risk for humans, animals and the environment, while Category 3 materials are in principle fit for human consumption and represent no higher health and environmental risks than disposed food. This is the reason why legislation demands for Category 2 materials, with a few exceptions, prior treatment with method 1 (133°C/3 bar/20 minutes)

considering that this treatment will also give additional safety concerning as yet unidentified pathogens and will remove residual transmissible spongiform encephalopathies (TSE) infectivity. The treatment of Category 3 materials in biogas or composting plants is based on the assumption that the risk of TSE infectivity, as well as other infectious agents, is low and the given requirements for a batch pasteurisation process before the biogas treatment would then be considered sufficiently robust. When unpasteurised material is composted, a closed composting reactor must be used and the given particle size as well as the time/temperature relationship (70°C/60 minutes) must be maintained throughout the material. The limitations of this treatment have already been considered in the 'Opinion on the safety vis-à-vis biological risks of biogas and compost treatment standards of animal by-products' (EFSA, 2005). In the case of dead-in-shell chicks as feeding material for the reactors, the presence of pathogens, as mentioned in Section 3.2, as well as aerobic and anaerobic spoilage organisms must be expected with a high probability.

Microorganisms, such as the heat-resistant picornaviruses and circoviruses and *Salmonella*, may cause active infection/disease in some birds belonging to the avifauna. However, several microorganisms also may be spread passively by the fauna and wild birds, rodents, foxes or flying insects, which may act as passive vectors and transport the agents over long distances (Langholz and Jay-Russell, 2013).

Moreover there is uncertainty on whether pathogenic and/or toxigenic clostridia, e.g. *C. botulinum*, may propagate in the biogas process and may present a risk to the environment (Böhnel and Gessler, 2012). Nevertheless, clostridial spores would be expected to survive in the treated animal by-products considered in this opinion (AVA, 2010).

The requirements for particle size, temperature/time relationships for treatment of Category 3 materials in the Regulation are, in principle, the same for both composting and biogas production even if the aerobic and the anaerobic processes are different in their biological and technical specifications. Generally, the given parameters are not based on an experimental validation of a typical pasteurisation unit or of a defined composting reactor with an animal by-product characterised by defined physical and chemical properties by the use of a heat-resistant pathogen. The given parameters are historical requirements based on the assumption that they are sufficient for hygienic safety of a material which is considered, in principle, to be fit for human consumption.

## 4. Uncertainty evaluation

### 4.1. Background

In the EFSA context, the term 'uncertainty' is intended to cover '*all types of limitations in knowledge, at the time it is collected*' in the risk assessment process (EFSA Scientific Committee, 2009). The need to address uncertainty is expressed in the Codex Working Principles for Risk Analysis. These state that '*constraints, uncertainties and assumptions having an impact on the risk assessment should be explicitly considered at each step in the risk assessment and documented in a transparent manner*' (CODEX, 2007). The Scientific Committee of EFSA explicitly endorsed this principle in its guidance on transparency in risk assessment (EFSA Scientific Committee, 2009). Therefore it is recognised that in the risk assessment process it is important to characterise, document and explain all types of uncertainty arising in the process.

Ideally, the analysis of the uncertainty in a risk assessment would require the following steps:

- a. identifying uncertainties;
- b. describing uncertainties;
- c. evaluating uncertainties around individual factors in their own scales;
- d. evaluating the impact of individual factors uncertainties on the assessment outcome;
- e. evaluating the combined impact of multiple uncertainties on the assessment outcome including evaluating how much the combined uncertainties downgrade the weight of the evidence.

The last three steps can be conducted at three levels: qualitative, deterministic and probabilistic.

The EFSA Working Group on Uncertainty in Risk Assessment<sup>6</sup> is currently formulating guidelines on how the uncertainty analysis should be performed in a harmonised and structured way. As this is still ongoing, this opinion addresses only the first two steps, i.e. identification and description of the uncertainty.

A summary of the main sources of uncertainty is provided in Appendix A.

## 4.2. Identification and description of the sources of uncertainty

The most common type of uncertainty identified for assessment components are 'Ambiguity', mainly because of scarcity of data on the extent of the variability in heat resistance of different strains of the target pathogens, substrate properties, limited data on numbers and thermoresistance of the pathogens and contaminants as well as the lack of practical experience in treating those substrates in different technical devices. This is followed by 'Extrapolation uncertainties' due to uncertainties relating to extrapolation from data generated under different test conditions and 'Sampling and Measurement uncertainty' due to the small-scale nature and lack of replication of the existing published experimental work. 'Distribution uncertainty' is also an issue as the survival and thermal tolerance of different genotypes within a group of pathogens is often very variable and experiments are usually based on a single or limited number of strains.

There is a large amount of information on the occurrence of pathogens in poultry as poultry breeding and production represents a massive global industry so there is a strong incentive to identify and control pathogens and many text books and scientific publications have been written on the subject. Similarly, those commensal organisms that may be found in poultry faeces and lead to contamination of eggs are well known because of the importance of reducing the risk of zoonotic infection and food poisoning associated with the large volume of poultry products that are traded and consumed.

There is little information on the rate of contamination of fertile eggs or avian embryos or on numbers of organisms present for any of the pathogens.

Quantitative data on the multiplication of organisms within embryonated eggs are also lacking, with available data relating to only highly artificial studies of experimentally inoculated table eggs with *Salmonella*. Numbers of organisms present before treatment is a very important consideration when considering a log reduction target if some organisms remain and can then multiply, often more efficiently than before if competing organisms have been reduced by the treatment.

A potentially significant concern is the impact of the specific matrix on thermal inactivation of the pathogens. Most inactivation studies are carried out on broth or suspensions of single organisms in liquid products, whereas in the solid material considered here there may be microcolonies of organisms embedded in biofilms or present intracellularly that are relatively protected from the direct impact of heat. The presence of fatty material and high osmotic pressure may also be protective. On the other hand, the time taken to reach the designated temperature should also be taken into account in the assessment of efficacy and there is normally significant additional reduction of pathogens during the time it takes for material to reach the final treatment temperature.

There is also considerable uncertainty about the efficiency of the different types of equipment that are used for compost and biogas product and the potential for errors or mechanical problems that could interfere with the decontamination process or lead to recontamination.

The combined effect of these areas of uncertainty indicates that caution should be applied to extrapolation of experimental data to the processes that will be used for the current application. This largely applies to only those pathogens that show a high level of heat resistance, but the risk associated with mechanical failure and consequent incomplete treatment or recontamination of some batches may be important for those organisms that can readily multiply outside the host, such as *Salmonella*.

The data limitations apply to all the pathogens under consideration but are not likely to affect the outcome of the assessment since the reduction of vegetative bacteria is substantial and any variation between strains and the protective effect of the matrix would not be expected to result in a lesser reduction than that required by the EU Regulation, in numbers of organisms. It is possible that such

<sup>6</sup> See [www.efsa.europa.eu/en/scer/scerwgs.htm](http://www.efsa.europa.eu/en/scer/scerwgs.htm) for details.



variability may influence the survival of thermotolerant agents, resulting in greater survival than anticipated. This may, however, be mitigated if the concentration of agents in the material before treatment is low because of limited occurrence in or on hatching eggs or ability to multiply in hatching eggs or waste derived from hatching eggs.

## 5. Conclusions

- No exposure assessment was carried out to assess the risks associated with survival of pathogens or toxins following treatment of dead in shell embryos during the biogas or compost production process.
- Where data were available, the probability of survival of pathogenic agents subjected to the processing conditions specified in Regulation (EU) No 142/2011 and the reduction in their concentration was assessed.
- Dead-in-shell chicks are, according to the basic rationale of the animal by-products regulations, classified as Category 2 materials because they are, in principle, not considered to be fit for human consumption, unlike Category 3 materials. They are considered to be more similar to fallen animals than to animal by-products from food production, because, in most cases, the reason for their death is unknown and infectious disease cannot be excluded.
- A wide range of pathogens can be associated with embryonated eggs and hatchery waste and may cause disease in poultry and other animal species. Some of them are zoonotic.
- There is a lack of quantitative data on the occurrence or survival of the relevant pathogens and effect of treatment conditions in the specific material and processes under consideration. Extrapolation from small-scale laboratory experiments using different matrices leads to uncertainty which is likely to overestimate the capability of the treatment in most cases if the material to be treated is highly contaminated.
- The most heat-resistant agents are bacterial spores of *C. botulinum* and *C. perfringens*, as well as thermoresistant viruses, such as parvoviruses and circoviruses.
- The approved treatment conditions for biogas and compost production derived from Category 3 materials may inactivate vegetative bacteria (e.g. *Salmonella*) and most virus species (e.g. AIV, NDV) in material derived from dead-in-shell chicks, but not the most heat-resistant agents. Treatment of dead-in-shell chicks, according to the biogas and composting standards for Category 3 material, is not able to reduce pathogenic agents, by 5 log for bacteria and 3 log for viruses, as required by Regulation (EU) No 142/2011.
- Although there is a lack of quantitative data on the concentration and reduction of the pathogenic agents considered in this opinion within the actual matrix, the resulting uncertainty does not affect the conclusions above regarding insufficient inactivation of thermoresistant agents.

## 6. Recommendations

The BIOHAZ Panel recommends:

- studies under field conditions to determine the occurrence and concentration of pathogens in hatchery waste, as well as the time and temperature combinations that would sufficiently reduce pathogenic agents;
- development and implementation of a protocol for use of representative test organisms to be added during the validation of the thermal processes considered in this opinion in order to demonstrate a required level of inactivation of pathogens in the end product.

## References

- Adair BM and Smyth JA, 2008. Egg drop syndrome. In: Diseases of poultry, 12th edn. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE. Blackwell Publishing, Ames, IA, USA, 266–276.
- Adam MR and Moss MO, 1995a. *Bacillus cereus*. In: Food microbiology. TRSo Chemistry. Redwood Books Ltd., Trowbridge, UK, 160.
- Adam MR and Moss MO, 1995b. *Campylobacter*. In: Food microbiology. TRSo Chemistry. Redwood Books Ltd., Trowbridge, UK, 164.
- Adam MR and Moss MO, 1995c. *Clostridium perfringens*. In: Food microbiology. TRSo Chemistry. Redwood Books Ltd., Trowbridge, UK, 174.
- Adam MR and Moss MO, 1995d. *Listeria monocytogenes*. In: Food microbiology. TRSo Chemistry. Redwood Books Ltd., Trowbridge, UK, 187.
- Adam MR and Moss MO, 1995e. *Staphylococcus aureus*. In: Food microbiology. TRSo Chemistry. Redwood Books Ltd., Trowbridge, UK, 205.
- Alexander DJ, 2008. *Orthomyxoviridae*-avian influenza. In: Poultry diseases, 6th edn. Eds Pattison M, McMullin PF, Bradbury JM and Alexander DJ, Saunders-Elsevier, Philadelphia, PA, USA, 317–332.
- Alexander DJ and Chettle NJ, 1998. Heat inactivation of serotype 1 infectious bursal disease virus. *Avian Pathology*, 27, 97–99.
- Alexander DJ and Manvell RJ, 2004. Heat inactivation of Newcastle disease virus (strain Herts 33/56) in artificially infected chicken meat homogenate. *Avian Pathology*, 33, 222–225.
- Alexander DJ and Senne DA, 2008. Newcastle disease. In: Diseases of poultry, 12th edn. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE. Blackwell Publishing, Ames, IA, USA, 75–100.
- Allen KJ and Griffiths MW, 2001. Use of luminescent *Campylobacter jejuni* ATCC 33291 to assess eggshell colonization and penetration in fresh and retail eggs. *Journal of Food Protection*, 64, 2058–2062.
- Al-Muffarej SI, Savage CE and Jones RC, 1996. Egg transmission of avian reoviruses in chickens: comparison of a trypsin-sensitive and a trypsin-resistant strain. *Avian Pathology*, 25, 469–480.
- Al-Sadi HI, Basher HA and Ismail HK, 2000. Bacteriologic and pathologic studies on dead-in-shell chicken embryos. *Iraqi Journal of Veterinary Sciences*, 13, 297–307.
- Alvarez-Ordóñez A, Fernández A, Bernardo A and López M, 2009a. A comparative study of thermal and acid inactivation kinetics in fruit juices of *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Senftenberg grown at acidic conditions. *Foodborne Pathogens and Disease*, 6, 1147–1155.
- Alvarez-Ordóñez A, Fernández A, Bernardo A and López M, 2009b. Relationship between membrane fatty acid composition and heat resistance of acid and cold stressed *Salmonella* Senftenberg CECT 4384. *Food Microbiology*, 26, 347–353.
- Andersen AA and van Rompay D, 2008. Avian chlamydiosis (Psittacosis, Ornithosis). In: Diseases of poultry, 12th edn. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE. Blackwell Publishing, Ames, IA, USA, 971–986.
- Andreasen CB, 2008. Staphylococcosis. In: Diseases of poultry, 12th edn. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE, Blackwell Publishing, Ames, IA, USA, 892–900.
- Ardrey WB, Peterson CF and Haggart M, 1968. Experimental colibacillosis and the development of carriers in laying hens. *Avian Diseases*, 12, 505–511.
- AVA (Agrar und Veterinär Akademie), 2010. Chronischer Botulismus. Botulinumtoxikosen bei Mensch und Tier—Chronischer Botulismus in Milchviehherden? Was ist dran? Available online: [http://www.ig-botulismus.de/index\\_htm\\_files/ava\\_np\\_rind\\_botulismus\\_leseprobe.pdf](http://www.ig-botulismus.de/index_htm_files/ava_np_rind_botulismus_leseprobe.pdf)

- Bagust TJ, Grimes TM and Ratnamohan N, 1981. Experimental infection of chickens with an Australian strain of reticuloendotheliosis virus 3. Persistent infection and transmission by the adult hen. *Avian Pathology*, 10, 375–385.
- Bailey JS, Cox NA and Berrang ME, 1994. Hatchery-acquired salmonellae in broiler chicks. *Poultry Science*, 73, 1153–1157.
- Baker RC, 1990. Survival of *Salmonella enteritidis* on and in shelled eggs, liquid eggs and cooked egg products. *Dairy, Food and Environmental Sanitation*, 10, 273–275.
- Bangun A, Tripathy DN and Hanson LE, 1981. Studies of *Pasteurella anatipestifer*: an approach to its classification. *Avian Diseases*, 25, 326–327.
- Barnes HJ and Nolan LK, 2008. Other bacterial diseases. In: *Diseases of poultry*, 12th edn. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE. Blackwell Publishing, Ames, IA, USA, 952–970.
- Barrow PA, 1994. The microflora of the alimentary tract and avian pathogens: translocation and vertical transmission. In: *Microbiology of the avian egg*. Eds Board RG and Fuller R. Springer, USA, 117–138.
- Beney L, Perrier-Cornet JM, Fine F and Gervais P, 2000. Combining heat treatment, control of water activity and pressure to preserve foods. In: *Food preservation techniques*. Eds Zeuthen P and Bøgh-Sørensen L. Woodhead Publishing Limited, Cambridge, UK, 179–203.
- Beninati C, Reich F, Muscolino D, Giarratana F, Panebianco A, Klein G and Atanassova V, 2015. ESBL-producing bacteria and MRSA isolated from poultry and turkey products imported from Italy. *Czech Journal of Food Sciences*, 33, 97–102.
- Berchieri A Jr, Murphy CK, Marston K and Barrow PA, 2001a. Observations on the persistence and vertical transmission of *Salmonella enterica* serovars Pullorum and Gallinarum in chickens: effect of bacterial and host genetic background. *Avian Pathology*, 30, 221–231.
- Berchieri A Jr, Wigley P, Page K, Murphy CK and Barrow PA, 2001b. Further studies on vertical transmission and persistence of *Salmonella enterica* serovar Enteritidis phage type 4 in chickens. *Avian Pathology*, 30, 297–310.
- Betts GD and Gaze JE, 1995. Growth and heat resistance of psychrotrophic *Clostridium botulinum* in relation to 'sous vide' products. *Food Control*, 6, 57–63.
- Bidín Z, Majnarić D, Lojkić I and Bidín M, 2013. Vertical transmission and possible role of viral infections in hatchability problems of duck embryos. *Proceedings of the XVIIIth Congress 2013 WVPA*, 19–23 August 2013, Nantes, France.
- Blümel J, Schmidt I, Willkommen H and Löwer J, 2002. Inactivation of parvovirus B19 during pasteurization of human serum albumin. *Transfusion*, 42, 1011–1018.
- Böhnel H and Gessler F, 2012. Hinweise zum Vorkommen von Rinderbotulismus in Deutschland anhand von Laboruntersuchungen der Jahre 1996–2010. *Tierärztliche Umschau*, 67, 1–6.
- Böhnel H and Lube K, 2000. *Clostridium botulinum* and bio-compost. A contribution to the analysis of potential health hazards caused by bio-waste recycling. *Journal of Veterinary Medicine*. B, 47, 785–795.
- Brackett RE, Schuman JD, Ball HR and Scouten AJ, 2001. Thermal inactivation kinetics of *Salmonella* spp. within intact eggs heated using humidity-controlled air. *Journal of Food Protection*, 64, 934–938.
- Bradbury JM and Morrow C, 2008. Avian mycoplasmas. In: *Poultry diseases*, 6th edn. Eds Pattison M, McMullin PF, Bradbury JM and Alexander DJ, Saunders-Elsevier, Philadelphia, PA, USA, 220–235.
- Bruce J and Drysdale EM, 1983. The bacterial flora of candling-reject and dead-in-shell turkey eggs. *British Poultry Science*, 24, 391–395.
- Bruzual JJ, Peak SD, Brake J and Peebles ED, 2000. Effects of relative humidity during incubation on hatchability and body weight of broiler chicks from young breeder flocks. *Poultry Science*, 79, 827–830.



- Butterfield WK, Helmboldt CF and Luginbuhl RE, 1969. Studies on avian encephalomyelitis. IV. Early incidence and longevity of histopathologic lesions in chickens. *Avian Diseases*, 13, 53–57.
- Caballero-Servín A, 1974. Congenital malformations in *Gallus gallus* induced by *Toxoplasma gondii* (article in Spanish). *Revista de Investigación en Salud Pública*, 34, 87–94.
- Calnek BW, 2008. Avian encephalomyelitis. In: *Diseases of poultry*, 12th edn. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE. Blackwell Publishing, Ames, IA, USA, 430–431.
- Cappucci DT Jr, Johnson DC, Brugh M, Smith TM, Jackson CF, Pearson JE and Senne DA, 1985. Isolation of avian influenza virus (subtype H5N2) from chicken eggs during a natural outbreak. *Avian Diseases*, 29, 1195–1200.
- Carnaghan RB, 1961. Egg transmission of infectious synovitis. *Journal of Comparative Pathology*, 71, 279–285.
- Cason JA, Cox NA and Bailey JS, 1994. Transmission of *Salmonella typhimurium* during hatching of broiler chicks. *Avian Diseases*, 38, 583–588.
- Cerf O, 1977. A Review. Tailing of survival curves of bacterial spores. *Journal of Applied Bacteriology*, 42, 1–19.
- Chettle NJ, Eddy RK, Wyeth PJ and Lister SA, 1989. An outbreak of disease due to chicken anaemia agent in broiler chickens in England. *Veterinary Record*, 124, 211–215.
- Chin RP, Ghazikhanian GY and Kempf I, 2008. *Mycoplasma meleagridis* Infection. In: *Diseases of poultry*, 12th edn. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE. Blackwell Publishing, Ames, Iowa, USA, 834–841.
- Chmielewski RA, Beck JR and Swayne DE, 2011. Thermal inactivation of avian influenza virus and Newcastle disease virus in a fat-free egg product. *Journal of Food Protection*, 74, 1161–1168.
- Christensen VL, 2001. Factors associated with early embryonic mortality. *World's Poultry Science Journal*, 57, 359–372.
- Clemmer DI, 1964. Characterization of agents isolated from market chickens in a quest for enteric viruses. *Journal of Infectious Diseases*, 114, 386–400.
- CODEX, 2007. Working Principles for Risk Analysis for Food Safety for Application by Governments. Available online: <ftp://ftp.fao.org/docrep/fao/010/a1550t/a1550t00.pdf>
- Cox NA, Bailey JS, Mauldin JM and Blankenship LC, 1990. Research note—presence and impact of salmonella contamination in commercial broiler hatcheries. *Poultry Science*, 69, 1606–1609.
- Cox NA, Bailey JS and Berrang ME, 1997. The presence of *Listeria monocytogenes* in the integrated poultry industry. *The Journal of Applied Poultry Research*, 6, 116–119.
- Craven SE, Cox NA, Stern NJ and Mauldin JM, 2001a. Prevalence of *Clostridium perfringens* in commercial broiler hatcheries. *Avian Diseases*, 45, 1050–1053.
- Craven SE, Stern NJ, Bailey JS and Cox NA, 2001b. Incidence of *Clostridium perfringens* in broiler chickens and their environment during production and processing. *Avian Diseases*, 45, 887–896.
- Dale E and Brown C, 2013. Zoonotic diseases from poultry. *Brazilian Journal of Veterinary Pathology*, 6, 76–82.
- Darlix JL, Gabus C and Allain B, 1992. Analytical study of avian reticuloendotheliosis virus dimeric RNA generated *in vivo* and *in vitro*. *Journal of Virology*, 66, 7245–7252.
- Das KC, Minkara MY, Melear ND and Tollner EW, 2002. Effect of poultry litter amendment on hatchery waste composting. *Journal of Applied Poultry Research*, 11, 282–290.
- Davies RH and Wray C, 1994. An approach to reduction of *Salmonella* infection in broiler chicken flocks through intensive sampling and identification of cross-contamination hazards in commercial hatcheries. *International Journal of Food Microbiology*, 24, 147–160.
- Davis D, 1987. Temperature and pH stability of duck hepatitis virus. *Avian Pathology*, 16, 21–30.

- Diao MM, André S and Membré J-M, 2014. Meta-analysis of D-values of proteolytic *Clostridium botulinum* and its surrogate strain *Clostridium sporogenes* PA 3679. *International Journal of Food Microbiology*, 174, 23–30.
- Dierikx CM, van der Goot JA, Smith HE, Kant A and Mevius DJ, 2013. Presence of ESBL/AmpC-producing *Escherichia coli* in the broiler production pyramid: a descriptive study. *PLoS One*, 8, e79005.
- Dimmiok NJ, 1967. Differences between the thermal inactivation of picornaviruses at “high” and “low” temperatures. *Virology*, 31, 338–353.
- Dixon BR, 1992. Prevalence and control of toxoplasmosis—a Canadian perspective. *Food Control*, 3, 68–75.
- Dougherty RM, 1961. Heat inactivation of Rous sarcoma virus. *Virology*, 14, 371–372.
- Doyle MP, 1984. Association of *Campylobacter jejuni* with laying hens and eggs. *Applied and Environmental Microbiology*, 47, 533–536.
- Doyle MP and Marth EH, 1975. Thermal inactivation of conidia from *Aspergillus flavus* and *Aspergillus parasiticus*. I. Effects of moist heat, age of conidia, and sporulation medium. *Journal of Milk and Food Technology*, 38, 678–682.
- Dubey JP, 2010. *Toxoplasma gondii* infections in chickens (*Gallus domesticus*): prevalence, clinical disease, diagnosis and public health significance. *Zoonoses Public Health*, 57, 60–73.
- Dubey JP, Kotula AW, Sharar A, Andrews CD and Lindsay DS, 1990. Effect of high temperature on infectivity of *Toxoplasma gondii* tissue cysts in pork. *Journal of Parasitology*, 76, 201–204.
- Dumke J, Hinse D, Vollmer T, Schulz J, Knabbe C and Dreier J, 2015. Potential transmission pathways of *Streptococcus gallolyticus* subsp. *gallolyticus*. *PLoS One*, 10, e0126507.
- EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on biological hazards (BIOHAZ) on vis-à-vis biological risks of biogas and compost treatment standards of animal by-products (ABP). *The EFSA Journal* 2005, 264, 1–21. doi:10.2903/j.efsa.2005.264
- EFSA (European Food Safety Authority), 2009. Scientific Opinion of the Panel on Biological Hazards on Quantitative estimation of the impact of setting a new target for the reduction of Salmonella in breeding hens of *Gallus gallus*. *The EFSA Journal* 2009, 1036, 1–68. doi:10.2903/j.efsa.2009.1036
- EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014. Scientific Opinion on the public health risks of table eggs due to deterioration and development of pathogens. *EFSA Journal* 2014;12(7):3782, 147 pp. doi:10.2903/j.efsa.2014.3782
- EFSA Panel on Biological Hazards (BIOHAZ) and EFSA Panel on Animal Health and Welfare (AHAW), 2011. Scientific Opinion on Hatchery Waste as animal by-products. *EFSA Journal* 2011;9(7):2321, 35 pp. doi:10.2903/j.efsa.2011.2321
- EFSA Scientific Committee, 2009. Guidance of the Scientific Committee on Transparency in the Scientific Aspects of Risk Assessments carried out by EFSA. Part 2: General Principles. *The EFSA Journal* 2009, 1051, 1–22. doi:10.2903/j.efsa.2009.1051
- Eggert MJ and Barnhart JV, 1953. A case of egg-borne aspergillosis. *Journal of the American Veterinary Medical Association*, 122, 225.
- El-Gohary AA, 1998. *Ornithobacterium rhinotracheale* (ORT) associated with hatching problems in chicken and turkey eggs. *Veterinary Medical Journal Giza*, 46, 183–191.
- Engström BE, 1999. Prevalence of antibody to chicken anaemia virus (CAV) in Swedish chicken breeding flocks correlated to outbreaks of blue wing disease (BWD) in their progeny. *Acta Veterinaria Scandinavica*, 40, 97–107.
- Eriksson H, Jansson DS, Johansson KE, Baverud V, Chirico J and Aspan A, 2009. Characterization of *Erysipelothrix rhusiopathiae* isolates from poultry, pigs, emus, the poultry red mite and other animals. *Veterinary Microbiology*, 137, 98–104.

- Estes MK, Graham DY, Smith EM and Gerba CP, 1979. Rotavirus stability and inactivation. *Journal of General Virology*, 43, 403–409.
- Fadly AM and Nair V, 2008. Leukosis/sarcoma group. In: *Diseases of poultry*, 12th edn. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE. Blackwell Publishing, Ames, IA, USA, 514–568.
- Fernández A, Alvarez-Ordóñez A, López M and Bernardo A, 2009. Effects of organic acids on thermal inactivation of acid and cold stressed *Enterococcus faecium*. *Food Microbiology*, 26, 497–503.
- Fitzgerald SD, 2013. Introduction. Chapter 9. Adenovirus Infections. In: *Diseases of Poultry*, 13th edn. Eds Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL and Nair VL. Wiley-Blackwell, 289–332.
- French EL, St George TD and Percy JJ, 1967. Infection of chicks with recently isolated Newcastle disease viruses of low virulence. *Australian Veterinary Journal*, 43, 404–409.
- Fry DM, 1995. Reproductive effects in birds exposed to pesticides and industrial chemicals. *Environmental Health Perspectives*, 103(Suppl. 7), 165–171.
- Fulton RM and Sanchez S, 2008. Tuberculosis. In: *Diseases of poultry*, 12th edn. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE. Blackwell Publishing, Ames, IA, USA, 940–951.
- Fung DY, Steinberg DH, Miller RD, Kurantnick MJ and Murphy TF, 1973. Thermal inactivation of staphylococcal enterotoxins B and C. *Applied Microbiology*, 26, 938–942.
- Gast RK, 2008. Paratyphoid infections. In: *Diseases of poultry*, 12th edn. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE. Blackwell Publishing, Ames, IA, USA, 636–665.
- Gast RK and Beard CW, 1990. Production of *Salmonella enteritidis*-contaminated eggs by experimentally infected hens. *Avian Diseases*, 34, 438–446.
- Gaze JE and Brown KL, 1988. The heat resistance of spores of *Clostridium botulinum* 213B over the temperature range 120 to 140°C. *International Journal of Food Science and Technology*, 23, 373–378.
- Glisson JR and Kleven SH, 1985. *Mycoplasma gallisepticum* vaccination: further studies on egg transmission and egg production. *Avian Diseases*, 29, 408–415.
- Gough RE, 2008. Parvovirus infections. In: *Diseases of poultry*, 12th edn. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE. Blackwell Publishing, Ames, IA, USA, 397–404.
- Gough RE, Spackman D and Collins MS, 1981. Isolation and characterisation of a parvovirus from goslings. *Veterinary Record*, 108, 399–400.
- Grgic H, Philippe C, Ojkic D and Nagy E, 2006. Study of vertical transmission of fowl adenoviruses. *Canadian Journal of Veterinary Research*, 70, 230–233.
- Grieco MH and Sheldon C, 1970. *Erysipelothrix rhusiopathiae*. *Annals of the New York Academy of Sciences*, 174, 523–532.
- Guo H, Zhou EM, Sun ZF and Meng XJ, 2007. Egg whites from eggs of chickens infected experimentally with avian hepatitis E virus contain infectious virus, but evidence of complete vertical transmission is lacking. *Journal of General Virology*, 88, 1532–1537.
- Guy JS, McNulty MS and Hayhow CS, 2008. Avian entero-like viruses. In: *Diseases of poultry*, 12th edn. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE. Blackwell Publishing, Ames, IA, USA, 365–361.
- Hafez HM and Hauck R, 2015. Zoonoses with Public Health Relevance in Poultry. In: *Zoonoses— infections affecting humans and animals*. Ed. Sing A, Springer Netherlands, 103–123.
- Harry EG, 1957. The effect on embryonic and chick mortality of yolk contamination with bacteria from the hen. *Veterinary Record*, 69, 1433–1441.

- Heier BT and Jarp J, 2001. An epidemiological study of the hatchability in broiler breeder flocks. *Poultry Science*, 80, 1132–1138.
- Hoop RK, 1992. Persistence and vertical transmission of chicken anaemia agent in experimentally infected laying hens. *Avian Pathology*, 21, 493–501.
- Humphrey TJ, Baskerville A, Mawer S, Rowe B and Hopper S, 1989. *Salmonella enteritidis* phage type 4 from the contents of intact eggs: a study involving naturally infected hens. *Epidemiology and Infection*, 103, 415–423.
- Iannuzzi L and Renieri G, 1971. The egg in the epidemiology of toxoplasmosis. Tests of experimental infections by injection through the shell (article in Italian). *Acta Medica Veterinaria (Napoli)*, 17, 311–317.
- ICTV (International Committee on Taxonomy of Virus), online. Virus Taxonomy Reports, online. Available online: <http://www.ictvonline.org/>
- Jacobs L and Melton M L, 1966. Toxoplasmosis in chickens. *The Journal of parasitology*, 52, 1158–1162.
- Jin T, Zhang H, Boyd G and Tang J, 2008. Thermal resistance of *Salmonella enteritidis* and *Escherichia coli* K12 in liquid egg determined by thermal-death-time disks. *Journal of Food Engineering*, 84, 608–614.
- Jones RC, 2013. Chapter 11. Reovirus Infections. In: *Diseases of poultry*, 13th edn. Eds Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL and Nair VL. Wiley-Blackwell, 351–374.
- Jordan FT and Amin MM, 1980. A survey of Mycoplasma infections in domestic poultry. *Research in Veterinary Science*, 28, 96–100.
- Jordan FTW and Hampson DJ, 2008. Chapter 22—Some other bacterial diseases. In: *Poultry diseases*. 6th edn. Eds Pattison M, McMullin PF, Bradbury JM and Alexander DJ. Saunders-Elsevier, Philadelphia, PA, USA, 243–256.
- Kalita N, Pathak N, Ahmed M and Saikia GK, 2013. Various causes related to dead-in-shell embryos of crossbred (PB-2 × Indigenous) chicken egg. *Veterinary World*, 6, 774–777.
- Kamolsiripichaiporn S, Subharat S, Udon R, Thongtha P and Nuanualsuwan S, 2007. Thermal inactivation of foot-and-mouth disease viruses in suspension. *Applied and Environmental Microbiology*, 73, 7177–7184.
- Kang SY, Nagaraja KV and Newman JA, 1988. Physical, chemical, and serological characterization of avian rotaviruses. *Avian Diseases*, 32, 195–203.
- Kearns AM, Freeman R and Lightfoot NF, 1995. Nosocomial enterococci: resistance to heat and sodium hypochlorite. *Journal of Hospital Infection*, 30, 193–199.
- Keller LH, Benson CE, Krotec K and Eckroade RJ, 1995. *Salmonella enteritidis* colonization of the reproductive tract and forming and freshly laid eggs of chickens. *Infection and Immunity*, 63, 2443–2449.
- King AMQ, Adams MJ, Carstens EB and Lefkowitz EJ 2012. *Virus Taxonomy. Classification and Nomenclature of Viruses*. Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, CA, USA.
- King'ori AM, 2011. Review of the factors that influence egg fertility and hatchability in poultry. *International Journal of Poultry Science*, 10, 483–492.
- Kingsley DH, Chen H and Hoover DG, 2004. Inactivation of selected picornaviruses by high hydrostatic pressure. *Virus Research*, 102, 221–224.
- Kizerwetter-Świda M and Binek M, 2008. Bacterial microflora of the chicken embryos and newly hatched chicken. *Journal of Animal and Feed Sciences*, 17, 224–232.
- Kleven SH and Ferguson-Noel N, 2008. *Mycoplasma synoviae* infection. In: *Diseases of poultry*, 12th edn. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE. Blackwell Publishing, Ames, IA, USA, 845–856.

- Knight GC, Nicol RS and McMeekin TA, 2004. Temperature step changes: a novel approach to control biofilms of *Streptococcus thermophilus* in a pilot plant-scale cheese-milk pasteurisation plant. *International Journal of Food Microbiology*, 93, 305–318.
- Kyeremateng-Amoah E, Nowell J, Luty A, Lees PSJ and Silbergeld EK, 2014. Laceration injuries and infections among workers in the poultry processing and pork meatpacking industries. *American Journal of Industrial Medicine*, 57, 669–682.
- Langholz JA and Jay-Russell MT, 2013. Potential role of wildlife in pathogenic contamination of fresh produce. *Human–Wildlife Interactions*, 7, 140–157.
- Lederer P and Muller R, 1999. Ornithosis—studies in correlation with an outbreak. *Gesundheitswesen*, 61, 614–619.
- Lewith S, 1890. Ueber die Ursache der Widerstandsfähigkeit der Sporen gegen hohe Temperaturen. *Archiv für experimentelle Pathologie und Pharmakologie*, 26, 341–354.
- Li J, Adams V, Bannam TL, Miyamoto K, Garcia JP, Uzal FA, Rood JI and McClane BA, 2013. Toxin plasmids of *Clostridium perfringens*. *Microbiology and Molecular Biology Reviews*, 77, 208–233.
- Li Z, Wang X, Zhang R, Chen J, Xia L, Lin S, Xie Z and Jiang S, 2014. Evidence of possible vertical transmission of duck circovirus. *Veterinary Microbiology*, 174, 229–232.
- Liljebjelke KA, Hofacre CL, Liu T, White DG, Ayers S, Young S and Maurer JJ, 2005. Vertical and horizontal transmission of salmonella within integrated broiler production system. *Foodborne Pathogens Diseases*, 2, 90–102.
- Losikoff ME, 1978. Establishment of a heat inactivation curve for *Clostridium botulinum* 62A toxin in beef broth. *Applied and Environmental Microbiology*, 36, 386–388.
- Lublin A, Shudari G, Mechani S and Weisman Y, 1996. Egg transmission of *Chlamydia psittaci* in turkeys. *Veterinary Record*, 139, 300.
- Lund B, Jensen VF, Have P and Ahring B, 1996. Inactivation of virus during anaerobic digestion of manure in laboratory scale biogas reactors. *Antonie Van Leeuwenhoek*, 69, 25–31.
- Magnus CA, McCurdy AR and Ingledew WM, 1988. Further studies on the thermal resistance of *Streptococcus faecium* and *Streptococcus faecalis* in pasteurized ham. *Canadian Institute of Food Science and Technology Journal*, 21, 209–212.
- Malladi S, Weaver JT, Alexander CY, Middleton JL, Goldsmith TJ, Snider T, Tilley BJ, Gonder E, Hermes DR and Halvorson DA, 2015. Quantitative estimation of the number of contaminated hatching eggs released from an infected, undetected turkey breeder hen flock during a highly pathogenic avian influenza outbreak. *Avian Diseases*, 59, 355–367.
- Maluta RP, Logue CM, Casas MR, Meng T, Guastalli EA, Rojas TC, Montelli AC, Sadatsune T, de Carvalho Ramos M, Nolan LK and da Silveira WD, 2014. Overlapped sequence types (STs) and serogroups of avian pathogenic (APEC) and human extra-intestinal pathogenic (ExPEC) *Escherichia coli* isolated in Brazil. *PLoS One*, 9, e105016.
- Mañas P, Pagán R, Alvarez I and Condón Usón S, 2003. Survival of *Salmonella senftenberg* 775 W to current liquid whole egg pasteurization treatments. *Food Microbiology*, 20, 593–600.
- Martínez S, López M and Bernardo A, 2003. Thermal inactivation of *Enterococcus faecium*: effect of growth temperature and physiological state of microbial cells. *Letters in Applied Microbiology*, 37, 475–481.
- Mathers AJ and Guerrant RL, 2014. Dissecting the evolutionary stealth of our flora against antibiotics. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 108, 121–122.
- Matthews REF, 1982. Classification and nomenclature of viruses. Fourth report of the International Committee on Taxonomy of Viruses. *Intervirology*, 17, 1–199.
- Mayes FJ and Takeballi MA, 1983. Microbial contamination of the hen's egg: a review. *Journal of Food Protection*, 46, 1092–1098.



- Mazaheri A, Philipp HC, Bonsack H and Voss M, 2006. Investigations of the vertical transmission of *Erysipelothrix rhusiopathiae* in laying hens. *Avian Diseases*, 50, 306–308.
- Mazzette R, Sanna E, De Santis EPL, Pisanu S and Leoni A, 1991. Experimental listeriosis in chickens microbiological and anatomic histopathological examinations and food hygiene considerations. *Bollettino della Società Italiana di Biologia Sperimentale*, 67, 569–576.
- McClenaghan M, Bradbury JM and Howse JN, 1981. Embryo mortality associated with avian *Mycoplasma* serotype I. *Veterinary Record*, 108, 459–460.
- McDonel JL, 1980. *Clostridium perfringens* toxins (type A, B, C, D, E). *Pharmacology and Therapeutics*, 10, 617–655.
- McFerran JB and Adair BM, 1977. Avian adenoviruses—a review. *Avian Pathology*, 6, 189–217.
- McGaving D, 2008. Inactivation of canine parvovirus by disinfectants and heat. *Journal of Small Animal Practice*, 23, 523–525.
- McNulty MS and Reynolds DL, 2008. Rotavirus infections. In: *Diseases of poultry*, 12th edn. Eds Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL and Nair VL, Wiley-Blackwell, 338–350.
- Meissner G and Anz W, 1977. Sources of *Mycobacterium avium* complex infection resulting in human diseases. *The American Review of Respiratory Disease*, 116, 1057–1064.
- Meliopoulos VA, Kayali G, Burnham A, Oshansky CM, Thomas PG, Gray GC, Beck MA and Schultz-Cherry S, 2014. Detection of antibodies against Turkey astrovirus in humans. *PLoS One*, 9, e96934.
- Menendez NA, Calnek BW and Cowen BS, 1975. Experimental egg-transmission of avian reovirus. *Avian Diseases*, 19, 104–111.
- Meng XJ and Shivaprasad HL, 2013. Avian Hepatitis E Virus Infections. Chapter 14. Other Viral Infections. In: *Diseases of poultry*, 13th edn. Eds Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL and Nair VL. Wiley-Blackwell, 465–512.
- Merkal RS and Crawford JA, 1979. Heat inactivation of *Mycobacterium avium-Mycobacterium intracellulare* complex organisms in aqueous suspension. *Applied and Environmental Microbiology*, 38, 827–830.
- Murphy DW and Handwerker TS, 1988. Preliminary investigations of composting as a method of dead bird disposal. *Proceedings of the first national poultry waste management symposium*, Columbus, OH, USA, 65–72.
- Nair VL and Fadly AM, 2013. Leukosis/sarcoma group. In: Chapter 15. Neoplastic diseases. *Diseases of poultry*, 13th edn. Eds Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL and Nair VL, Wiley-Blackwell, 513–674.
- Nair VL, Zavala G and Fadly AM, 2013. Reticuloendotheliosis. Chapter 15. Neoplastic Diseases. In: *Diseases of poultry*, 13th edn. Eds Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL and Nair VL. Wiley-Blackwell, 513–674.
- Nettleton PF, Davies MJ and Rweyemamu MM, 1982. Guanidine and heat sensitivity of foot-and-mouth disease virus (FMDV) strains. *Journal of Hygiene*, 89, 129–138.
- Neuhaus J, Schrodli W, Shehata AA and Kruger M, 2015. Detection of *Clostridium botulinum* in liquid manure and biogas plant wastes. *Folia Microbiologica (Praha)*, 60, 451–456.
- Newell DG and Fearnley C, 2003. Sources of *Campylobacter* colonization in broiler chickens. *Applied and Environmental Microbiology*, 69, 4343–4351.
- Niederwohmeier B, 1985. Microwave treatment as an alternative pasteurisation process for the disinfection of sewage sludge—experiences with the treatment of liquid manure. In: *Microorganisms in sewage sludge by stabilization processes*. Eds Strauch D, Niederwohrmeier B and Bohm R, Elsevier Applied Science, London, UK, 135–147.
- Nolan LK, Barnes HJ, Vaillancourt J-P, Abdul-Aziz T and Logue CM, 2013. Chapter 18. Colibacillosis. In: *Diseases of poultry*, 13th edn. Eds Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL and Nair VL. Wiley-Blackwell, 751–806.

- O'Dea MA, Hughes AP, Davies LJ, Muhling J, Buddle R and Wilcox GE, 2008. Thermal stability of porcine circovirus type 2 in cell culture. *Journal of Virological Methods*, 147, 61–66.
- Page LA, 1959. Thermal inactivation studies on a turkey ornithosis virus. *Avian Diseases*, 3, 67–79.
- Pattison M, McMullin PF, Bradbury JM and Alexander DJ, 2008. *Poultry diseases*, 6th edn. Saunders-Elsevier, Oxford, UK, 632 pp.
- Patzschke W, 1916. Über die Widerstandsfähigkeit von Bakterien gegenüber hohen Temperaturen und das Lobecksche Biorisierverfahren. *Zeitschrift für Hygiene und Infektionskrankheiten*, 81, 227–256.
- Payne LN, Holmes AE, Howes K, Pattison M, Pollock DL and Walters DE, 1982. Further studies on the eradication and epizootiology of lymphoid leukosis virus infection in a commercial strain of chickens. *Avian Pathology*, 11, 145–162.
- Peck MW, Lund BM, Fairbairn DA, Kaspersson AS and Undeland PC, 1995. Effect of heat treatment on survival of, and growth from, spores of nonproteolytic *Clostridium botulinum* at refrigeration temperatures. *Applied and Environmental Microbiology*, 61, 1780–1785.
- Peck MW, Goodburn KE, Betts RP and Stringer SC, 2008. Assessment of the potential for growth and neurotoxin formation by non-proteolytic *Clostridium botulinum* in short shelf-life commercial foods designed to be stored chilled. *Trends in Food Science and Technology*, 19, 207–216.
- Peleg M and Pechina CM, 2000. Modeling microbial survival during exposure to a lethal agent with varying intensity. *Critical Reviews in Food Science and Nutrition*, 40, 159–172.
- Pérez de Val B, Grau-Roma L, Segales J, Domingo M and Vidal E, 2014. Mycobacteriosis outbreak caused by *Mycobacterium avium* subsp. *avium* detected through meat inspection in five porcine fattening farms. *Veterinary Record*, 174, 96.
- Petersen A, Christensen JP, Kuhnert P, Bisgaard M and Olsen JE, 2006. Vertical transmission of a fluoroquinolone-resistant *Escherichia coli* within an integrated broiler operation. *Veterinary Microbiology*, 116, 120–128.
- Pilipcinec E, Tkacikova L, Naas HT, Cabadaj R and Mikula I, 1999. Isolation of verotoxigenic *Escherichia coli* O157 from poultry. *Folia Microbiologica (Praha)*, 44, 455–456.
- Promkuntod N, Antarasena C, Prommuang P and Prommuang P, 2006. Isolation of avian influenza virus A subtype H5N1 from internal contents (albumen and allantoic fluid) of Japanese quail (*Coturnix coturnix japonica*) eggs and oviduct during a natural outbreak. *Annals of the New York Academy of Sciences*, 1081, 171–173.
- Rajkovic A, Uyttendaele M, Vermeulen A, Andjelkovic M, Fitz-James I, in 't Veld P, Denon Q, Vérhe R and Debevere J, 2008. Heat resistance of *Bacillus cereus* emetic toxin, cereulide. *Letters in Applied Microbiology*, 46, 536–541.
- Raju D, Waters M, Setlow P and Sarker MR, 2006. Investigating the role of small, acid-soluble spore proteins (SASPs) in the resistance of *Clostridium perfringens* spores to heat. *BMC Microbiology*, 6, 50.
- Reboli AC and Farrar WE, 1989. *Erysipelothrix rhusiopathiae*: an occupational pathogen. *Clinical Microbiology Reviews*, 2, 354–359.
- Rodriguez-Calleja JM, Cebrian G, Condon S and Manas P, 2006. Variation in resistance of natural isolates of *Staphylococcus aureus* to heat, pulsed electric field and ultrasound under pressure. *Journal of Applied Microbiology*, 100, 1054–1062.
- Rosenberger JK and Cloud SS, 1998. Chicken anemia virus. *Poultry Science*, 77, 1190–1192.
- Russell SM, 2003. The effect of electrolyzed oxidative water applied using electrostatic spraying on pathogenic and indicator bacteria on the surface of eggs. *Poultry Science*, 82, 158–162.
- Saif YM, 2013. Introduction. Chapter 12. Viral Enteric Infections. In: *Diseases of poultry*, 13th edn. Eds Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL and Nair VL. Wiley-Blackwell, 375–416.

- Sandhu TS, 2008. *Riemerella anatipestifer* infection. In: Diseases of poultry, 12th edn. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE. Blackwell Publishing, Ames, Iowa, USA, 758–764.
- Sapru V, Teixeira AA, Smerage GH and Lindsay JA, 1992. Predicting thermophilic spore population dynamics for UHT sterilization processes. *Journal of Food Science*, 57, 1248–1257.
- Sasipreeyajan J, Halvorson DA and Newman JA, 1987. Effect of *Mycoplasma gallisepticum* bacterin on egg-transmission and egg production. *Avian Diseases*, 31, 776–781.
- Sauerbrei A and Wutzler P, 2009. Testing thermal resistance of viruses. *Archives of Virology*, 154, 115–119.
- Schat KA and van Santen VL, 2008. Chicken infectious anaemia. In: Diseases of poultry, 12th edn. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE, Blackwell Publishing, Ames, IA, USA, 209–251.
- Senne DA, Panigrahy B and Morgan RL, 1994. Effect of composting poultry carcasses on survival of exotic avian viruses: highly pathogenic avian influenza (HPAI) virus and adenovirus of egg drop syndrome-76. *Avian Diseases*, 38, 733–737.
- Shane SM, 1992. The significance of *Campylobacter jejuni* infection in poultry: a review. *Avian Pathology*, 21, 189–213.
- Sherman JM and Stark P, 1931. Streptococci which grow at high temperatures. *Journal of Bacteriology*, 22, 275–285.
- Shimada K and Matsushita S, 1980. Thermal coagulation of egg albumin. *Journal of Agricultural and Food Chemistry*, 28, 409–412.
- Shivaprasad HL and Barrow PA, 2008. Pullorum disease and Fowl typhoid. In: Diseases of poultry, 12th ed. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE, Blackwell Publishing, Ames, IA, USA, 620–636.
- Smith JP, Toupin C, Gagnon B, Voyer R, Fiset PP and Simpson MV, 1990. A hazard analysis critical control point approach (HACCP) to ensure the microbiological safety of sous vide processed meat/pasta product. *Food Microbiology*, 7, 177–198.
- Smyth JA and McNamee PT, 2008. Staphylococci, streptococci and enterococci. In: Poultry diseases, 6th edn. Eds Pattison M, McMullin PF, Bradbury JM and Alexander DJ. Saunders-Elsevier, Philadelphia, PA, USA, 191–199.
- Snoeyenbos GH, 1991. Pullorum disease. In: Diseases of poultry, 9th edn. Eds Calnek BW, Barnes HJ, Beard CW, Reed WM and Yoder JH Jr, Iowa State University Press, Ames, IA, USA, 73–86.
- Sobel J, Hirshfeld AB, McTigue K, Burnett CL, Altekruise S, Brenner F, Malcolm G, Mottice SL, Nichols CR and Swerdlow DL, 2000. The pandemic of *Salmonella enteritidis* phage type 4 reaches Utah: a complex investigation confirms the need for continuing rigorous control measures. *Epidemiology and Infection*, 125, 1–8.
- Sörqvist S, 2003. Heat resistance in liquids of *Enterococcus* spp., *Listeria* spp., *Escherichia coli*, *Yersinia enterocolitica*, *Salmonella* spp. and *Campylobacter* spp. *Acta Veterinaria Scandinavica*, 44, 1–19.
- Spicher G, Peters J, Nürnberg M and Schwebke I, 2002. Microbicidal efficacy of superheated steam. II. Studies involving *E. faecium* and spores of *B. xerothermodurans* and *B. coagulans*. *International Journal of Hygiene and Environmental Health*, 204, 309–316.
- Suarez DL, 2013. Chapter 14. Other Viral Infections. Avian Encephalomyelitis. In: Diseases of poultry, 13th edn. Eds Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL and Nair VL. Wiley-Blackwell, 465–512.
- Swayne DE and Beck JR, 2004. Heat inactivation of avian influenza and Newcastle disease viruses in egg products. *Avian Pathology*, 33, 512–518.



- Swayne DE and Halvorson DA, 2008. Influenza. In: Diseases of poultry, 12th edn. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE. Blackwell Publishing, Ames, IA, USA, 153–184.
- Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL and Nair VL, 2013. Diseases of poultry, 13th edn. Wiley-Blackwell, 1408 pp.
- Takase K, Shinohara K, Tsuneyoshi M, Yamamoto M and Yamada S, 1989. Isolation and characterisation of cytopathic avian enteroviruses from broiler chicks. *Avian Pathology*, 18, 631–642.
- Tannock GA and Shafren DR, 1994. Avian encephalomyelitis: A review. *Avian Pathology*, 23, 603–620.
- Taylor LW, Lowry DC and Raggi LG, 1955. Effects of an outbreak of avian encephalomyelitis (epidemic tremor) in a breeding flock. *Poultry Science*, 34, 1036–1045.
- Theil KW and Saif YM, 1987. Age-related infections with rotavirus, rotaviruslike virus, and atypical rotavirus in turkey flocks. *Journal of Clinical Microbiology*, 25, 333–337.
- Thomas C and Swayne DE, 2007. Thermal inactivation of H5N1 high pathogenicity avian influenza virus in naturally infected chicken meat. *Journal of Food Protection*, 70, 674–680.
- Todd D and Imada T, 2013. Avian Nephritis. Chapter 14. Other viral infections. In: Diseases of poultry, 13th edn. Eds Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL and Nair V. Wiley-Blackwell, 465–512.
- Todd D, Mawhinney KA, McAlinden VA and Douglas AJ, 1993. Development of an enzyme-linked immunosorbent assay for the serological diagnosis of big liver and spleen disease. *Avian Diseases*, 37, 811–816.
- Tona K, Onagbesan O, De Ketelaere B, Decuypere E and Bruggeman V, 2004. Effects of age of broiler breeders and egg storage on egg quality, hatchability, chick quality, chick weight, and chick posthatch growth to forty-two days. *Journal of Applied Poultry Research*, 13, 10–18.
- Tullett SG, 1990. Science and the art of incubation. *Poultry Science*, 69, 1–15.
- Urlings HA, de Boer GF, van Roozelaar DJ and Koch G, 1993. Inactivation of chicken anaemia virus in chickens by heating and fermentation. *Veterinary Quarterly*, 15, 85–88.
- Varga J, Fodor L and Makrai L, 2001. Characterisation of some *Ornithobacterium rhinotracheale* strains and examination of their transmission via eggs. *Acta Veterinaria Hungarica*, 49, 125–130.
- Vidal AB, Davies RH, Rodgers JD, Ridley A and Clifton-Hadley F, 2014. Epidemiology and control of campylobacter in modern broiler production. In: *Campylobacter ecology and evolution*. Eds Sheppard SK and Meric G, Caister Academic Press, Norfolk, 287–312.
- Welch J, Bienek C, Gomperts E and Simmonds P, 2006. Resistance of porcine circovirus and chicken anemia virus to virus inactivation procedures used for blood products. *Transfusion*, 46, 1951–1958.
- Williams A, Davies AC, Wilson J, Marsh PD, Leach S and Humphrey TJ, 1998. Contamination of the contents of intact eggs by *Salmonella typhimurium* DT104. *Veterinary Record*, 143, 562–563.
- Wittenbrink MM, Mrozek M and Bisping W, 1993. Isolation of *Chlamydia psittaci* from a chicken egg: evidence of egg transmission. *Journal of Veterinary Medicine. Series B*, 40, 451–452.
- Yamaguchi S, Imada T, Kawamura H, Taniguchi S, Saio H and Shimamatsu K, 1981. Outbreaks of egg-drop syndrome-1976 in Japan and its etiological agent. *Avian Diseases*, 25, 628–641.
- Yamamoto R, Bigland CH and Peterson IL, 1966. Egg transmission of *Mycoplasma meleagridis*. *Poultry Science*, 45, 1245–1257.
- Yoder HW Jr, 1970. Preincubation heat treatment of chicken hatching eggs to inactivate mycoplasma. *Avian Diseases*, 14, 75–86.

- Yuasa N and Yoshida I, 1983. Experimental egg transmission of chicken anemia agent. National Institute of Animal Health Quarterly (Japan), 23, 99–100.
- Zhang H, 2008. Campylobacteriosis. In: Diseases of poultry, 12th edn. Eds Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK and Swayne DE. Blackwell Publishing, Ames, IA, USA, 675–691.

## Abbreviations

AEV	avian encephalomyelitis virus
AHAW Panel	EFSA Panel on Animal Health and Welfare
AIV	avian influenza virus
ANV	avian nephritis virus
$a_w$	water activity
BIOHAZ Panel	EFSA Panel on Biological Hazards
CAV	chicken anaemia virus
EC	European Commission
EDSV	egg drop syndrome virus
EID <sub>50</sub>	mean embryo infectious dose
FMDV	foot-and-mouth disease virus
HEV	hepatitis E virus
HPAI	Highly pathogenic avian influenza
HPAIV	Highly pathogenic avian influenza virus
INDV	lentogenic Newcastle disease virus
LPAIV	low pathogenicity avian influenza virus
L/S	leucosis/sarcoma
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
ND	Newcastle disease
NDV	Newcastle disease virus
REV	Reticuloendotheliosis virus
TSE	Transmissible Spongiform Encephalopathy
STEC	Shiga toxin-producing <i>E. coli</i>
vNDV	virulent Newcastle disease virus

## Appendix A – Examples of the major sources of uncertainty

Assessment components		Sources of uncertainty	Types of uncertainty	Potential impact of the uncertainty
Assessment/ sub-assessment	Assessment inputs			
<b>Hazard identification</b>	<b>Pathogens present in poultry</b>	There is a high degree of confidence in the list of pathogens as there is extensive literature on diseases in poultry, but detailed information is not available on the occurrence of pathogens in individual Member States	Ambiguity (incomplete information)	It is possible, although unlikely, that some pathogens could have been missed, or not recognised as potential threats in the context of exposure of other hosts to recycled avian material. For this to have an impact on the assessment, such hazards would have to be more resistant to the treatments applied than those identified which may be the case for contaminants which have not been identified and regarded here. The occurrence of an avian vertically transmitted TSE prion-based agent that has not yet been discovered would be an example of an agent that could survive treatment, besides thermoresistant viruses and bacterial spores. The direction of this uncertainty could suggest a higher potential risk than that identified in this assessment
<b>Description of the hazard</b>	<b>Transmission rate from birds to eggs</b>	No data for many of the agents Although the occurrence of the listed agents is well recognised, the transmission rate within and on the surface of the eggs is unknown and likely to be very variable according to circumstances, such as the immunity of the birds and flocks and hatchery management systems	Ambiguity (incomplete information)	Most of the agents that are likely to be present on the egg surface or able to transmit via vertical transmission to egg contents are theoretically susceptible to the proposed temperatures and times for heat treatment. Since no results of experimental validation with the substrate to be treated are available, a verification of the microbicidal effects of the processes to be regarded here will not be based on actual data. The direction of this uncertainty could suggest a higher potential risk than that identified in this assessment for those pathogens that are considered to be susceptible to the treatment. Several pathogens are considered to be capable of withstanding the treatment and such ambiguity will not affect this assessment
	<b>Occurrence and concentration of pathogens inside infected eggs and on their surface</b>	Insufficient experimental investigation  There is a small amount of information regarding the concentration of <i>Salmonella</i> in table eggs No data are available for <i>Salmonella</i> in hatching eggs or for the other listed pathogens	Ambiguity (incomplete information)	Those agents that are transmitted vertically into the egg contents, as well as those on their surface, may be variable in concentration. During collection and storage a mixture of broken and intact eggs is generated, further complicating the assessment of the final concentration of pathogens and contaminants in the substrate to be treated under practical conditions. The direction of this uncertainty could suggest a higher or lower potential risk than that identified in this assessment, according to the ability of the pathogen to survive or multiply in the material or to the likelihood of being outcompeted by non-pathogenic spoilage organisms

Assessment components		Sources of uncertainty	Types of uncertainty	Potential impact of the uncertainty
Assessment/ sub-assessment	Assessment inputs			
	<b>Potential for multiplication in eggs</b>	Insufficient experimental investigation There is a small amount of information regarding the multiplication of <i>Salmonella</i> in table eggs	Ambiguity (incomplete information)	Several agents that are transmitted vertically into the egg contents may propagate them up to an unknown level. During collection and storage a mixture of broken and intact eggs is generated, further complicating the assessment of the final concentration of pathogens in the substrate to be treated under practical conditions. The direction of this uncertainty could suggest a higher or lower potential risk than that identified in this assessment, according to the ability of the pathogen to survive or multiply in the material or to the likelihood of being outcompeted by non-pathogenic spoilage organisms
		No data are available for <i>Salmonella</i> in hatching eggs or for the other listed pathogens		
	<b>Thermal resistance of the agent</b>	Missing basic data on thermal resistance of poultry pathogens	Ambiguity (incomplete information)	The impact would be relevant only if it leads to an underestimation of the resistance of the pathogens. The greatest concern is the lack of specific information of the listed agents within the specific matrix of embryonated eggs. It is likely that the tissues of the embryo will be partially protective against the effect of heat on pathogens. In particular, agents that have become intracellular following vertical transmission may be more difficult to inactivate and the presence of yolk and egg proteins from the yolk sac and any infertile eggs that may be present in the bulk material may offer thermal protection
		Sometimes only data on related pathogens of other animal species were available	Extrapolation uncertainty	
		Information on the variability of the thermal resistance among the different isolates within the species is very limited	Distribution uncertainty	
		There are no data available on thermal resistance in dead-in-shell chicks	Ambiguity (incomplete information)	
		Data on other matrices, such as liquid egg, have been used for the assessment. It is unclear to what extent these matrices may influence the thermal resistance compared with dead-in-shell chicks	Extrapolation uncertainty	
		Many of the studies describing the thermal resistance values have been performed at a small scale in the laboratory. No validation of the inactivation of the most heat-resistant agents identified in this assessment has been carried out in industrial scale conditions of composting and biogas plants	Sampling and measurement uncertainties	



Assessment components		Sources of uncertainty	Types of uncertainty	Potential impact of the uncertainty
Assessment/ sub-assessment	Assessment inputs			
		The accuracy of the detection methods used to identify and quantify the organisms and viruses is uncertain because most of the studies are old and the methods are not validated	Sampling and measurement uncertainties	
<b>Inactivation ability</b>	<b>Process parameters approved for biogas and compost</b>	Legislation provides only a rough and very general description of the treatment in biogas and compost plants	Ambiguity (incomplete information)	The impact may lead to an unrealistic assumption concerning the microbicidal and virucidal effectiveness of the treatment, because each type of composting reactor and each type of pasteurisation unit for anaerobic treatment has different technical specifications, and distribution of temperature in the substrate is influenced by many factors
		Uncertainty results from the fact that different types of technical equipment used in those processes may have different abilities to inactivate agents, which can only be determined by an experimental validation. No experimental data are available showing whether the most heat-resistant agents identified in this assessment would be inactivated in specific types of equipment up to the required level	Extrapolation uncertainty	
	<b>D-values z-values</b>	Although there is some information on the heat resistance of the main viral hazards identified there is a lack of data on D-values/z-values that could be used to estimate the level of reduction achieved with the method	Ambiguity (incomplete information)	The impact could be assessed only if the process had been validated with test organism with defined D-values/z-values in the same range as those of the specific pathogens. If such information is missing, it is impossible to predict if the treatment will be sufficient to destroy the relevant pathogens and contaminants
	<b>Level of reduction</b>	All the factors mentioned as being associated with uncertainty in the thermal resistance Section will have an impact on the level of uncertainty in the estimated level of reduction	Ambiguity (incomplete information)	It is not possible to assess the expected level of reduction of pathogens under the proposed conditions in this matrix, although those pathogens that are relatively susceptible to heat inactivation would be expected to be eliminated. Since no results of experimental validation with the substrate to be treated are available, a verification of the microbicidal and virucidal effects of the processes to be regarded here will not be based on actual data and could overestimate the efficacy of the process