

SCIENTIFIC OPINION

Scientific Opinion on Hatchery Waste as animal by-products¹

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ABSTRACT

The risk posed by the possible use of dead-in-shell chicks for the production of processed petfood was assessed. According to current legislation two processing methods were considered i) treatment to a minimum Fc value of 3; and ii) treatment of at least 90°C throughout the substance of the final product. A list of the possible pathogens potentially present in the material to be treated was compiled and the available literature data was used to assess the ability of the processing methods to inactivate the most resistant pathogens identified. The processing methods were assessed assuming that the heat treatments would be performed in a moist environment. 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 respectively as the most resistant viruses and non-sporulating bacterium to heat treatment. Moreover, depending on storage conditions, the generation of bacterial toxins could be possible. Consequently, the processing methods considered were assessed against their ability to inactivate those hazards. The risk related to the use of dead-in-shell chicks, submitted to a conventional heat treatment to a minimum Fc value of 3 in a moist environment, for the production of canned petfood was considered negligible. No indication is given in the current regulation on the processing time and heating method needed for the treatment at 90°C throughout the substance of the final product. A treatment lasting 18 seconds can assure a rapid destruction of the non-sporeforming bacteria identified as hazards. However, this treatment is not able to inactivate other relevant hazards such as bacterial spores, thermoresistant viruses and some toxins. The final risk posed by the agents that may survive this treatment additionally depends on several factors and cannot be considered to be negligible.

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KEY WORDS

Animal By-Products, Hatchery, Dead-in-shell chicks, Petfood, Hazards, Thermal Inactivation

SUMMARY

Following a request from the European Commission, 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.

Article 9(f)(iv) of the Animal By-Product (ABP) Regulation (Reg. (EC) 1069/2009⁴) classifies dead-in-shell chicks as Category 2 ABP material and according to article 13 of the same regulation they can not be used for the manufacturing of petfood. Other hatchery by-products and egg shells are classified under article 10(k)(ii) of the Regulation as Category 3 material and according to article 14 of the Regulation may be used for the production of petfood in approved establishments.

In 2009 the European Commission received a request from the Department of Food, Livestock and Consumer Affairs of the Dutch Ministry of Agriculture, Nature and Food Quality to change the categorisation of dead-in-shell chicks from Category 2 to Category 3 ABP material.

In order to be able to consider whether a change in categorisation could be envisaged for dead-in-shell chicks the European Commission asked EFSA for a Scientific Opinion on the risk posed by the possible use of dead-in-shell chicks for the production of petfood under the provisions currently applicable for processed petfood. It was clarified that the processing methods to be considered were the ones described under Reg. (EC) 142/2011⁵, Annex XIII, Chapter II, point 3, letters (a) and (b): i) treatment to a minimum Fc value of 3 for canned petfood; and ii) treatment of at least 90°C throughout the substance of the final product for processed petfood other than canned petfood.

Considering the mandate received, it was agreed that the scope of this assessment 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 potentially present in the material and the BIOHAZ Panel used the available literature 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 respectively as the most resistant viruses and non-sporulating bacterium to heat treatment. Moreover, depending on the storage conditions of the material to be processed the generation of bacterial toxins was considered as possible. Consequently, the currently approved standards for processed petfood were assessed against their ability to inactivate those hazards. Furthermore, considering that moist heat treatment is the commonly applied heating method for food and feed processing, the assessment was based on the assumption that the heat treatment would be performed in a moist environment.

⁴ 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).

⁵ 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).

It was pointed out that the feed composition and intrinsic and extrinsic factors can play a major role in determining the growth and survival of the target microorganisms and the final risk they may pose. Moreover, the method and conditions of heat treatment are known to affect the microbial inactivation.

It was concluded that the risk related to the use of dead-in-shell chicks, submitted to a conventional heat treatment to a minimum Fc value of 3 in a moist environment, for the production of canned petfood is negligible.

It was highlighted that there is no indication given in the ABP regulation of the processing time and heating method needed for the treatment at 90°C throughout the substance of the final product. A treatment lasting 18 seconds can assure a rapid destruction of the non-sporeforming bacteria identified as hazards. However, this treatment is not able to inactivate other relevant hazards such as bacterial spores, thermoresistant viruses and some toxins. The final risk posed by the agents that may survive this treatment additionally depends on several factors and cannot be considered to be negligible.

It was recommended that in order to assess the ability of the processing method at 90° C throughout the substance of the product to inactivate possible biological hazards, the treatment time and other additional parameters such as particle size, heating method and water content of the matrix should be provided.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Under Regulation (EC) No 1774/2002⁶ on animal by-products (ABP), dead-in-shell chicks are designated by Article 5(1)(e) as Category 2 material. According to Article 5(2), animal by-products of Category 2 must not be used in the production of feed for farmed animals or in petfood. Other waste material from hatcheries is classified as Category 3 animal by-products according to Article 6(1)(j) and may therefore be processed further into feed for farmed animals or into petfood. This interpretation has also been confirmed by a Guidance Note from the Commission⁷.

The new Animal By-products Regulation (EC) No 1069/2009⁸, which will apply from 4 March 2011, maintains the current categorisation. Under Article 9(f)(iv), dead-in-shell chicks are still classified as Category 2 and by reference to Article 12 must not be used in the production of feed for farmed animals and processed petfood. Other hatchery by-products and egg shells are classified under Article 10(k)(ii) as Category 3 material and may be processed into feed for farmed animals or into petfood in approved establishments, subject to further requirements laid down in the Animal By-products Regulation.

Under the new Animal By-products Regulation, changes of categorisation may be made. The first sentence of Article 7(3) states "Articles 8, 9 and 10 may be amended in order to take into account scientific progress as regards the assessment of the level of risk, provided such progress can be identified on the basis of a risk assessment carried out by the appropriate scientific institution".

The Commission has received a request from the Department of Food, Livestock and Consumer Affairs of the Dutch Ministry of Agriculture, Nature and Food Quality to change the categorisation of dead-in-shell chicks.

In support of its request, the Department of Food, Livestock and Consumer Affairs has provided two documents:

- the Opinion of the Director of the Dutch Office for Risk Assessment. Ref.: VWA/BuR/2009/21889 of 9 July 2009 (Opinion on risks of hatchery by-products);
- a *Qualitative and Quantitative Risk Analysis of Hatchery Waste* dated June 2009 from the Dutch Animal Health Service.

The Opinion on risks of hatchery by-products presents the assessment of *Qualitative and Quantitative Risk Analysis of Hatchery Waste*, which indicates that the occurrence of the relevant infectious agents in hatching eggs ranges from very low (in the case of *Salmonella* spp.) to negligible (in the case of Newcastle disease virus and avian influenza virus). The afore-mentioned infectious agents are already covered by the EU veterinary legislation. The *Qualitative and Quantitative Risk Analysis of Hatchery Waste* indicates that other micro-organisms are found quite regularly. They include Cocci bacteria on the egg shell and gut bacteria in hatchery eggs, particularly in cases of 'dead-in-shell chicks'. Furthermore, the *Qualitative and Quantitative Risk Analysis of Hatchery Waste* report presents an assessment of certain specific avian vertical transmissible diseases such as Avian Leucosis (ALV), Chicken Infectious Anemia Virus, Avian adenoviruses (Egg drop syndrome), Reticuloendotheliosis (REV), Avian Encephalomyelitis, *Mycoplasma* (*M. gallisepticum* and *M. synoviae*), Avian psittacosis

⁶ Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption (OJ L 273, 10.10.2002, p.1).

⁷ Guidance note Interpretation of Regulation 1774/2002/EC, Questions arising from FVO inspections to Member States (2004-2005) (SANCO/10098/2006 Rev.1)
http://ec.europa.eu/food/food/biosafety/animalbyproducts/guidancefvomission_en.pdf.

⁸ 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).

and other possible infections in hatchery waste such as campylobacteriosis, Erysipelas (*Erysipelothrix rhusiopathiae*), botulism toxins (*Clostridium botulinum*) and Toxoplasmosis, although these are considered to be very rarely present in hatchery animal by-products.

In addition, the Dutch documents refer to the processing standards for pet food. Article 35(a) of the new Animal by-products Regulation lays down criteria for sourcing of animal by-products intended for the production of pet food.

Former provisions for the processing of petfood are laid down in point B.2 of Chapter II of Annex VIII to Regulation (EC) No 1774/2002, canned petfood must be subjected to heat treatment to a minimum Fc value of 3. Such heat treatment destroys practically all vegetative and spore forms of pathogens, even the most resistant strains. According to point B.3 of the same Chapter, processed petfood other than canned petfood must be subjected to a heat treatment of at least 90°C throughout its substance. After such treatment there is no risk to animal and public health. The implementing Regulation (EU) No 142/2011 lays down in Chapter II of Annex XII rules which covers this topic in the similar way as the former legislation.⁹

In order to be able to consider whether on the basis of Article 7(3) of the new Animal by-products Regulation, a change in categorisation could be considered for dead-in-shell chicks, EFSA is requested to assess the level of the risk posed by changing the categorisation of dead-in-shell chicks from Category 2 to Category 3 and by using these animal by-products as raw material for the production of feed for farmed animals or for petfood.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In view of the above, and in accordance with Article 29 of Regulation (EC) 178/2002, the Commission asks EFSA for a scientific opinion on the risk posed by changing the categorisation of dead-in-shell chicks from Category 2 to Category 3 and in particular:

1. to assess the risk to animal and public health of transmission of the most important infectious agents in hatchery by-products, such as *Salmonella spp.*, Avian Influenza Virus and Newcastle Disease Virus from dead-in-shell chicks through animal feed;
2. to assess the risk of transmission of vertically transmissible avian diseases, such as Avian Leucosis, Chicken Infectious Anemia Viruses, Avian adenoviruses (Egg drop syndrome), Reticuloendotheliosis, Avian Encephalomyelitis, Mycoplasmosis and Avian psittacosis from dead-in-shell chicks through animal feed;
3. to assess the risk to animal and public health of the transmission of other biological hazards from dead-in-shell chicks, such as *Campylobacter*, *Enterobacteriaceae*, Erysipelas, Botulism toxins, Toxoplasmosis through animal feed;
4. in the case of identified risk in points 1-3, to indicate the most important factors which would have to be monitored in the production of feed for farmed animals and processed petfood, should dead-in-shell chicks be categorised as Category 3 materials.

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:

⁹ Following an e-mail received from the European Commission on 11th May 2011 this paragraph has been amended in comparison with the original mandate received.

- The Commission asks EFSA for a Scientific Opinion on the risk posed by the possible use of dead-in-shell chicks for the production of petfood under the provisions currently applicable for processed petfood.

Moreover, it was clarified that that the requested risk assessment should only consider the processing methods for canned petfood (minimum Fc value of 3) and those for processed petfood (treatment at 90°C throughout the substance).

APPROACH TAKEN TO ANSWER THE TERMS OF REFERENCE

After having received this request from the European Commission, EFSA assigned the mandate to the Panel on Biological Hazards (BIOHAZ Panel) and the Panel on Animal Health and Welfare (AHAW Panel). AHAW Panel drafted and adopted the Chapter 2 about the hazard identification, whereas the BIOHAZ Panel drafted and adopted all the other chapters, the conclusions and recommendations.

ASSESSMENT

1. Introduction

This opinion focuses on the possible use of dead-in-shell chicks sourced from the hatchery industry for the production of petfood under the provisions currently applicable for processed petfood. This document considers all the relevant poultry species produced in hatcheries in the European Union.

According to Article 9(f)(iv) of the Animal By-Product (ABP) Regulation (Reg. (EC) 1069/2009¹⁰) dead-in-shell chicks are classified as Category 2 ABP material and consequently they can not be used for the manufacturing of petfood.

The assessment considers the ability of the processing standards currently approved for the manufacturing of processed petfood to inactivate the possible biological hazards present in such material. Exposure assessment to any of the hazards identified was excluded from the current opinion.

According to Reg. (EC) 142/2011¹¹, Annex XIII, Chapter II, point 3, letters (a) and (b) two standards are approved for the manufacturing of processed petfood:

1. Treatment to a minimum Fc value of 3

This treatment has to be used for the production of canned petfood. Under practical conditions an Fc value of 3 is obtained by a heat treatment at 121°C at the core of the can for 3 minutes or by a heat treatment achieving an equivalent temperature/time combination.

2. Treatment of at least 90°C throughout the substance of the final product

This treatment can be used for processed petfood other than canned petfood. No indication on the time needed and heating method used for this treatment is given in the ABP regulation.

Considering the mandate received it was agreed that the scope of this assessment 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 consequently, all relevant microorganisms have been included in the assessment also those not presently occurring in the EU as such organism may be introduced in eggs/hatching eggs from Third countries.

In this context it was agreed that:

- the EFSA Scientific Panel on Animal Health and Welfare (AHAW) would compile a list of the possible pathogens potentially present in the material;
- the EFSA Scientific Panel on Biological Hazards (BIOHAZ) would assess the ability of the processing methods to inactivate the possible pathogens potentially present in the material. The assessment will make use of the available literature to assess the ability of the processing methods to inactivate the most resistant pathogens identified.

¹⁰ 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).

¹¹ 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).

2. Hazard Identification (AHAW Panel)

2.1. Approach

A search of infectious agents that may be found in eggs has been conducted by accessing textbooks of avian diseases and specific references. Basic information has been collected on the infectious agent, as well as on relevant physicochemical properties, such as thermal resistance, when available. Also, poultry species affected by these agents have been indicated. Information related to the likelihood of finding the different agents in eggs from several poultry species and the initial level of contamination were also searched and, whenever found, included. These data refer to identified hazards posed by known infectious agents at the time of preparing this opinion. If new hazards should appear in the future, the risk assessment should be reviewed.

Microorganisms that may be present in the chicken egg

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

The agents which may be found in the chicken egg, either transmitted vertically or as a result of horizontal transmission or contamination, are discussed below, following an aetiological classification (as viruses, bacteria, and fungi). 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.

2.1.1. Viruses

2.1.1.1. *Retroviridae Oncovirinae*

- The leukosis/sarcoma group of avian type C oncoviruses (Rubin et al., 1961; Rubin et al., 1962)
- Reticuloendotheliosis viruses of the REV group (Motha and Egerton, 1987)
- Other oncogenic viruses (rarely) (Calnek, 1992)

With few exceptions, virus from the leukosis/sarcoma group of avian type C oncoviruses (L/S group) can be isolated from any flock of commercial chickens (Fadly and Nair, 2008). Chickens are the natural hosts for all viruses in the L/S group and in addition to chickens they have been isolated only 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. 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.

Likelihood of presence in eggs: Embryo infection with the L/S group is strongly related to infection in the oviduct and egg albumen. Depending on the infection/immune status of the flock very high numbers of eggs may be positive – up to 60% (Payne et al., 1982). REV virus 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 min. and at 60 °C is 0.7 min. (Dougherty, 1961). No information on REV was found but as it is the same class of viruses the resistance probably could be similar.

2.1.1.2. *Picornaviridae*

Avian enteroviruses - *Infectious avian encephalomyelitis virus* (AEV) (Calnek et al., 1960). Limited host range: Chickens, pheasants, coturnix quail, pigeons and turkeys. AEV occurs worldwide (Tannock and Shafren, 1994).

- Avian enterovirus- like viruses - baby chick nephritis (Spackman et al., 1984)

Turkeys, chickens, guinea fowl and psittacines. No evidence suggesting that they are transmissible from avian species to mammals or humans. This type of viruses has been isolated from all over the world including several EU member states (UK, Italy, France, Belgium, Holland and Germany) (Guy et al., 2008).

Likelihood of presence in eggs: When susceptible flocks are exposed to AEV after sexual maturity, the hens transmit the infection to a variable proportion of their eggs. Some reports have recorded 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 may be infected (up to 60%) if the breeders are fully susceptible (Calnek, 2008).

No information was found concerning avian enterovirus-like viruses.

Thermal resistance: Infectivity of AEV and avian enterovirus-like viruses has not been reduced following heating at 56 °C for 1 hour (Butterfi et al., 1969; Takase et al., 1989).

2.1.1.3. *Reoviridae*

- Avian reoviruses (Al Muffarej et al., 1996; Menendez et al., 1975)

Avian reoviruses have been found in many avian hosts including chickens, turkeys, ducks, pigeons, geese and psittacine birds. Attempts to establish active infection in the canary, pigeon, guinea pig, rat, mouse, hamster and rabbit have failed. The group of viruses is considered to be ubiquitous in commercial poultry (Jones, 2008).

Likelihood of presence in eggs: Egg transmission rates in infected commercial flocks are considered low – in one experiment approximately 2%, but shell contamination was not found likely (Menendez et al., 1975). Higher transmission rates for some strains of virus have been reported (AlMuffarej et al., 1996) but under natural conditions the rate is considered low.

Thermal resistance: Quite heat resistant – able to withstand 60 °C for 8-10 hours (Matthews, 1982).

- Avian rotaviruses (Theil and Saif, 1987)

Turkeys, chickens, pheasants, partridges, ducks, guinea fowl, pigeons and lovebirds. Rotaviruses have been isolated from poultry all over the world including several EU member states (UK, France, Belgium and Germany) (McNulty and Reynolds, 2008).

Likelihood of presence in eggs: Egg transmission has not been demonstrated conclusively but due to infection of very young birds it has been speculated that shell infection (virus excreted in large numbers in faeces) may be responsible for a low transmission rate (Theil and Saif, 1987).

Thermal resistance: little information available concerning avian rotaviruses but heating at 56°C for 30 min. decrease infectivity 100 fold (Kang et al., 1988).

2.1.1.4. *Adenoviridae*

- Avian group I adenoviruses (McFerran and Adair, 1977)

Chicken adenoviruses are ubiquitous in fowl populations, as demonstrated in antibody surveys. 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.

Likelihood of presence in eggs: No transmission rates have been reported but the transmission to eggs is considered common (McFerran and Adair, 1977).

Thermal resistance: Major variation concerning heat resistance seems to exist. Strains have been observed to be viable following 30 min. at 70 °C, but this seems to be an exception. Treatment at 80°C for 30 min has destroyed all infectivity (Clemmer, 1964). However, several reports have indicated that the stability of these viruses to heat is greater when they are suspended in mono-valent cations compared to divalent cations, a property which adenoviruses have in common with other DNA viruses.

- *Egg drop syndrome virus* (group III) (McFerran et al., 1978)

Chickens, ducks and geese are affected. Turkeys and pheasants may be infected experimentally. EDS virus has been isolated from several EU member states (Belgium, UK, Italy and Denmark) (Adair and Smyth, 2008).

Likelihood of presence in eggs: The number of infected embryos is considered “low” but no exact figures have been obtained.

Thermal resistance: The virus is inactivated by heating for 30 min. at 60 °C (Yamaguchi et al., 1981).

2.1.1.5. *Orthomyxoviridae*

- Avian influenza virus (AIV) (Cappucci et al., 1985)

AI viruses have been isolated from more than 90 species of free-living birds representing 13 different orders. Several mammalian species including humans may be infected naturally or experimentally. The Low pathogenic avian influenza viruses (LPAIV) are widespread globally (including Europe), primarily in wild birds but they are occasionally introduced into commercial production without major clinical signs necessarily appear. In contrast, the highly pathogenic avian influenza viruses (HPAIV) 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 H5N1 crisis starting in 2003, this view on the epidemiology may need to be reconsidered. Now, HPAIV (H5N1) is endemic in several countries, primarily in Asia, and it seems as if the virus is circulating and is being maintained primarily in waterfowl (also migrating species of birds). Thus, the possibility for introduction of HPAIV into commercial poultry production globally has become more likely (Alexander, 2008). EU has

implemented an "early warning system" for HPAI - which ensures that outbreaks will be detected early (EC, 2005)¹². Consequently, the risk of introducing the virus to the hatcheries is extremely low.

Likelihood of presence in eggs: Final proof of vertical transmission of the disease to eggs is lacking (it has only been demonstrated experimentally). However, AIV virus infection of hens has resulted in virus recovery from the egg shell 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 virus positive – 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: Standard industry pasteurization protocols are effective for inactivation in homogenized whole egg – e.g. 60 °C for 2 min (Swayne and Beck, 2004).

2.1.1.6. *Caliciviridae*

- New classification: Avian hepevirus (Crerar and Cross, 1994)

Under field conditions, chickens are the only known host for avian hepatitis E virus (HEV) infections. Turkeys have been infected experimentally. Attempts to infect monkeys and mice have failed. Most reports concerning prevalence of the virus concern the USA and Australia but serological evidence has also been obtained from the UK (Todd et al., 1993). However, it seems that few investigations have been performed in order to assess the occurrence of the virus.

Likelihood of presence in eggs: No exact figures have been reported – but large numbers of virus are present in faeces and vertical transmission also has been suggested. Following experimental infection of hens, avian HEV can be found in egg whites, but evidence of vertical transmission is lacking (Guo et al., 2007).

Thermal resistance: Most data are from human hepatitis E virus but avian and human HEV are likely similar in susceptibility to inactivation (Meng et al., 2008). Human HEV is about 50% inactivated at 56 °C and almost totally inactivated (96%) at 60 °C (Emerson et al., 2005).

¹² 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).

2.1.1.7. *Circoviridae*

- Chicken anaemia virus (CAV) (Chettle et al., 1989; Engstrom, 1999)

CAV infection has been recognized only in chickens, turkeys and perhaps Japanese quail and some European Corvids but circoviruses or circo-like viruses have been found in other species of birds and mammals. Serological data suggest that CAV is ubiquitous in all major chicken producing countries of the world and virus isolation has been demonstrated in all continents (Schat and van Santen, 2008).

Likelihood of presence in eggs: 7.5 % of the eggs were infected in one study with experimentally infected hens (Yuasa and Yoshida, 1983) whereas approximately 3 % were positive in another study (Hoop, 1992).

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 min. and is completely destroyed within 15 min. at 100°C. However, inactivation of CAV in infected chicken by-products requires a core temperature of 95°C for 35 min. or 100°C for 10 min. (Urlings et al., 1993). CAV has been shown to be almost completely resistant to dry heat treatment up to 120°C for 30 min (mean log infectivity reduction 0.6) (Welch et al., 2006). Circovirus 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 up to 120°C for 30 min and moist heat at 75°C for 15 min (O'Dea et al., 2008).

2.1.1.8. *Parvovirus*

- Derszy's disease in Geese and Muscovy ducks only (Derzsy, 1967; Kisary, 1986)

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

Likelihood of presence in eggs: Vertical transmission is well documented but no transmission rates have been reported.

Thermal resistance: Avian strains are highly resistant to inactivation. No loss of titre is observed following heating to 65°C for 30 min. (Gough et al., 1981). Bovine parvovirus also has been demonstrated to be highly heat resistant. The infectivity of this virus is 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 (McGavin, 1987).

2.1.1.9. *Paramyxoviridae*: Paramyxovirinae – Avulavirus

- Newcastle disease virus (NDV)

In Western Europe, sporadic epizootics occur on a fairly regular basis despite the widespread use of vaccination (Alexander and Senne, 2008). However, the true distribution of the virus is difficult to assess due to vaccination. The main reason that ND is not considered a truly vertically transmitted disease is that flocks infected with ND stop laying eggs and that infected embryos probably will die during incubation. However, 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 wild type virus may be present in eggs from vaccinated flocks (French et al., 1967). The thermal resistance of NDV is not high. In artificially infected chicken meat homogenate the following D values were obtained: 65°C 120 secs, 70°C 82 secs. 80°C 29 secs (Alexander and Manvell, 2004). D values for liquid whole egg has been deduced to be 38 secs. at 64.4 °C for one

particular strain (Alexander and Chettle, 1998). Surveillance programmes and several control measures have been set in place for many years by EC. Outbreaks of Newcastle disease in holdings need to be notified and control measures are put in place including destruction of the eggs (even hatching eggs)¹³. Consequently, the risk of introducing and maintenance of the virus to the hatcheries is extremely low.

2.1.2. Bacteria

2.1.2.1. *Salmonella*

- Serovar Gallinarum/Pullorum (only birds; wide range of species) (Berchieri et al., 2001)
- Serovar Enteritidis (Gast and Beard, 1990; Keller et al., 1995)
- Serovar Typhimurium (Cason et al., 1994; Liljebjelke et al., 2005)
- Other serovars primarily fecal contamination (Gast, 2008)

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 the environment. Most European countries have a low incidence or absence of *S. Gallinarum* and *S. Pullorum* (Shivaprasad and Barrow, 2008). The paratyphoid serotypes are widespread in Europe but with varying incidence in different countries (Gast, 2008).

Likelihood of presence in eggs: Infection of egg is an insignificant part of the epidemiology for most of the > 2000 serovars of *Salmonella*, with the following major exceptions. Genuine vertical transmission appears to be the main mechanism for *S. Gallinarum* and *S. Pullorum* where up to 33% of the eggs may be infected (Shivaprasad and Barrow, 2008).

S. Enteritidis and *S. Typhimurium* may be vertically transmitted by internal infection of the egg, and the vertical transmission of *S. Enteritidis* was a major reason for the pandemic spread of this serovars starting at the end of 1980s (Sobel et al., 2000), although, only a very small percentage will be in the egg – less than 1 % (Humphrey et al., 1989; Williams et al., 1998). Shell contamination may be very high in the case of progeny being hatched from salmonella-positive flocks – figures ranging from 17% to 75% have been reported depending on the serotypes involved, immune status of the flocks, etc. (Bailey et al., 1994; Cox et al., 1990).

Thermal resistance: Generally, *S. Gallinarum* and *S. Pullorum* are less resistant to heat than members of the paratyphoid groups and are killed within 10 min. at 60°C (Snoeyenbos, 1991).

Heating at 57°C for at least 70 min. can eliminate *Salmonella* inside intact eggs (Brackett et al., 2001). Liquid whole egg can be pasteurized successfully at 60°C for 3.5 min (Baker, 1990).

2.1.2.2. *E. coli* (Barnes et al., 2008; Petersen et al., 2006)

Most, if not all, avian species are susceptible to colibacillosis. Clinical disease is reported most often in chickens, turkeys and ducks. Natural infection of quail, pheasants, pigeons, guinea fowl, waterfowl, ostriches, and emus has been reported. *E. coli* is part of the normal flora in all individual birds, however, little is known about the carriage of potentially virulent types. Most avian *E. coli* isolates are specific clonal types that are pathogenic only for birds and represent a low risk of disease for people

¹³ Council Directive 92/66/EEC of 14 July 1992 introducing Community measures for the control of Newcastle disease (OJ L 260, 5.9.1992).

or other animals. However, chickens are readily infected experimentally with *E. coli* O157:H7. Also ducks, pigeons and turkeys can carry this serovar.

Serotypes, virulence factors, and antimicrobial resistance are often shared between avian strains and strains from other sources. Thus, avian strains potentially can be a source of genes and plasmids that encode antimicrobial resistance and virulence factors. Avian *E. coli* often carries virulence factors which are identical to the ones found in human uropathogenic *E. coli* and plasmids from avian strains can contribute to uropathogenicity of *E. coli* in a murine model of human disease.

Likelihood of presence in eggs: In one study 2.7% of the eggs contained *E. coli* inside following colonization of the oviduct (*via* oral infection)(Ardrey et al., 1968). However, commercial eggs may contain *E. coli* (in the yolk-sac) – up to 6% has been reported. In addition, experimental infections of hens have resulted in shedding in up to 26% of their eggs (Harry, 1957). It must be remembered that not all *E. coli* strains appear to be pathogenic.

Thermal resistance: Inactivation of most strains will occur at temperatures ranging from 60°C for 30 min. to 70°C for 2 min. (Barnes et al., 2008).

2.1.2.3. *Staphylococcus, several species* (Kizerwetter-Swida and Binek, 2008; Smyth and McNamee, 2008)

All avian species are susceptible to staphylococcal infections and all *staphylococcus* spp. 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 chicken meat. MRSA has been reported in poultry sampled in Korea and Japan.

Likelihood of presence in eggs: *S. aureus* is ubiquitous and conditions in incubators and hatchers are ideal for bacterial growth. Fecal contamination of eggs may cause embryo mortality and recently hatched and hatching chicks may easily be infected (Smyth and McNamee, 2008). 20% of 25, 18 day old chickens embryos were *Staphylococcus* positive in one study (Kizerwetter-Swida and Binek, 2008). In case *S. aureus* is observed as a major problem, management problems must have occurred somewhere in the system.

Thermal resistance: Some strains are quite resistant to inactivation but significant strain variation occurs. D_{72} of 4.1 s has been reported when measured in milk (stationary-phase) (Adam and Moss, 1995e).

2.1.2.4. *Streptococcus spp., several species* (Kizerwetter-Swida and Binek, 2008; Smyth and McNamee, 2008)

Several species have been isolated from a variety of bird species. Chickens, turkeys, ducks, pigeons and psittacines may be all 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 streptococci should be regarded as zoonotic agents.

Likelihood of presence in eggs: As taxonomy is still quite confusing with many species being reclassified over the years it is difficult to assess egg transmission rates. But it is recognized that streptococci can be isolated from hatchers and that spread may be direct through the egg (Smyth and McNamee, 2008).

Thermal resistance: Variation between species – but some may survive 63°C for 30 min. and others 60°C for 30 min. Streptococci are intestinal inhabitants of birds and mammals and can be recovered from the environment of poultry ((Kizerwetter-Swida and Binek, 2008; Smyth and McNamee, 2008).

2.1.2.5. *Enterococcus spp., several species (Kizerwetter-Swida and Binek, 2008; Smyth and McNamee, 2008)*

As with the streptococci, enterococci can be recovered from the environment of any poultry flock.

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.

Likelihood of presence in eggs: Common in both embryos and on the surface of eggs (Kizerwetter-Swida 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-Swida and Binek, 2008).

Thermal resistance: Probably as with the streptococci – many strains survive 60 °C for 30 min. In one study, *E. faecium* isolates survived 80°C for 3 min. (Kearns et al., 1995). D_{70} values obtained for *E. faecium* (ATCC 49624) varies from 0.33 to 1.73 min as a function of culture temperature and physiological state of cells. However, z values calculated were not significantly influenced by these factors. A mean value of $4.50 \pm 0.39^\circ\text{C}$ was found (Martinez et al., 2003).

2.1.2.6. *Mycobacterium avium (avian tuberculosis) (Schalk et al., 1935)*

There are several reports of the isolation of *Mycobacterium avium* from different European countries (Finland, Norway, Denmark, Germany and UK) (Fulton and Sanchez, 2008). However, much of the information relies on old investigations and it is difficult to provide solid data on current incidence and distribution.

All species of birds may be infected with *M. avium*. *M. avium* infections have been common in patients with AIDS. *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. *M. avium* serovar 2, the organism most frequently isolated from chickens, is rarely isolated from humans.

Likelihood of presence in eggs: *M. avium* has been isolated from eggs of naturally infected chickens – no transmission rates have been reported. However, it has been reported that the bacilli occur “rarely” (Jordan and Hampson, 2008).

Thermal resistance: *M. avium* does not survive in eggs after 6 min. of boiling (Fulton and Sanchez, 2008).

2.1.2.7. *Campylobacter species (Newell and Fearnley, 2003)*

Many prevalence studies have been conducted in Europe which have reported campylobacter-positive flocks ranging from 3%-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).

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. They are common in chickens, turkeys and ducks and have also been found in various game birds, pigeons and several wild bird species.

Likelihood of presence in eggs: Still quite a controversial subject. In early studies, *C. jejuni* was recovered from approximately 1% of eggs from colonized 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).

Thermal resistance: *Campylobacter* are delicate organisms and are easily destroyed by heating (e.g. pasteurization – D₅₅~1 min.) (Adam and Moss, 1995b)

2.1.2.8. *Ornithobacterium rhinotracheale*, many strains (El-Gohary, 1998)

O. rhinotracheale has been reported from throughout the world (Chin et al., 2008b).

O. rhinotracheale has been isolated world-wide from numerous bird species, including chickens, chukar partridge, duck, goose, guinea fowl, gull, pheasant, pigeon, quail and turkey.

Likelihood of 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., 2008b). However, no transmission rates have been indicated.

Thermal resistance: Available data indicate that at 37°C, *O. rhinotracheale* did not survive on eggshells for more than 24 hours (Varga et al., 2001).

2.1.2.9. *Rimerella anatipestifer*

The disease occurs world-wide and has been recognized in countries that have intensive duck production (Sandhu, 2008).

R. 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.

Likelihood of 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 3-4 days at 37°C or room temperature. Incubation at 55°C for 12-16 hours inactivates the organism (Bangun et al., 1981).

2.1.2.10. *Mycoplasma gallisepticum* (Glisson and Kleven, 1985), *M. meleagridis* (Yamamoto et al., 1966), *M. iowae* (McClenaghan et al., 1981), *M. synoviae* (Carnaghan, 1961)

- *M. gallisepticum*: infections naturally occur primarily in gallinaceous birds, particularly chickens and turkeys in commercial production. 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 recently has been isolated from clinically unaffected birds of prey in Germany. The organism is considered to occur world-wide (Chin et al., 2008a).

- *M. iowae*: natural host is the turkey, but isolation of *M. iowae* from chickens is not uncommon, and it has also been isolated from geese. In addition, *M. iowae* has been isolated from Amazon parrots and from wild and exotic birds. The organism is considered to occur world-wide (Jordan and Amin, 1980).
- *M. synoviae*: Chickens and turkeys are natural hosts. Ducks, geese, guinea fowl, pigeons, quail, pheasants and partridges have been found to be naturally infected. *M. synoviae* has also been isolated from house sparrows in Spain. *M. synoviae* is worldwide in distribution but primary breeding companies is largely free of infection due to control programmes (Kleven and Ferguson-Noel, 2008).

Likelihood of presence in eggs: *M. gallisepticum*: more than 50% of the eggs may be infected due the acute phase of the infection (Sasipreeyajan et al., 1987).

M. meleagridis: The egg transmission rate among individual hens may vary from 10-60% (Yamamoto et al., 1966).

M. iowa: No data recorded – however, some individuals in a flock may lay “many” infected eggs (McClenaghan et al., 1981).

M. synoviae: No data recorded, but major variation is expected as observed with the other species.

Thermal resistance: Mycoplasmas are sensitive to heating – 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).

2.1.2.11. *Chlamydophila psittaci* (Lublin et al., 1996; Wittenbrink et al., 1993)

Antibodies to *C. psittaci* have been found in more than 400 wild avian species. It is recognized that probably any species of bird may be infected although the susceptibility to infection may vary considerable. 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 in recent years (Andersen and van Rompay, 2008; Lederer and Muller, 1999).

Likelihood of presence in eggs: Its occurrence in the egg is considered to be “fairly low” (Andersen and van Rompay, 2008).

Thermal resistance: Dilute suspensions (20%) of infectious tissue homogenates are inactivated by incubation for 5 min. at 56 °C (Page, 1959).

2.1.3. Fungi

2.1.3.1. *Aspergillus* spp.

A. fumigatus and other *Aspergillus* species may also be egg-transmitted (Eggert and Barnhaet, 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 world-wide. 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.

Likelihood of 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 eg. in-ovo vaccination as the puncture facilitates entry and dissemination of spores.

Thermal resistance: The fungi are not considered highly resistant to heat (in contrast to various disinfectants). D values for conidia from various strains at 60°C ranged from 8 to 59 s. (Doyle and Marth, 1976).

2.1.4. Spoilage organisms

Several organisms have been isolated from chicken and turkey eggs including Gram-positive cocci, *Proteus* spp., coliforms, *Pseudomonas*, *Klebsiella* spp., *Enterobacter* spp., and clostridial infections (organisms may be transmitted through the egg, including spores) (Jordan and Hampson, 2008; Kizerwetter-Swida and Binek, 2008), but no reports have been found that toxins have contaminated hatching eggs (egg shells) (Kizerwetter-Swida and Binek, 2008). Most of these organisms have a broad host range but may be regarded as opportunistic pathogens occurring world-wide.

2.1.5. Other organisms of relevance

The organisms listed below are not considered as being truly vertically transmitted. However, as they may cause important infections in animals and/or humans they will be briefly discussed in relation to their possible appearance in hatching eggs.

2.1.5.1. *Listeria monocytogenes*

Outbreaks of listeriosis occur sporadically in chickens, turkeys and waterfowl (occasionally other species). In one study, the organism was not shed in eggs of heavily inoculated laying hens (Mazzette et al., 1991). However, the organism may be present in faeces and consequently a theoretical risk of surface contamination of the egg exists. Despite some conflicting data in the literature, it appears that the heat resistance of *L. monocytogenes* is similar to that of other non-sporeforming Gram-positive organisms with a typical D_{60} of a few min. and a D_{70} of a few secs (Adam and Moss, 1995d).

2.1.5.2. *Toxoplasma gondii* – toxoplasmosis

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 confined chickens have little exposure to *T. gondii* oocysts in contrast to free-range birds. 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-Servin, 1974). Generally it is recognized that cooking of e.g. pork to between 70° and 75°C provides a wide safety margin (Dixon, 1992). Thermal death times of 3, 12, 94 and 1.116 secs. for exposure at 67, 61, 55 and 49°C, respectively, were reported by Dubey et al. (1990).

2.1.5.3. *Erysipelothrix rhusiopathiae* - Erysipelas

E. rhusiopathiae is worldwide in distribution. There is no evidence of vertical transmission of *E. rhusiopathiae* (Mazaheri et al., 2006). It is killed at 70°C in 5-10 min.

2.1.5.4. Spore-forming rods

Clostridial disease: Several species may be involved in disease development in poultry and different species have been isolated from egg-shell fragments, embryos and chick fluff from the hatchery (Craven et al., 2001; Kizerwetter-Swida and Binek, 2008). Much of the contamination of egg shells probably 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. 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 min. to more than 38 min. at 100°C (Adam and Moss, 1995c). *C. botulinum* may also produce heat resistant spores, D values of 0.6 to 3.3 min at 80°C have been reported dependent up on the strain. At 121°C values have ranged between 0.1 to 0.2 min. (Adam and Moss, 1995c; Betts and Gaze, 1995; Blackburn and McClure, 2001; Gaze and Brown, 1988). No reports have been found where *C. botulinum* has been associated eggs but this possibility exists.

Bacillus spp. including *B. cereus*, occasionally have been associated embryo mortality in chickens, turkeys and ducks (Barnes and Nolan, 2008). 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 between around 1 min up to 36 min. Resistance appears to vary with serovar (Adam and Moss, 1995a).

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

3.1. General considerations on thermobacteriology

The death of microbial populations exposed to lethal temperatures follows the kinetics of first-order reactions (Stumbo, 1973). This principle is widely used in thermal process calculations and accepted in the canning industry 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.

It should be pointed out that sometimes survival curves do not fit within first-order kinetics, showing shoulders and/or tails (Cerf, 1977), or downward and upward concavity phenomena. To adjust to those behaviors more sophisticated models have been proposed (Peleg and Penchina, 2000; Sapru et al., 1992). Some of them 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 understandably the cumulative form of the survival curves of the individual cells which present a biological variation in thermoresistance. This is reasonably supported since it is unlikely that all cells behave the same way and that the death of a single cell is due to one single event. The Weibull distribution equation is one of the most suitable functions to describe microbial survival from a probabilistic viewpoint (Peleg, 1999; van Boekel, 2002), although other distributions such as the log-logistic or beta can be also appropriate.

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 . Since in a commercial thermal process heating and cooling are infrequently instantaneous, the calculation of the number of organisms destroyed usually takes also into account the heat transfer rate. Therefore, to achieve a target preserving action in a product, heat penetration data must be coupled with kinetic data of microbial destruction, in order to know the processing time at a given temperature.

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 survival 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 (decimal reduction time): it is the measure of the relative heat resistance of a microorganism at a constant temperature and represents the time required for a 10-fold reduction in survivors.
- Z-value: it can be defined as the temperature change (in °C) required for a 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 of bacteria. 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

Salmonella enterica serovar Senftenberg in liquid whole eggs at 63 °C ranges from 1.2 to 3.1, and its Z-value is 5.2 °C (Manas et al., 2003).

- F-value: it is the temperature-time combination necessary to produce a certain sterilization effect. It can be defined as the time needed at a certain temperature to obtain a safe product (the product is considered to be safe when a 12-log reduction in the population of the most heat-resistant pathogenic microorganism is achieved).

3.2. Identification of the target biological hazards

Spores of *C. botulinum* have been identified as the most resistant hazard potentially present in the material to be processed.

As regards viruses, circovirus and parvovirus are considered the most resistant ones.

Considering non-sporulating bacteria *Enterococcus faecium* is regarded as the most resistant agent to heat treatment.

Moreover, depending on the storage conditions of the material to be processed the production of bacterial toxins could be possible.

Consequently, the currently approved standards for processed petfood should be assessed against their ability to inactivate those hazards.

The feed composition and intrinsic and extrinsic factors can play a major role in determining the growth and survival of the target microorganisms and the final risk they may pose. For instance, in pelleted processed petfood submitted to a heat treatment at 90°C at the substance of the product *Cl. botulinum* may not represent a major risk since aerobic conditions, low water content and competition with other bacteria can impede toxin production. It is important to bear in mind that the method and conditions of heat treatment are known to affect the microbial inactivation. In terms of microbial inactivation it is well recognized that dry heat is less effective than moist heat since it is less penetrating and proteins, which are an important component in maintaining cell viability, are more stable in low-moisture environments.

Considering that moist heat treatment is the commonly applied heating method for food and feed processing, the approved standards for processed petfood in this opinion have been assessed based on the assumption that the heat treatment would be performed in a moist environment.

3.3. Treatment to a minimum Fc value of 3

Traditionally, a thermal process requiring 12 log reduction for spores of *C. botulinum* has been used to assure safety in canned foods. *C. botulinum* spores may show D₁₂₁-values as high as 0.2 min in a moist heat treatment (Betts and Gaze, 1995; Blackburn and McClure, 2001; Brown, 2000; Gaze and Brown, 1988). Therefore, a 12-log reduction would be reached after a treatment at 121 °C for approximately 2.4 min, and a Fc value of 3, which indicates a 3 minute heating at 121.1 °C (or an equivalent temperature/time combination), incorporates a margin of safety, if the heating method is represented by a moist heat treatment. With a dry heat treatment there is the possibility of a possible survival of spores and very resistant microorganisms (Molin, 1977; Oag, 1940; Pflug, 1960; Rank and Pflug, 1977; Welch et al., 2006; Wood et al., 2010) but this condition is not normally used in food or feed processing.

Spores of *C. botulinum* have been identified as the most resistant biological hazard potentially present in the material to be processed. Despite the lack of studies assessing the thermal inactivation kinetics

of *C. botulinum* spores in eggs, and although food components may have adverse or positive effects on the thermal destruction of spores, the requirements currently approved for the manufacturing of canned pet food (Fc value of 3) are designed to guarantee the complete inactivation of *C. botulinum* spores.

Therefore, a conventional treatment to a minimum Fc value of 3 in a moist environment, would assure the safety of the final product if dead-in-shell chicks are used since the other targets identified in section 3.2 will also be destroyed by this treatment.

3.4. Treatment of at least 90°C throughout the substance of the product

Although no mention to the treatment time and heating method used is made in the European Regulation, some estimates can be made regarding the efficacy of this processing treatment for the inactivation of the main biological hazards potentially present in hatchery waste. Table 1 shows the inactivation parameters described in the literature for some pathogenic microorganisms in whole liquid eggs.

Table 1: Inactivation parameters for various microorganisms in whole liquid eggs

Microorganism	Dt-value (minutes)	Z-value (°C)	Time required at 90 °C for a 12-log reduction ^(a) (seconds)	Reference
<i>Enterococcus faecium</i>	D ₆₀ -value 17.0 – 21.7	10.2	17.9	Sörqvist (2003)
<i>Salmonella</i> Enteritidis	D ₆₀ -value 0.15 – 0.5	5.2	6.1x10 ⁻⁴	Jin et al. (2008) Sörqvist (2003)
<i>Salmonella</i> Senftenberg	D ₆₃ -value 1.2 – 3.1	5.2	0.014	Manas et al. (2003)
<i>Campylobacter</i> spp.	D ₆₀ -value 0.11 – 0.17	5.5	4.3x10 ⁻⁴	Sörqvist (2003)
Avian Influenza virus	D ₅₉ -value 0.36 – 0.37	3.2-3.6	6.5x10 ⁻⁷	Swayne and Beck (2004)
Newcastle disease virus	D ₅₉ -value 0.37 – 1.60	3.9-7.7	0.11	Swayne and Beck (2004)

^(a) Worst case scenario calculated on the basis of the information reported in the references provided

The processing conditions prevailing are equivalent to a pasteurization treatment, where the vegetative forms of pathogenic microorganisms are killed, while most spores remain viable. *Enterococcus faecium* is considered to be the most heat-tolerant non-sporeforming bacterium (Fernandez et al., 2009; Martinez et al., 2003). Its high tolerance to adverse conditions and its ubiquitous nature makes *E. faecium* a reference microorganism appropriate to evaluate the efficacy of pasteurization 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 D₆₀-values ranging from 17.0 to 21.7 min and a mean Z-value of 10.2 °C. This would result in a D₉₀-value of 0.024 min in the worst case scenario, where an 18 s treatment at 90 °C would be required for a 12-log inactivation. The thermal tolerance of other microorganisms belonging to the *Enterobacteriaceae* is well recognized to be lower than that of *E. faecium*. As an example, *Salmonella enterica* serovar Enteritidis, one of the main biological hazards linked to hatchery waste, shows D₆₀-values in the range 0.15 to 0.5 min (Jin et al., 2008; Sörqvist, 2003). Taking into account that the mean Z-value described for *Salmonella* spp. is 5.2 °C (Doyle and Mazzotta, 2000), a 12-log reduction in bacterial population would be achieved after a treatment at 90 °C for 6.1x10⁻⁴ seconds in the worst case scenario. With regard to *S. Senftenberg*, the *Salmonella* serovar with the greatest thermal tolerance (Alvarez-Ordóñez et al., 2009a; Alvarez-Ordóñez et al., 2009b), D₆₃-values in the range 1.2-3.1 min have been reported (Manas et al., 2003). Thus, a treatment for 0.014 s at 90 °C would assure a 12-log inactivation.

Among the viruses identified in section 2.2.1 as potential hazards that may be present in the material to be thermally processed, circovirus (e.g. chicken anaemia virus) and parvovirus are the most

thermo-resistant ones. Although, to the best of our knowledge, there are no studies determining the thermal inactivation kinetics and describing D and Z-values for both families of viruses, various research articles show that they may be able to survive under the treatment conditions prevailing (90 °C throughout the substance of the product). 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 byproducts requires a core temperature of 95 °C for 35 min, or 100 °C for 10 min. It is important to note that the nature of the atmosphere surrounding microorganisms during heat treatments influences the effectiveness of the process, and that microbial thermal tolerance is known to be higher under dry environments. This fact explains the extreme heat resistance described for circoviruses by Welch et al. (2006). Parvovirus are also highly resistant to thermal inactivation. Thus, it has been previously shown that their infectivity is not significantly influenced by exposure to 95 °C for 2 hours (Sauerbrei and Wutzler, 2009). Other relevant viral hazards linked to hatchery waste, Avian influenza (AI) and Newcastle disease (ND) viruses are less heat tolerant. Their thermal tolerance in homogenized whole egg has been assessed by Swayne and Beck (2004), who reported D₅₀-values ranging from 0.36 to 0.37 min and 0.37 to 1.60 min for AI and ND viruses, respectively. These authors also described Z-values ranging from 3.2 to 3.6 °C and 3.9 to 7.7 °C for AI and ND viruses, respectively. This renders times to achieve a 12-log reduction at 90 °C of 6.5×10^{-7} and 0.11 s for AI and ND viruses in the worst case scenarios, respectively.

Spores of *C. botulinum* and *B. cereus* able to survive mild thermal treatments can germinate and multiply at the low temperatures prevailing in the final product and produce toxins, responsible for foodborne illness.

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 thermo-stable. That is the case of cereulide, the emetic toxin produced by *Bacillus cereus* strains, which has been recently shown to remain stable after more than 2 h at 121°C (dry heat treatment) (Rajkovic et al., 2008). Also *Staphylococcus aureus* produces a highly resistant enterotoxin able to show activity after a treatment at 100°C (Fung et al., 1973). In summary, some spore-forming bacteria able to survive the thermal treatment at 90°C throughout the substance of the product, may germinate, multiply and produce toxins.

The values reported in Table 1 are calculated from experiments in liquid egg products, the time needed for a 12 log reduction at 90°C calculated in the table could be different if the microbiological agents are in a matrix with different physico-chemical properties. For example a lower water activity of the matrix would lead to an increase in the resistance of the organisms to the heat treatment.

To sum up, a 90 °C treatment throughout the substance of the product will not be able to inhibit the main bacterial spores and thermo-resistant viruses and to inactivate some toxins present in the waste material, but it will assure a rapid destruction of non-sporeforming bacteria – i.e. a 18 s treatment at 90 °C would reduce by a 12-log factor the population of *Enterococcus faecium*, the most thermotolerant non-sporeforming bacterium.

4. Risk posed by the possible use of dead-in-shell chicks for the production of processed petfood

4.1. Treatment to a minimum Fc value of 3

The risk related to the use of dead-in-shell chicks, submitted to a conventional heat treatment to a minimum Fc value of 3 in a moist environment, for the production of canned petfood is negligible.

4.2. Treatment of at least 90°C throughout the substance of the product

The current ABP Regulation does not specify the processing time and heating method needed for the treatment at 90°C throughout the substance of the final product. A treatment for 18 seconds can assure a rapid destruction of the non-sporeforming bacteria identified as hazards. However, this treatment is not able to inactivate other relevant hazards such as bacterial spores, thermoresistant viruses and some toxins.

The final risk posed by the agents that may survive this treatment additionally depends on a number of factors including the following ones:

- the extent to which the physico-chemical properties of the final feed facilitate the survival and growth of the organisms;
- the extent to which the surviving viruses represent a risk for human and animal health;
- the possible cross-contaminations that could arise from this material;
- the pet species consuming the petfood (the risk for avian pet species is likely to be higher than for mammalian pets);
- the possible pathways that could lead to the exposure of people and animals.

For the above-mentioned reasons the risk related to the use of dead-in-shell chicks, submitted to a heat treatment of at least 90°C throughout the substance of the product, for the production of processed petfood cannot be considered to be negligible.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- The risk related to the use of dead-in-shell chicks, submitted to a conventional heat treatment to a minimum Fc value of 3 in a moist environment, for the production of canned petfood is negligible.
- There is no indication given in the ABP regulation of the processing time and heating method needed for the treatment at 90°C throughout the substance of the final product.
- The treatment at 90°C throughout the substance of the final product for 18 seconds can assure a rapid destruction of the non-sporeforming bacteria identified as hazards. However, this treatment is not able to inactivate other relevant hazards such as bacterial spores, thermoresistant viruses (parvoviruses and circoviruses) and some toxins (the ones produced by *Staphylococcus aureus* and *Bacillus cereus*). The final risk posed by the agents that may survive this treatment additionally depends on several factors (e.g. the level of hazards in the raw material, the physico-chemical properties of the final feed, the pet species consuming the petfood, the way of handling the petfood) and cannot be considered negligible under all circumstances.

RECOMMENDATIONS

- In order to assess the ability of the processing method at 90°C throughout the substance of the product to inactivate possible biological hazards, the treatment time and other additional parameters such as particle size, heating method and water content of the matrix should be provided.

DOCUMENTATION PROVIDED TO EFSA

1. Opinion of the Director of the Dutch Office for Risk Assessment. Ref.: VWA/BuR/2009/21889 of 9 July 2009 (Opinion on risks of hatchery by-products). Submitted by the European Commission.
2. Qualitative and Quantitative Risk Analysis of Hatchery Waste dated June 2009 from the Dutch Animal Health Service. Submitted by the European Commission.

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