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## Assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) No 2016/429): avian mycoplasmosis (*Mycoplasma gallisepticum*, *M. meleagridis*)

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### Abstract

Avian mycoplasmosis (*Mycoplasma gallisepticum*, *Mycoplasma meleagridis*) has been assessed according to the criteria of the Animal Health Law (AHL), in particular criteria of Article 7 on disease profile and impacts, Article 5 on the eligibility of avian mycoplasmosis to be listed, Article 9 for the categorisation of avian mycoplasmosis according to disease prevention and control rules as in Annex IV and Article 8 on the list of animal species related to avian mycoplasmosis. The assessment has been performed following a methodology composed of information collection and compilation, expert judgement on each criterion at individual and, if no consensus was reached before, also at collective level. The output is composed of the categorical answer, and for the questions where no consensus was reached, the different supporting views are reported. Details on the methodology used for this assessment are explained in a separate opinion. According to the assessment performed, avian mycoplasmosis can be considered eligible to be listed for Union intervention as laid down in Article 5 (3) of the AHL. The disease would comply with the criteria as in Sections 4 and 5 of Annex IV of the AHL, for the application of the disease prevention and control rules referred to in points (d) and (e) of Article 9(1). The assessment here performed on compliance with the criteria as in Section 3 of Annex IV referred to in point (c) of Article 9(1) is inconclusive. The animal species to be listed for avian mycoplasmosis according to Article 8(3) criteria are mainly domestic and wild birds of the order Galliformes, and also Passeriformes for *M. gallisepticum*.

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## 1. Introduction

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

The background and Terms of Reference (ToR) as provided by the European Commission for the present document are reported in Section 1.2 of the scientific opinion on the *ad hoc* methodology followed for the assessment of the disease to be listed and categorised according to the criteria of Article 5, Annex IV according to Article 9, and 8 within the Animal Health Law (AHL) framework (EFSA AHAW Panel, 2017).

### 1.2. Interpretation of the Terms of Reference

The interpretation of the ToR is as in Section 1.2 of the scientific opinion on the *ad hoc* methodology followed for the assessment of the disease to be listed and categorised according to the criteria of Article 5, Annex IV according to Article 9, and 8 within the AHL framework (EFSA AHAW Panel, 2017).

The present document reports the results of assessment on avian mycoplasmosis (*Mycoplasma gallisepticum*, *Mycoplasma meleagridis*) according to the criteria of the AHL articles as follows:

- Article 7: avian mycoplasmosis (*M. gallisepticum*, *M. meleagridis*) profile and impacts
- Article 5: eligibility of avian mycoplasmosis (*M. gallisepticum*, *M. meleagridis*) to be listed
- Article 9: categorisation of avian mycoplasmosis (*M. gallisepticum*, *M. meleagridis*) according to disease prevention and control rules as in Annex IV
- Article 8: list of animal species related to avian mycoplasmosis (*M. gallisepticum*, *M. meleagridis*).

## 2. Data and methodologies

The methodology applied in this opinion is described in detail in a dedicated document about the *ad hoc* method developed for assessing any animal disease for the listing and categorisation of diseases within the AHL framework (EFSA AHAW Panel, 2017).

## 3. Assessment

### 3.1. Assessment according to Article 7 criteria

This section presents the assessment of avian mycoplasmosis (*M. gallisepticum*, *M. meleagridis*) according to the Article 7 criteria of the AHL and related parameters (see Table 2 of the opinion on methodology (EFSA AHAW Panel, 2017)), based on the information contained in the fact-sheet as drafted by the selected disease scientist (see Section 2.1 of the scientific opinion on the *ad hoc* methodology) and amended by the AHAW Panel.

#### 3.1.1. Article 7(a) Disease Profile

##### 3.1.1.1. Article 7(a)(i) Animal species concerned by the disease

*Susceptible animal species*

Parameter 1 – Naturally susceptible wildlife species (or family/orders)

#### ***M. gallisepticum***

Naturally *M. gallisepticum* infections occur in several wild avian species. In Europe, the main wild avian species affected by *M. gallisepticum* infections are game birds (belonging to the order Galliformes): pheasants, grey partridges, chukar partridges, etc.; often because of captive rearing conditions resembling those of domestic Galliformes such as chickens and turkeys (Nicholas, 2012). But other bird orders can also be affected: a recent Belgian study detected *M. gallisepticum* in one wood pigeon (Columbiformes), two grey herons (Pelecaniformes), one mallard (Anseriformes) and one Eurasian magpie (Passeriformes) (Michiels et al., 2016). In the USA, *M. gallisepticum* was detected in birds belonging to the order Galliformes (wild turkeys, peafowls, peacocks, bobwhite quail) and Passeriformes (house finches, rooks, American goldfinches, pine grosbeaks, evening grosbeaks, purple finch and blue jay) (for review, see Stipkovits and Kempf, 1996; Raviv and Ley, 2013). Cases were

reported for Japanese quails (Passeriformes) and parrots (Psittaciformes). Antibodies were also detected in several other bird species (Luttrell et al., 2001; Dhondt et al., 2014).

### ***M. meleagridis***

*M. meleagridis* is a specific pathogen of turkeys (order Galliformes) (for review see Chin, 2013) and wild turkeys in America are therefore susceptible to this disease (Charlton, 2000). No other data are available about another susceptible wildlife species (clinical signs or lesions). However, seropositivity is often reported in several wild bird species: antibodies were found in sera of other bird species belonging to the order Galliformes (lesser prairie chickens and peafowls in the USA (Hagen et al., 2002; Hollamby et al., 2003) and of a scaled quail in Mexico (Aguirre et al., 1992)). Occurrence of *M. meleagridis* in peacocks and Japanese quail (Galliformes), and pigeons (Columbiformes) was also reported (Yamamoto, 1991). *M. meleagridis* was also isolated from birds of prey (Falconiformes) without clinical signs or histopathological alterations in air sac biopsies in Germany (Aguirre et al., 1992; Lierz et al., 2000).

#### Parameter 2 – Naturally susceptible domestic species (or family/orders)

### ***M. gallisepticum***

*M. gallisepticum* infections occur mostly in domestic Galliformes, mainly chickens and turkeys (Stipkovits and Kempf, 1996; Raviv and Ley, 2013), but has also been described in geese and ducks (Jordan and Amin, 1980; Buntz et al., 1986; Bencina et al., 1987, 1988).

### ***M. meleagridis***

*M. meleagridis* was thought to be very host-specific and only restricted to turkeys but *M. meleagridis* was recently isolated from chicken breeders with respiratory symptoms and poor performances reared near a turkeys breeding unit in Tunisia (Bejaoui Khiari et al., 2011).

#### Parameter 3 – Experimentally susceptible wildlife species (or family/orders)

### ***M. gallisepticum***

Experimental infections were carried out on several wild species of birds. House sparrows and pigeons are none to mildly susceptible (Dhondt et al., 2008; Gharaibeh and Hailat, 2011), whereas house finches are very susceptible (Sydenstricker et al., 2006; Dhondt et al., 2008). American goldfinches develop intermediate clinical signs (Dhondt et al., 2008). Chukar partridges were also used as experimental models with development of clinical signs and lesions (McMartin et al., 1996).

### ***M. meleagridis***

No reports available on experimentally infected wildlife species for *M. meleagridis*.

#### Parameter 4 – Experimentally susceptible domestic species (or family/orders)

### ***M. gallisepticum***

Most of experimental studies were performed on chickens and turkeys (for review see Stipkovits and Kempf, 1996; Raviv and Ley, 2013) which are susceptible for the infection. However, studies were also carried out on budgerigars and canaries with development of clinical signs and lesions (Brown and Butcher, 1991; Hawley et al., 2011).

### ***M. meleagridis***

Experimental infections were conducted on turkeys and turkey embryos, leading to airsacculitis, deciliation of trachea, induced curved toes, fissures of the cartilage, alterations of the eggshell membranes (Lam et al., 2003a,b, 2004).

One experiment performed on chicken embryos resulted in abnormal-shaped toes and severely denuded tracheae (Lam, 2004).

#### *Reservoir animal species*

#### Parameter 5 – Wild reservoir species (or family/orders)

### ***M. gallisepticum***

As described below in Section 3.1.1.6 Parameter 1, airborne transmission is a major way of transmission of *M. gallisepticum* between birds. Wild birds described in Section 3.1.1.1 can therefore



be reservoir animals (Ferguson et al., 2003) as *M. gallisepticum* infections do not always lead to clinical signs. However, wild birds probably play a limited role as a reservoir compared to domestic species such as chickens and turkeys.

### ***M. meleagridis***

As described below in Section 3.1.1.6 Parameter 1, airborne transmission is apparently of little significance for *M. meleagridis* transmission compared to vertical transmission (Stipkovits and Kempf, 1996; Chin, 2013). Therefore, even if this *Mycoplasma* species can be isolated or detected by serology in some other avian species described below, it is quite unlikely that these species may act as a reservoir for *M. meleagridis* infection in turkeys.

As described in Section 3.1.1.6 Parameter 1, *M. meleagridis* was isolated from birds of prey in Germany (Aguirre et al., 1992; Lierz et al., 2000) and antibodies were also found in sera of lesser prairie chickens, peafowls, a scaled quail, peacocks, pigeons and Japanese quails (Yamamoto, 1991; Hagen et al., 2002). However, no reports showed evidence of turkey flock recontamination from these bird species.

### Parameter 6 – Domestic reservoir species (or family/orders)

#### ***M. gallisepticum***

Backyard flocks and multiage flocks (especially laying-hen flocks) can be reservoir for *M. gallisepticum* infections (Mohammed et al., 1987; McBride et al., 1991; Haesendonck et al., 2014).

#### ***M. meleagridis***

*M. meleagridis* was isolated from chicken and turkey flocks near turkey breeding units or meat turkey flocks (McBride et al., 1991; Bejaoui Khiari et al., 2011).

### **3.1.1.2. Article 7(a)(ii) The morbidity and mortality rates of the disease in animal populations**

#### *Morbidity*

#### Parameter 1 – Prevalence/incidence

#### ***M. gallisepticum***

*M. gallisepticum* infections have a worldwide distribution. They resulted in important flock health problems before implementation of control programmes, which succeeded in reducing the prevalence of *M. gallisepticum* in all areas of commercial productions, especially in the USA and Europe (Levisohn and Kleven, 2000). However, outbreaks of *M. gallisepticum* infections continue to occur in production flocks and *M. gallisepticum* is endemic in many multiage commercial egg production farms (Levisohn and Kleven, 2000; Raviv and Ley, 2013). Recent studies on prevalence of *M. gallisepticum* in Europe are scarce. A seroprevalence of 2.4% was found in Latvia (Zute and Valdovska, 2015). Two studies performed in France in 1998 and in 2003 showed a low seroprevalence of *M. gallisepticum* (between 0% and 2%) in laying hen flocks (Kermorgant, 1999; Dufour-Gesbert et al., 2006). Likewise, in Germany, Kohn et al. (2009) did not detect *M. gallisepticum* infection in laying hens from different housing systems. However, antibodies against *M. gallisepticum* were found in 36.7% of birds from backyard and fancy poultry flocks in Belgium (Haesendonck et al., 2014), highlighting the possible risk of transmission to commercial flocks.

Seroprevalence of *M. gallisepticum* in other countries outside Europe is often much higher: 69.9% in broiler and laying chickens in Algeria (Heleili et al., 2012) and 56.1–64.5% in Bangladesh (Ali et al., 2015). *M. gallisepticum* was also detected by polymerase chain reaction (PCR) in 18.7% of samples from breeder, broiler and layer flocks (Faisal et al., 2011).

#### ***M. meleagridis***

Studies performed before 1980 showed that *M. meleagridis* was a common pathogen of turkeys with a worldwide distribution (Vlaovic and Bigland, 1971; Rosenfeld and Grimes, 1972; Shimizu and Yagihashi, 1980). However, little recent data is available on prevalence/incidence of this disease as intensive eradication programmes were conducted to eliminate *M. meleagridis* from turkey breeder flocks (Chin, 2013). These programmes succeeded in reducing the prevalence of *M. meleagridis* infections in the major producing areas of the world. Studies performed in Germany and Belgium on several turkey flocks showed that *M. meleagridis* infections could not be detected (Van Loock et al., 2005).

More recently, *M. meleagridis* was detected by PCR in 3/624 (0.5%) meat-type turkeys randomly selected in Turkey (Ongor et al., 2009).

#### Parameter 2 – Case-morbidity rate (% clinically diseased animals out of infected ones)

##### ***M. gallisepticum***

*M. gallisepticum* causes chronic respiratory disease in chickens and infectious sinusitis in turkeys characterised by rales, coughing, nasal discharge, sinusitis and the development of severe airsacculitis (Stipkovits and Kempf, 1996; Raviv and Ley, 2013). *M. gallisepticum* infections usually affect nearly all chickens in a flock but severity and duration of disease are variable. It affects males and young birds more severely than females and adult birds. The disease is also more severe in winter than in summer.

In broilers, *M. gallisepticum* infections lead to retarded growth with a reduction in weight gain up to 20–30% and to a 10–20% decrease in food conversion efficiency. Clinical signs and lesions are also source of 10–20% of condemnations or downgrading of carcasses in slaughterhouses (Stipkovits and Kempf, 1996).

In breeders and laying hens, *M. gallisepticum* infection is responsible for a 10–20% decrease in egg production.

*Escherichia coli* and Infectious Bronchitis virus (IBV) infections lead to complicated diseases with more severe clinical signs and lesions and a higher morbidity rate. Moreover, vaccination programmes against infectious bursal disease (IBD), IBV, laryngotracheitis and infectious coryza significantly increase economic losses due to *M. gallisepticum* infection (Stipkovits and Kempf, 1996; Raviv and Ley, 2013).

Turkeys are more susceptible to *M. gallisepticum* infections than chickens, developing more severe clinical signs such as marked swelling of infraorbital sinuses: partial to complete closure of the eyes can affect 1–70% of birds in affected flocks and can lead to weight losses when birds cannot see to eat (Raviv and Ley, 2013). However, clinical signs may be highly variable within a flock or between flocks.

##### ***M. meleagridis***

*M. meleagridis* causes late incubation mortality (from 25 to 28 days of incubation) in infected turkey embryos (Carpenter et al., 1981), with a loss of hatchability of 5–6% of fertile eggs, but does not affect egg production or fertility in adult birds (Chin, 2013).

Most of problems are seen in young birds: 10–25% of young turkeys under 15–16 weeks can show *M. meleagridis*-associated air sacculitis; 5–10% may show *M. meleagridis*-associated skeletal abnormalities such as wry necks, twisting and shortening of the tarso-metatarsal bones (Wise et al., 1973; Chin, 2013). Reduction of growth rate is not always observed in *M. meleagridis*-infected flocks compared to *M. meleagridis*-free flocks (Wise et al., 1973; Carpenter et al., 1982).

##### *Mortality*

#### Parameter 3 – Case-fatality rate

##### ***M. gallisepticum***

Experimental inoculation of *M. gallisepticum* in embryos usually results in embryo deaths within 5–7 days. Natural infection leads to a 5–10% increase in embryo mortality (Stipkovits and Kempf, 1996; Raviv and Ley, 2013). In broilers, the mortality may range from low in uncomplicated disease to as much as 30% in severe outbreaks due to concurrent infections (*E. coli* or viruses) and environmental factors (ammoniac, low or high temperatures).

*Mycoplasma*, and especially *M. gallisepticum* infections are source of morbidity (sinusitis and conjunctivitis) and mortality rates of 5–10% in game birds housed in high density (Nicholas, 2012). According to several studies, *M. gallisepticum* was responsible for up to 60% of population decline in house finches in the USA (Luttrell et al., 2001; Sydenstricker et al., 2006; Raviv and Ley, 2013).

##### ***M. meleagridis***

Except embryo mortality, *M. meleagridis* infections do not lead to direct mortality, which is due primarily to cannibalism of affected birds (Chin, 2013).

### 3.1.1.3. Article 7(a)(iii) The zoonotic character of the disease

#### Presence

Parameter 1 – Report of zoonotic human cases (anywhere)

#### ***M. gallisepticum***

No reports of zoonotic human cases have been published. *M. gallisepticum* infects a relatively narrow range of exclusively avian host species and has no public health significance.

#### ***M. meleagridis***

No reports of zoonotic human cases have been published. *M. meleagridis* infects a very narrow range of exclusively avian host species as listed in Section 3.1.1.1.

### 3.1.1.4. Article 7(a)(iv) The resistance to treatments, including antimicrobial resistance

Parameter 1 – Resistant strain to any treatment even at laboratory level

#### ***M. gallisepticum***

Because *M. gallisepticum* is a wall-less bacteria, it is assumed that most of the commonly employed chemical disinfectants are effective against this *Mycoplasma* species (Brunner and Laber, 1985; Raviv and Ley, 2013).

*M. gallisepticum*, as other *Mycoplasma* species, is naturally resistant to penicillin and other antibiotics acting on cell-wall synthesis. Several studies showed that most of field strains are susceptible *in vitro* and *in vivo* to several antibiotics of the tetracycline, macrolide, pleuromutilin and fluoroquinolone families (for review see Brunner and Laber, 1985; Raviv and Ley, 2013). However, recent studies showed that *M. gallisepticum* can develop resistance and cross-resistance mechanisms to several antimicrobials (within an antimicrobial family) (Raviv and Ley, 2013; Ammar et al., 2016). However, no strain was found to be resistant to all treatments *in vivo* or *in vitro*.

#### ***M. meleagridis***

There are a very low number of publications on *M. meleagridis* and resistance to treatments. It is assumed that most chemical disinfectants would be effective on *Mycoplasma* species as these bacteria do not have a cell wall (Brunner and Laber, 1985).

As occurrence of *M. meleagridis* infections is very low, due to eradication programmes, no recent studies were performed on minimum inhibitory concentration (MIC) determination. Most studies on efficacy of different antibiotics (gentamycin, tetracyclines, macrolides, spectinomycin, fluoroquinolones) against *M. meleagridis*-induced clinical signs were carried out *in vivo* or *in ovo* before or at the beginning of eradication programs between 1970 and 1982 (for review see Brunner and Laber, 1985; Chin, 2013). A publication on antibiotic MIC determination against *M. meleagridis* in 1989 showed that the four strains tested were susceptible to enrofloxacin, tylosin and tiamulin (Jordan et al., 1989). However, *M. meleagridis* strains resistant to tylosin have been reported in 1969 (Chin, 2013).

### 3.1.1.5. Article 7(a)(v) The persistence of the disease in an animal population or the environment

#### Animal population

Parameter 1 – Duration of infectious period in animals

#### ***M. gallisepticum***

Transmission may be more likely during the acute phase of infection (usually 4–8 weeks after infection) when a large quantity of *Mycoplasma* may be excreted by nasal discharge, breathing or coughing. However, once a bird is infected by *M. gallisepticum*, it is considered chronically infected for life and it is a source of infection for other birds. Thus, infected flocks are often sources of new infections (in the same farm or in neighbouring farms).

#### ***M. meleagridis***

Several old studies showed that once established, *M. meleagridis* reproductive tract infections in turkey hens and males persist for long periods (Kumar and Pomeroy, 1969; Rhoades, 1969, 1971; Chin, 2013), with variation during the laying period (Kumar and Pomeroy, 1969; Rott et al., 1989).



These genital infections are the main way of transmission: once contaminated, a turkey (male or female) remains a source of contamination (infected eggs and poults for the hens, contaminated semen for the males). Tracheal infections are observed only in the first 14 weeks of age (Stipkovits and Kempf, 1996; Chin, 2013).

#### Parameter 2 – Presence and duration of latent infection period

##### ***M. gallisepticum***

*M. gallisepticum* incubation period varies from 6 to 21 days, but development of clinical signs can be highly variable depending on strain virulence, concomitant infections (other bacteria or viruses) and other stresses (Raviv and Ley, 2013).

##### ***M. meleagridis***

Isolation of *M. meleagridis* and development of air-sac lesions are observed after an incubation period of 1–2 weeks after infection in poults (Rhoades, 1971). *M. meleagridis* can be isolated from respiratory and reproductive tracts of experimentally infected adult turkeys from 2 weeks after exposure.

#### Parameter 3 – Presence and duration of the pathogen in healthy carriers

##### ***M. gallisepticum***

In general, *Mycoplasma* infections, once established, are known to persist for all of the flock's life: persistence of *M. gallisepticum* in infected chickens and turkey has been described (Stipkovits and Kempf, 1996; Reinhardt et al., 2005; Raviv and Ley, 2013). Chickens and turkeys often develop clinical signs near the onset of egg production, or after a vaccination (or another operation on animals), suggesting a low level of subclinical infection without antibody response (healthy carrier state) that becomes clinical in response to a stress. At later stages of infection, the number of *M. gallisepticum* organisms in chronically infected birds, such as commercial layers or backyard poultry, may be so low that *M. gallisepticum* may not be detected by usual sampling and culture methods (Raviv and Ley, 2013). However these birds still remain a possible source of infection since additional stresses (bad environmental conditions, vaccinations, etc.) or infection with other microorganisms can increase excretion of *M. gallisepticum* in these birds (Stipkovits and Kempf, 1996).

##### ***M. meleagridis***

In general, *Mycoplasma* infections, once established, are known to persist for all the flock's life. As described in Section 3.1.1.5 Parameter 1, persistence of *M. meleagridis* in the genitalia of adult turkeys has been reported for several weeks without clinical signs and lesions (Kumar and Pomeroy, 1969; Rhoades, 1969, 1971; Chin, 2013). Cloacal infection detected in the male at the time of hatch can persist through sexual maturity. Infection may occur without clinical signs and lesions in adult birds. Moreover, adult males can be serologically negative carriers of *M. meleagridis* (Rhoades, 1971).

#### *Environment*

#### Parameter 4 – Length of survival (dpi) of the agent and/or detection of DNA in selected matrices (soil, water, air) from the environment (scenarios: high and low T)

##### ***M. gallisepticum***

Several studies were performed on *M. gallisepticum* survival in the environment (for review see (Stipkovits and Kempf, 1996; Raviv and Ley, 2013)). The survival time of *M. gallisepticum* outside of a host varies from 1 to 14 days and depends upon environmental conditions (mainly substrate (faeces, feathers, dust, clothes and human hair, etc.) on which *M. gallisepticum* cells are found, but also temperature, humidity and pH). The longest survival time was found in egg material: up to 3 weeks in allantoic fluid, and up to 18 weeks in egg yolk. PCR is more sensitive than culture for assessing dissemination of *M. gallisepticum* in poultry environment, but it detects viable and non-viable bacteria (Marois et al., 2002a).

##### ***M. meleagridis***

Very few studies were performed on *M. meleagridis* survival in the environment as the major way of transmission is the vertical one. *M. meleagridis* was recovered from an artificially created aerosol in gradually decreasing amounts during a 6-h period (Beard and Anderson, 1967). *M. meleagridis* is able to survive in turkey semen during cryopreservation and subsequent thawing (Ferrier et al., 1982).

Studies on other avian *Mycoplasma* species have shown that these bacteria are able to survive on different matrices from several hours to several days (Christensen et al., 1994; Marois et al., 2002b).

### **3.1.1.6. Article 7(a)(vi) The routes and speed of transmission of the disease between animals, and, when relevant, between animals and humans**

#### *Routes of transmission*

#### Parameter 1 – Types of routes of transmission from animal to animal (horizontal, vertical)

##### ***M. gallisepticum***

The main route for the spread of *M. gallisepticum* infection is vertical transmission through eggs (Stipkovits and Kempf, 1996; Raviv and Ley, 2013). Even if a proportion of infected embryos die during incubation (5–10% increase in embryo mortality), other embryos will hatch, carrying the infection to the progeny flock. Consequently, infection can be carried on long distances by *M. gallisepticum*-infected eggs or 1-day-old chicks.

Horizontal transmission occurs readily by direct or indirect contact of susceptible birds with infected birds (clinical or subclinical carriers) (Stipkovits and Kempf, 1996; Raviv and Ley, 2013): a large quantity of mycoplasma can be excreted by nasal discharge, breathing or coughing during the acute phase of infection. Transmission of infection may also occur through artificial insemination since *M. gallisepticum* can be found in semen. Additional transmission and more widespread disease outbreaks may occur via fomites (*M. gallisepticum* found on dust, feathers, etc.) and suboptimal biosecurity measures and personnel practices (*M. gallisepticum* found on human hair and clothes, in the nasal passage). Egg debris in incubators is also essential in spreading infection (Stipkovits and Kempf, 1996).

##### ***M. meleagridis***

Direct horizontal transmission of *M. meleagridis* can occur by the airborne route within the hatchery and flock and between flocks separated by 400 meters (Kumar and Pomeroy, 1969; Stipkovits and Kempf, 1996; Chin, 2013). Airborne transmission in mature turkey results in infections localised in the sinus and trachea, whereas airborne infections of young birds can lead to genitalia localisation.

Indirect transmission can occur at any time of the bird's life during interventions (sexing, palpation of hens, vaccination) with contaminated hands, clothing and equipment. Adult females can also be infected by insemination with *M. meleagridis*-contaminated semen from infected males (Kumar and Pomeroy, 1969; Stipkovits and Kempf, 1996; Chin, 2013).

The horizontal spread of *M. meleagridis* is of little significance (except for contamination via artificial insemination) compared to vertical egg transmission. Embryos become infected following ingestion or inhalation of infected amniotic fluid and infection of the female reproductive tract occurs during embryonic development (Rhoades, 1971; Chin, 2013).

Very few organisms are necessary to produce infection: inoculation of embryos with as few as 0.685 CFU resulted in air-sac lesions (Rhoades, 1971).

#### Parameter 2 – Types of routes of transmission between animals and humans (direct, indirect, including food-borne)

##### ***M. gallisepticum***

No transmission from animals to humans reported for *M. gallisepticum*. This bacterium was only shown to be able to survive in the human nasal passage for 24 h (Christensen et al., 1994).

##### ***M. meleagridis***

No transmission from animals to humans have been reported for *M. meleagridis*.

#### *Speed of transmission*

#### Parameter 3 – Incidence between animals and, when relevant, between animals and humans

##### ***M. gallisepticum***

Since recent precise field epidemiological studies on *M. gallisepticum* are missing, the incidence cannot be calculated.

Airborne transmission leads to highly variable infection rate depending on the strain infectivity and virulence. Some strains spread very quickly by contact (100% of birds positive in a naïve flock after

4 weeks) while others spread very slowly (with a serologic response after 16 weeks). Variant atypical strains have been isolated, producing poor or none antibody response.

Horizontal transmission of *M. gallisepticum* from very few infected eggs and 1-day-old chicks is likely to involve entire flocks that receive any infected chicks (Raviv and Ley, 2013).

### ***M. meleagridis***

Since recent precise epidemiological studies are missing, the incidence cannot be calculated. Airborne transmission usually leads to a high infection rate (up to 100% of birds), resulting in a genital localisation in 5% of young birds, but not in adults.

Parameter 4 – Transmission rate (beta) (from  $R_0$  and infectious period) between animals and, when relevant, between animals and humans

### ***M. gallisepticum***

Feberwee et al. (2005) described an experimental model to quantify *M. gallisepticum* horizontal transmission:  $R_0$  was estimated to be greater than 1 and the estimated beta was 0.22 per day.

The egg transmission rate among individual hens may vary considerably under natural conditions. Under experimental conditions, the highest transmission rates were found during the acute phase of infection with 25–50% infected eggs 3–6 weeks after challenge. The egg transmission rate then declined after the acute phase: transmission rates of 3–5% after 8–25 weeks post-infection were reported (Raviv and Ley, 2013). Egg transmission probably occurs at lower levels during chronic infections.

Horizontal transmission of *M. gallisepticum* by direct or indirect contact between one infected bird and a naïve flock can lead to the infection of 100% the birds (development of clinical signs or serologic response to infection) within 3–19 weeks (Raviv and Ley, 2013).

### ***M. meleagridis***

There is no precise information about the transmission rate (beta and  $R_0$ ) for *M. meleagridis*.

The egg transmission rate among individual turkey hens may vary from 10% to 60%, with variation during the laying season: transmission starts at low rate at the beginning and reach a maximum at midseason. Egg transmission does not occur in animals only infected in the upper respiratory tract (Kumar and Pomeroy, 1969; Chin, 2013). Infected eggs result in a widespread distribution of infection in young turkeys and increase risk of further vertical transmission.

Insemination with *M. meleagridis* contaminated semen also plays a major role in infecting the genital tract of turkey hens and therefore in sustaining the egg-transmission rate during the laying season (Kumar and Pomeroy, 1969; Chin, 2013).

Egg transmission does not occur in non-infected females reared with infected ones.

## **3.1.1.7. Article 7(a)(vii) The absence or presence and distribution of the disease in the Union, where the disease is not present in the Union, the risk of its introduction into the Union**

### *Presence and distribution*

Parameter 2 – Type of epidemiological occurrence (sporadic, epidemic, endemic) at MS level

### ***M. gallisepticum***

Control programmes at the breeding flock level, with the availability of *M. gallisepticum*-free eggs and 1-day-old chicks or poults, reduce the risk of egg-borne infection and transmission between flocks by introduction of infected birds (Levisohn and Kleven, 2000; Raviv and Ley, 2013).

Sporadic cases are reported in broilers or meat turkeys in several Member States (MSs) (cases not published, personal communications from different research laboratories). However *M. gallisepticum* may be endemic in some large multiage laying-hen farms (Levisohn and Kleven, 2000) and vaccination programmes are therefore implemented to reduce clinical signs and losses.

### ***M. meleagridis***

No recent cases of infection with *M. meleagridis* have been published in the Union. Control programmes at the breeding flock level, with the availability of *M. meleagridis*-free eggs, reduce the risk of egg-borne infections.

## Risk of introduction

### Parameter 3 – Routes of possible introduction

#### ***M. gallisepticum***

A route of possible introduction of the disease would be an infection in a chicken or turkey breeder flock as egg-borne infection is a major route of infection: introduction of birds (males or females) infected by *M. gallisepticum*, artificial inoculation of hens by contaminated semen.

Other routes of introduction would be infection of birds (at any age) by an airborne transmission from an infected flock nearby, by fomites, or by contaminated wild birds (since peridomestic wild birds such as magpies or house-sparrows for example can be infected).

#### ***M. meleagridis***

A route of possible introduction of the disease would be an infection in a turkey breeder flock since egg-borne infection is the major route of infection: introduction of birds (males or females) infected by *M. meleagridis* or artificial inoculation of hens by contaminated semen.

Another route would be infection of young turkeys (before sexual maturity) by an airborne transmission from wild birds but this possibility seems highly unlikely.

### Parameter 4 – Number of animal moving and/or shipment size

#### ***M. gallisepticum***

Member States import hatching eggs and day-old chicks from the US and Canada. However, they apply the European Union (EU) import rules in Regulation (EC) 798/2008<sup>1</sup> and have equivalent measures for *Mycoplasma* in place (see certificates in that regulation).

#### ***M. meleagridis***

Turkey production in the EU is concentrated in a small number of MSs. Five countries (Germany, France, Italy, the UK and Poland) produce more than 80% of all EU turkey meat. The number of companies in the turkey primary breeding sector is even smaller as only a few companies in a limited number of MSs are involved (A.V.E.C., 2015). Movements/shipments of animals from non-European countries are probably not significant and subjected to control measures.

### Parameter 5 – Duration of infectious period in animal and/or commodity

#### ***M. gallisepticum*/*M. meleagridis***

See Section 3.1.1.5 Parameters 1 and 4.

### Parameter 6 – List of control measures at border (testing, quarantine, etc.)

#### ***M. gallisepticum***

Measures concerning *M. gallisepticum* are described in the Council Directive 2009/158/EC<sup>2</sup> and related import rules in Commission Regulation (EC) No 798/2008 on animal health conditions governing intra-Community trade in, and imports from third countries of, poultry and hatching eggs; with the Commission Decision 2011/214/EU<sup>3</sup> amending annexes II to IV of this directive:

- The presence of infection must be tested by validated serological and/or bacteriological and/or molecular tests. The presence of airsacculitis lesions in day-old birds suggests that a *Mycoplasma* infection is present and must be investigated.
- Samples for testing for the presence of *M. gallisepticum* infection must be taken, as appropriate, from blood, day-old chicks or turkey poults, sperm or swabs taken from the trachea, the cloaca or air sacs.

<sup>1</sup> Commission Regulation (EC) No 798/2008 of 8 August 2008 laying down a list of third countries, territories, zones or compartments from which poultry and poultry products may be imported into and transit through the Community and the veterinary certification requirements (Text with EEA relevance). OJ L 226, 23.8.2008, p. 1–94.

<sup>2</sup> Council Directive 2009/158/EC of 30 November 2009 on animal health conditions governing intra-Community trade in, and imports from third countries of, poultry and hatching eggs. OJ L 343, 22.12.2009, p. 74–113.

<sup>3</sup> 2011/214/EU: Commission Decision of 1 April 2011 amending Annexes II to IV to Council Directive 2009/158/EC on animal health conditions governing intra-Community trade in, and imports from third countries of, poultry and hatching eggs. OJ L 90, 6.4.2011, p. 27–49.

- Tests for detecting *M. gallisepticum* must be performed on a representative sample (usually including 60 animals) in order to allow continuous surveillance of the infection during rearing and laying, namely just before the start of laying and every 3 months thereafter.

### ***M. meleagridis***

Measures concerning *M. meleagridis* are described in the Council Directive 2009/158/EC on animal health conditions governing intra-Community trade in, and imports from third countries of, poultry and hatching eggs; with the Commission Decision 2011/214/EU amending annexes II to IV of this directive:

- The presence of infection must be tested by validated serological and/or bacteriological and/or molecular tests. The presence of airsacculitis lesions in day-old turkey poults suggests that a *Mycoplasma* infection is present and must be investigated.
- Samples for testing for the presence of *M. meleagridis* infection must be taken, as appropriate, from blood, day-old turkey poults, sperm, or swabs taken from the trachea, the choanae, cloaca or air sacs and in particular for the detection of *M. meleagridis* samples must be taken from oviduct and penis of turkeys.
- Tests for detecting *M. meleagridis* must be performed on a representative sample (usually 60 animals) in order to allow continuous surveillance of the infection during rearing and laying, namely just before the start of laying and every 3 months thereafter.

#### Parameter 7 – Presence and duration of latent infection and/or carrier status

### ***M. gallisepticum*/*M. meleagridis***

See Section 3.1.1.5. Parameters 2 and 3.

### **3.1.1.8. Article 7(a)(viii) The existence of diagnostic and disease control tools**

#### *Diagnostic tools*

#### Parameter 1 – Existence of diagnostic tools

### ***M. gallisepticum***

Several diagnostic tools are available to detect *M. gallisepticum* infections:

- Bacteriological isolation and identification (Raviv and Ley, 2013): *M. gallisepticum* can be isolated on specific media (commercially available or laboratory-prepared). However, isolation can be difficult: specific growth requirements, slow growth of *M. gallisepticum*, and poor recovery in case of contamination by other bacteria or bad transport conditions. Isolation is therefore mainly performed by specialised laboratories and might require up to 3–4 weeks for detectable growth.
- Serology: Antibodies can be detected from birds hatched from infected eggs in 3 weeks and from birds infected by contact in 2–3 weeks. Detection is usually performed by rapid serum agglutination (RSA) or enzyme-linked immunosorbent assay (ELISA). Several commercial antigens (for RSA) and tests (for ELISA) are available in EU.
- PCR: DNA-based detection has been developed for direct detection of *M. gallisepticum*. These techniques are rapid and highly specific and sensitive, and they allow detection of *M. gallisepticum* in contaminated samples or in antibiotic-treated flocks. These tests are however able to detect both viable and non-viable organisms (except with reverse-transcriptase PCR tests based on RNA detection (Tan et al., 2014)). Several conventional and real-time PCR tests have been described (for review see (Raviv and Ley, 2013)). Several PCR kits for the detection of *M. gallisepticum* are available in EU.

### ***M. meleagridis***

Several diagnostic tools are available to detect *M. meleagridis* infections:

- Bacteriological isolation and identification: like other avian *Mycoplasma* species, *M. meleagridis* can be isolated on specific media (commercially available or laboratory-prepared). However, isolation can be difficult: specific growth requirements, slow growth of *M. meleagridis*, poor recovery in case of contamination by other bacteria (especially with cloacal samples) or bad transport conditions. Isolation is therefore mainly performed by specialised laboratories.



- Serology: Antibodies can be detected from poult hatched from infected eggs in 3 weeks and from turkeys infected by contact in 4–5 weeks (Chin, 2013). Detection is usually performed by RSA (OIE, 2008) or ELISA (Dufour-Gesbert et al., 2001; Chin, 2013). Several commercial antigens (for RSA) and tests (for ELISA) are available in EU.
- PCR: DNA-based detection has been developed for direct detection of *M. meleagridis*. These techniques are rapid, specific and sensitive, and they allow detection of *M. meleagridis* in contaminated samples (such as cloacal swabs) or in antibiotic-treated flocks. These tests are however able to detect both viable and non-viable organisms. Several conventional PCR tests and one real-time PCR test have been described (for review see (Chin, 2013)).

### Control tools

#### Parameter 2 – Existence of control tools

##### ***M. gallisepticum***

Several disease control tools can be used:

- Because *M. gallisepticum* is egg-transmitted, maintaining commercial flocks free of *M. gallisepticum* is only possible by starting with infection-free breeding flocks reared with adequate biosecurity measures to avoid introduction of the organism (Levisohn and Kleven, 2000). Test and slaughter of *M. gallisepticum*-positive animals in these flocks is effective for disease control since most of breeding stocks are free of *M. gallisepticum* infections;
- Purchase of uninfected eggs (for hatcheries) and birds (for producers) as one the major transmission route is the vertical one, by egg-borne transmission; infected eggs result in widespread distribution of infection and increased risk of further vertical or horizontal transmission;
- All-in/all-out production (to avoid contamination of young birds by older ones in another building on the same site);
- Biosecurity measures (to avoid contamination of birds by airborne transmission from another farm or by horizontal transmission by contaminated materials or clothes from another farm);
- Where control of *M. gallisepticum* infection is more difficult, vaccination of flocks can be performed (Levisohn and Kleven, 2000; Raviv and Ley, 2013). Vaccination programmes are applied in commercial laying hen-flocks, but not in broiler flocks (short-lived birds) or turkey flocks (no commercial vaccine available for the moment). However several studies performed on different commercial vaccines (inactivated or live-attenuated vaccines) showed that vaccination can reduce expression of clinical signs and lesions, but cannot prevent colonisation with a virulent strain (Raviv and Ley, 2013);
- Decrease of the severity of clinical signs and better performances have also been observed with antimicrobial treatments (for review see (Raviv and Ley, 2013)). However, even if treatments are able to decrease the bacterial load, persistence of *M. gallisepticum* has been described (Raviv and Ley, 2013) even without resistance selection (Reinhardt et al., 2005).

##### ***M. meleagridis***

Several disease control tools can be used:

- Purchase of uninfected eggs (for hatcheries) and poult (for producers) as the major transmission route is the vertical one, by egg-borne transmission; infected eggs result in widespread distribution of infection and increased risk of further vertical transmission;
- All-in/all-out production (to avoid contamination of young birds by older ones in another building on the same site);
- Biosecurity measures (to avoid contamination of young birds by airborne transmission from another farm, or by horizontal transmission by contaminated materials or clothes from another farm);
- Males may warrant special attention as infected males are particularly prone to transmit infection (*M. meleagridis*-infected semen transmitted to hens through artificial insemination);
- Experimental studies showed that administration of antibiotics into eggs either by dipping or by inoculation were useful methods to reduce the egg-transmission rate and these methods were used in the past for eradication programmes (for review see Chin, 2013);
- Test and slaughter of *M. meleagridis*-positive animals may nowadays be effective for disease control since breeding stocks are free of *M. meleagridis* infections;

- Several antibiotics were tested for their *in vitro* activity against *M. meleagridis* strains, but these studies were performed more than 20 years ago with unstandardised methods (Chin, 2013). No recent data is available on antibiotic treatments against *M. meleagridis* infections.

### 3.1.2. Article 7(b) The impact of diseases

#### 3.1.2.1. Article 7(b)(i) The impact of the disease on agricultural and aquaculture production and other parts of the economy

*The level of presence of the disease in the Union*

Parameter 1 – Number of MSs where the disease is present

##### ***M. gallisepticum***

No precise data have been identified, but *M. gallisepticum* outbreaks are probably present in nearly all member states at low level (sporadic cases) because of control programmes in breeder flocks. For example, the French National Reference Laboratory recorded five reports of *M. gallisepticum* infections in 2016, and isolated two strains from commercial laying hens. But the occurrence is probably underestimated, especially for backyard poultry and production flocks.

##### ***M. meleagridis***

No data have been identified, but the level is probably very low because of control programmes in breeder flocks since the middle of the 1980s. For example, the French National Reference Laboratory did not receive any strains of *M. meleagridis* from France during the last 20 years.

*The loss of production due to the disease*

Parameter 2 – Proportion of production losses (%) by epidemic/endemic situation

##### ***M. gallisepticum***

In broilers, *M. gallisepticum* infections lead to retarded growth with a reduction in weight gain up to 20–30% and to a 10–20% decrease in food conversion efficiency. Clinical signs and lesions are also source of 10–20% of condemnations or downgrading of carcasses in slaughterhouses (Stipkovits and Kempf, 1996).

In breeders and laying hens, *M. gallisepticum* infection may be responsible for a 10–20% decrease in egg production.

Mortality in broilers may range from 1% in uncomplicated disease to as much as 30% in complicated outbreaks with other bacteria or viruses.

##### ***M. meleagridis***

During the early 1980s (when prevalence of *M. meleagridis* infections was very high in all turkey breeders), the cost to the US turkey industry resulting from reduced hatchability due to *M. meleagridis* and the cost of egg treatment to reduce egg-borne transmission of the pathogen was estimated at \$9.4 million per year (Carpenter et al., 1981).

Egg-transmission rates may vary from 10% to 60% (between hens and during the laying season), with a loss of hatchability of 5–6% of *M. meleagridis*-infected fertile eggs (late mortality between 25 and 28 days) (Chin, 2013).

Air-sacculitis, one of the major cause of condemnation of turkeys in the 1960s, was reported to rate 10–25% in *M. meleagridis*-infected flocks.

Skeletal abnormalities observed in 5–10% of the poults hatched from infected eggs can also lead to condemnation at slaughter or to reduced growth rates.

In case of a *M. meleagridis* infection in a breeder flock, even at a low level, semen and eggs cannot be sold (because of control programmes against *M. meleagridis* infections).

#### 3.1.2.2. Article 7(b)(ii) The impact of the disease on human health

##### ***M. gallisepticum***

No zoonotic cases have been recorded. *M. gallisepticum* infects exclusively avian host species as listed in Section 3.1.1.1.

### ***M. meleagridis***

No zoonotic cases have been recorded. *M. meleagridis* infects a very narrow range of exclusively avian host species as listed in Section 3.1.1.1.

#### **3.1.2.3. Article 7(b)(iii) The impact of the disease on animal welfare**

Parameter 1 – Severity of clinical signs at case level and related level and duration of impairment

##### ***M. gallisepticum***

Infection with *M. gallisepticum* may have a wide diversity of clinical manifestations. Infection alone is often mild to subclinical in chickens, but may cause respiratory disease in turkeys (sinusitis, respiratory distress, listlessness) (Stipkovits and Kempf, 1996; Levisohn and Kleven, 2000; Raviv and Ley, 2013). Feed consumption is reduced and birds lose weight. In commercial layer, flocks egg production is decreased and maintained at a lowered level. Intercurrent infections (*E. coli*, avian viruses) may cause severe outbreaks with high morbidity and mortality. Partial to complete closure of the eyes sometimes results from severe swelling of the sinuses.

A high level of morbidity and mortality was also observed in wild house finches in the USA with severe conjunctivitis (Raviv and Ley, 2013).

Respiratory distress and intercurrent infections causing morbidity and mortality can cause pain and distress to infected animals and therefore decrease their welfare.

##### ***M. meleagridis***

*M. meleagridis* do not induce significant clinical signs and commonly occur as a silent infection in adult turkeys (Chin, 2013). Young turkeys are more sensitive than older birds to *M. meleagridis* infections, with development of air-sacculitis, but respiratory signs are rarely observed. *M. meleagridis*-induced airsacculitis can be more severe in case of co-infection with *Mycoplasma iowae* or *E. coli* (Chin, 2013) and therefore decrease animal welfare.

Severe clinical signs are mainly associated with egg-borne infection and are observed in young birds: irreversible skeletal abnormalities such as bowing, twisting and shortening of the tarsometatarsal bones, wry necks and hock joint swelling (Cardona and Bickford, 1993; Stipkovits and Kempf, 1996; Chin, 2013). These signs can lead to reduction in growth rate, may cause pain to animals and decrease animal welfare. Moreover, they can also lead to indirect mortality by cannibalism of affected birds.

#### **3.1.2.4. Article 7(b)(iv) The impact of the disease on biodiversity and the environment**

*Biodiversity*

Parameter 1 – Endangered wild species affected: listed species as in CITES and/or IUCN list

##### ***M. gallisepticum***

Very little information is available on *M. gallisepticum* infections in endangered wild bird species. Hagen et al. (2002) found a low seroprevalence against *M. gallisepticum* in lesser prairie-chickens in Kansas but could not rule out the hypothesis of a non-specific reaction. Straub et al. (2015) reported a 1% seroprevalence for *M. gallisepticum* in free-flying Californian condors and a 57% seroprevalence for captive birds. However, these authors did not report observation of clinical signs on these birds. No other wild endangered species affected by *M. gallisepticum* infections were reported.

##### ***M. meleagridis***

No wild endangered species affected by *M. meleagridis* infections. As seen in Section 3.1.1.1 Parameters 1 and 5, very few studies evidenced a *M. meleagridis* infection in wild species, and if so, without clinical signs.

Parameter 2 – Mortality in wild species

##### ***M. gallisepticum***

According to several studies, *M. gallisepticum* can cause mortality in house finches (Luttrell et al., 2001; Sydenstricker et al., 2006; Raviv and Ley, 2013). Other species were found contaminated with sometimes expression of clinical signs, but without marked mortality.

### ***M. meleagridis***

No mortality recorded in wild species as *M. meleagridis* is very host specific and do not lead to direct mortality (except late embryo mortality in turkey eggs).

#### *Environment*

#### Parameter 3 – Capacity of the pathogen to persist in the environment and cause mortality in wildlife

### ***M. gallisepticum***

This pathogen is able to persist in the environment for several hours to several days depending on substrate, pH, temperature and humidity (see Section 3.1.1.5 Parameter 4). Contamination of wild naïve birds by *M. gallisepticum*-infected fomites (bird feeders for example) was reported (Dhondt et al., 2007).

### ***M. meleagridis***

The pathogen is probably able to persist in the environment for several days like other avian *Mycoplasma* species (see Section 3.1.1.5 Parameter 4), but without causing mortality in wildlife.

### **3.1.3. Article 7(c) Its potential to generate a crisis situation and its potential use in bioterrorism**

They are not listed.

### **3.1.4. Article 7(d) The feasibility, availability and effectiveness of the following disease prevention and control measures**

#### **3.1.4.1. Article 7(d)(i) Diagnostic tools and capacities**

#### *Availability*

#### Parameter 1 – Officially/internationally recognised diagnostic tool, OIE certified

### ***M. gallisepticum***

Diagnostic tools for *M. gallisepticum* are listed in the OIE terrestrial manual: RSA tests, isolation by culture and one PCR test are described in detail (OIE, 2008). The ELISA technology is not described in detail since several MG kits are available commercially.

Different antigens (for RSA) or kits (PCR, real-time PCR, ELISA) are used in Europe for the detection of *M. gallisepticum* in diagnostic and research laboratories.

### ***M. meleagridis***

Diagnostic tools for *M. meleagridis* are not listed in the OIE terrestrial manual but RSA tests and isolation by culture can be used in the same conditions as those described for *M. gallisepticum* and *Mycoplasma synoviae* (OIE, 2008).

No PCR or ELISA tests is known to have been officially/internationally recognised, but different tests or kits are used for the detection of *M. meleagridis* in diagnostic and research laboratories (for review see Chin, 2013).

#### *Effectiveness*

#### Parameter 2 – Se and Sp of diagnostic test

### ***M. gallisepticum*/*M. meleagridis***

Serological procedures are useful for flock monitoring in *M. gallisepticum*/*M. meleagridis* monitoring programmes: screening for infection is usually accomplished by RSA test or by ELISA. RSA test is highly efficient in detecting immunoglobulin M (IgM) antibodies, which is the first class of immunoglobulins produced in response to infection (first positive birds 1–2 weeks after infection), before IgY which are detected by RSA and ELISA (3–4 weeks after infection). Moreover, RSA test is quick, relatively inexpensive and sensitive. However, non-specific reactors may occur in some flocks infected with *M. synoviae* (due to cross-reactive antigens) or recently vaccinated with oil-emulsion vaccines. RSA positive results have therefore to be confirmed either by ELISA, culture or PCR tests. ELISA tests are in general slightly less sensitive but more specific than RSA tests (no cross-reaction).

Isolation and identification of the bacterium is the reference standard for *M. gallisepticum*/*M. meleagridis* diagnosis. However, culture is not a sensitive method, it is time-consuming and cannot be performed by non-specialised laboratories. PCR tests, which are highly sensitive (less than 1 CFU/ml for the most sensitive ones) and specific, represent a rapid alternative (positive results in hours instead of days or weeks) to traditional culture methods and are used to confirm serological results. However, PCR results should be interpreted with caution since detection of *M. gallisepticum*/*M. meleagridis* DNA represent the presence of viable organisms.

#### *Feasibility*

Parameter 3 – Type of sample matrix to be tested (blood, tissue, etc.)

#### ***M. gallisepticum***

Samples for testing for the presence of *M. gallisepticum* infection must be taken, as appropriate, from blood or vitellus (for RSA and ELISA tests), day-old chicks or turkey poults, sperm or swabs (or pieces of tissue) taken from trachea, choanae, cloaca, air sacs, oviduct and penis (for bacterial isolation or PCR tests).

#### ***M. meleagridis***

Samples for testing for the presence of *M. meleagridis* infection must be taken, as appropriate, from blood (for RSA and ELISA tests), day-old turkey poults, sperm, or swabs (or pieces of tissue) taken from trachea, choanae, cloaca, air sacs, oviduct and penis of turkeys (for bacterial isolation or PCR tests).

### **3.1.4.2. Article 7(d)(ii) Vaccination**

#### *Availability*

Parameter 1 – Types of vaccines available on the market (live, inactivated, DIVA, etc.)

#### ***M. gallisepticum***

Vaccination may be the most practical option in infected multiage commercial layer facilities when biosecurity measures fail to prevent the infection.

Both killed vaccines (bacterins) and live vaccines are currently in commercial use worldwide (Whithear, 1996; Levisohn and Kleven, 2000; Raviv and Ley, 2013; Jacob et al., 2014).

Bacterins usually contain an oil emulsion adjuvant to stimulate the bird's immune system. They have the advantage to be non-infectious but they are expensive to use (requiring large amounts of antigen and necessity of handling birds individually for intramuscular administration of the vaccine).

The F strain (Poulvac<sup>®</sup> Myco F, Zoetis for example) is considered to be of low to moderate virulence and transmissibility, but can induce respiratory signs in broilers and in turkeys. It persists in the upper respiratory tract of chickens for the flock's life and was shown to be transmitted between flocks.

The 6/85 strain (Nobilis MG 6/85, MSD Animal Health for example) is a modified *M. gallisepticum* strain which is avirulent for chickens and turkeys, and is not easily transmitted horizontally. This vaccine is authorised for use in the EU.

The ts-11 *M. gallisepticum* strain (Vaxsafe<sup>®</sup> MG strain ts-11, Bioproperties for example) was developed by chemical mutagenesis and was selected as a temperature-sensitive mutant. It is avirulent for chickens and turkeys, has a low propensity to spread from bird to bird and persists for the life of the flock in the upper respiratory tract.

Because of their superior safety characteristics of avirulence and low potential for spread to nearby flocks, both ts-11 and 6/85 vaccines are considered to be preferable to F strain.

#### ***M. meleagridis***

No vaccine available on the market.

Parameter 2 – Availability/production capacity (per year)

#### ***M. gallisepticum***

All vaccine strains described above are available, but only the 6/85 strain is authorised in EU and currently used in commercial layer farms. This vaccine strain is commercialised by MSD Animal Health (Intervet).



Autologous vaccines (bacterins) are also used in commercial laying flocks in EU, but their production is limited since large amounts of strains are necessary for the vaccination of one flock. Several laboratories in EU are specialised in production of these vaccines.

***M. meleagridis***

Not applicable.

*Effectiveness*

Parameter 3 – Field protection as reduced morbidity (as reduced susceptibility to infection and/or to disease)

***M. gallisepticum***

Inactivated *M. gallisepticum* bacterins have been shown to provide limited protection against field strains, to reduce the shedding of *M. gallisepticum*, but not to reduce the horizontal transmission of the bacterium between laying hens.

Vaccination with the F strain has been shown to reduce egg production losses, mortality and antibiotic requirement in commercial layers and to induce resistance against infection by wild-type strains or challenge infection. F strain can displace endemic strains but can also maintain itself in multi-age flocks after vaccination is discontinued.

Ts-11 and 6/85 strains are less protective, but are able to reduce losses associated with *M. gallisepticum* wild-type infections and confer significant protection against development of air-sac lesions.

***M. meleagridis***

Not applicable.

Parameter 4 – Duration of protection

***M. gallisepticum***

For all vaccine strains, immunity develops within 3–4 weeks.

F strain- and ts-11-vaccinated birds remain carriers of the strain for life and immunity lasts through the laying season.

The 6/85 strain can be detected in the upper respiratory tract for 4–8 weeks after vaccination and immunity was shown to last for at least 24 weeks.

***M. meleagridis***

Not applicable.

*Feasibility*

Parameter 5 – Way of administration

***M. gallisepticum***

Bacterins are administered by intramuscular injections.

The F strain can be administered at 8–14 weeks of age by several routes including intraocular and intranasal, and by coarse spray. Vaccination may induce mild respiratory signs and lesions.

The recommended route of administration for the 6/85 strain is by aerosol spray from 6 weeks of age. Two vaccinations can be performed (6 and 16 weeks) to improve efficacy of vaccination.

The ts-11 strain is administered by eye drop between 4 and 16 weeks of age.

***M. meleagridis***

Not applicable.

**3.1.4.3. Article 7(d)(iii) Medical treatments**

*Availability*

Parameter 1 – Types of drugs available on the market

***M. gallisepticum***

Several antibiotics can be used to treat mycoplasmal infections: macrolides, lincosamides, aminoglycosides, tetracyclines, fluoroquinolones and pleuromutilins.

***M. meleagridis***

Several antibiotics can be used to treat mycoplasma infections: macrolides, lincosamides, aminoglycosides, tetracyclines, fluoroquinolones and pleuromutilins. Several antibiotics were tested for their *in vitro* activity against *M. meleagridis* strains, but these studies were performed more than 20 years ago with unstandardised methods (Chin, 2013).

Parameter 2 – Availability/production capacity (per year)***M. gallisepticum*/*M. meleagridis***

All these antibiotic families are produced to treat animals against several diseases, including other mycoplasma infections and their availability should not be a problem.

*Effectiveness*Parameter 3 – Therapeutic effects on the field (effectiveness)***M. gallisepticum***

Most strains of *M. gallisepticum* are susceptible *in vitro* to a number of broad-spectrum antibiotics, including macrolides, tetracyclines, fluoroquinolones and others but not to penicillins or those that act on the cell wall (intrinsic resistance of the class Mollicutes) (Levisohn and Kleven, 2000; Bébéar and Kempf, 2005; Raviv and Ley, 2013). However, *M. gallisepticum* can develop resistance and cross-resistance to antibiotics commonly used in field conditions (Reinhardt et al., 2002; Bébéar and Kempf, 2005).

Tylosin or tetracyclines have been commonly used to reduce egg transmission or as prophylactic treatment to prevent respiratory disease in broilers and turkeys (Levisohn and Kleven, 2000; Raviv and Ley, 2013). Antibiotics may alleviate the clinical signs and lesions, reduce egg transmission and production losses, but do not eliminate infection. Several studies have shown persistence of *M. gallisepticum* after antimicrobial treatments (Raviv and Ley, 2013), but this persistence is not always linked to antibiotic resistance (Reinhardt et al., 2005). Medication should not be regarded as a long-term solution to the problem, but only as a method for short-term amelioration of signs and economic effect in poultry flocks.

***M. meleagridis***

No recent data is available on antibiotic treatments against *M. meleagridis* infections. However, avian mycoplasmas are usually susceptible to macrolides, lincosamides, aminoglycosides, tetracyclines, fluoroquinolones and pleuromutilins (Hannan, 2000; Wang et al., 2001; Gerchman et al., 2008). Recent studies evidenced *M. gallisepticum* and *Mycoplasma synoviae* resistant strains (Gerchman et al., 2008, 2011; Lysnyansky et al., 2013, 2015) and development of resistance was also recorded *in vitro* for *M. gallisepticum*, *M. synoviae* and *M. iowae* (Gautier-Bouchardon et al., 2002), and *in vivo* for *M. gallisepticum* and *M. synoviae* (Le Carrou et al., 2006; Gerchman et al., 2011).

Antibiotic treatments against susceptible strains of avian mycoplasmas lead to a decrease or disappearance of clinical signs but persistence of mycoplasmas is often observed (Reinhardt et al., 2005; Le Carrou et al., 2006).

*Feasibility*Parameter 4 – Way of administration***M. gallisepticum***

Antibiotic treatments are usually administered via drinking water (for flock treatments).

***M. meleagridis***

Antibiotic treatments are usually administered in drinking water (for flock treatments), but for *M. meleagridis*, as an egg-borne infection, treatments can also be administered by egg-dipping or inoculation into eggs (Chin, 2013).

#### 3.1.4.4. Article 7(d)(iv) Biosecurity measures

##### Availability

##### Parameter 1 – Available biosecurity measures

##### ***M. gallisepticum*/*M. meleagridis***

Most of breeder flocks have strong biosecurity measures such as:

- All-in/all-out production, allowing time for cleaning and disinfection of buildings and equipment between two flocks;
- In multiage farms, traffic through poultry houses should always flow from younger to older birds;
- Introduction of new animals, eggs or semen (potentially infected, healthy carrier) should be avoided without testing and/or quarantine;
- Visitors should be kept to a minimum and any visitors should wear protective covering such as boots, coveralls and headgear after hand-washing;
- Visits to other poultry farms should be limited unless absolutely necessary;
- Disinfection of transport trucks and loadout materials;
- All animals should be kept out of poultry houses (cats, dogs, wild animals and birds, insects) and sound rodent and pest control should be implemented;
- If possible, breeder farms should be far enough from production farms to avoid airborne contaminations;
- Environment of birds should be maximised (dry litter, good ventilation and temperature) to avoid development of diseases.

Requirements for establishments (Article 6 and conditions in Annex II) do not only apply to breeding poultry but also to productive poultry when traded between MSs.

##### Effectiveness

##### Parameter 2 – Effectiveness of biosecurity measures in preventing the pathogen introduction

##### ***M. gallisepticum***

These biosecurity measures have been applied for more than 30 years, together with control programmes (regularly performed diagnostic tests) to avoid contamination of chicken and turkey breeder flocks by *M. gallisepticum* after eradication programmes implemented in the 1980s. Since only sporadic cases of *M. gallisepticum* infections are reported in EU, these biosecurity measures are effective in preventing introduction in breeder flocks.

##### ***M. meleagridis***

These biosecurity measures have been applied for more than 30 years, together with control programmes (regularly performed diagnostic tests) to avoid contamination of turkey breeder flocks by *M. meleagridis* after eradication programmes in the 1980s. The lack of data about *M. meleagridis* prevalence in EU is a good indicator of the effectiveness of these measures.

##### Feasibility

##### Parameter 3 – Feasibility of biosecurity measures

##### ***M. gallisepticum*/*M. meleagridis***

Most of these biosecurity measures are easy to put in place in all farms. All European breeder flocks provide *M. gallisepticum*/*M. meleagridis*-free eggs.

#### 3.1.4.5. Article 7(d)(v) Restrictions on the movement of animals and products

##### Availability

##### Parameter 1 – Available movement restriction measures

##### ***M. gallisepticum***

The only restriction movement measure available for *M. gallisepticum* is applied for breeder flocks and hatcheries (as described in the Council Directive 2009/158/EC on animal health conditions):

positive flocks cannot sell animals or eggs, and hatcheries have to buy *M. gallisepticum*-free certified eggs.

### ***M. meleagridis***

The only restriction movement measure available for *M. meleagridis* is applied for breeder flocks (as described in the Council Directive 2009/158/EC on animal health conditions): positive flocks cannot sell animals or eggs.

#### *Effectiveness*

#### Parameter 2 – Effectiveness of restriction of animal movement in preventing the between farm spread

### ***M. gallisepticum***

Restriction of animal and egg movement for *M. gallisepticum* positive flocks is preventing vertical spread of disease since this measure prevents contamination of farms free of *M. gallisepticum*. However, *M. gallisepticum* can also be readily transmitted horizontally by contaminated birds and fomites (Stipkovits and Kempf, 1996; Raviv and Ley, 2013). This transmission is not entirely taken into account in the current measures since surveillance measures and restriction of animal movement are not compulsory in production flocks. Sporadic cases recorded in breeder flocks or production flock may find their origin in cases from backyard poultry or production flocks (commercial laying hen flocks for example) of unknown status for *M. gallisepticum*.

### ***M. meleagridis***

Restriction of animal and egg movement for *M. meleagridis*-positive flocks is preventing disease spread since this measure prevents contamination of farms free of *M. meleagridis*. This mycoplasma infection is mainly transmitted vertically not only through contaminated egg, but also by contaminated poults (young turkeys being more sensitive to infection than adults).

#### *Feasibility*

#### Parameter 3 – Feasibility of restriction of animal movement

### ***M. gallisepticum***

This measure is already applied in case of outbreaks of *M. gallisepticum* infection in breeder flocks. Since this disease is only sporadically observed nowadays, it is not a highly infectious disease and it is mainly transmitted vertically, this measure should apply to very few flocks if the outbreak is detected early by regularly performed diagnostic tests.

### ***M. meleagridis***

This measure is already applied in case of outbreaks of *M. meleagridis* infection. As this disease is rarely observed nowadays, is not a highly infectious disease and is mainly transmitted vertically, this measure should apply to very few flocks if the outbreak is detected early by regularly performed diagnostic tests.

#### **3.1.4.6. Article 7(d)(vi) Killing of animals**

#### *Availability*

#### Parameter 1 – Available methods for killing animals

### ***M. gallisepticum*/*M. meleagridis***

Since *M. gallisepticum*/*M. meleagridis* is not a zoonotic and highly contagious agent, infected birds can be killed in slaughterhouses and killed animals can enter human consumption if they don't harbour clinical signs or lesions.

Animals may also be killed on farm (electrical stunning and bleeding in trucks, CO<sub>2</sub> culling in barns, or lethal injection).

## Parameter 2 – Effectiveness of killing animals (at farm level or within the farm) for reducing/stopping spread of the disease

### ***M. gallisepticum*/*M. meleagridis***

In general, Mycoplasma infections, once established, are known to persist for all the flock's life, even after antibiotic treatments. Killing animals of positive flocks is a good measure to remove this permanent source of infection for other lots or flocks. However, since *M. gallisepticum*/*M. meleagridis* infection is not a highly infectious disease (like avian influenza for example), massive culling of birds is not necessary. Killing can be applied at farm level or at flock level (within a farm) if good biosecurity measures can ensure the non-spread of *M. gallisepticum* to other flocks in or outside the farm.

#### *Feasibility*

### Parameter 3 – Feasibility of killing animals

#### ***M. gallisepticum***

This measure is already applied in case of outbreaks of *M. meleagridis* infection in breeder flocks. As this disease is rarely observed in these flocks, this measure should apply to very few flocks if the outbreak is detected early by regularly performed diagnostic tests.

It would be more difficult to apply this measure to production flocks and backyard poultry flocks to remove all sources of infection. The first step would be to implement an epidemiological survey to have recent data about the real prevalence of *M. gallisepticum* infections in poultry flocks in the EU. *M. gallisepticum* may be endemic in several multiage commercial laying hen farms.

#### ***M. meleagridis***

This measure is already applied in case of outbreaks of *M. meleagridis* infection. As this disease is rarely observed nowadays and is mainly transmitted vertically, this measure should apply to very few flocks if the outbreak is detected early by regularly performed diagnostic tests.

### **3.1.4.7. Article 7(d)(vii) Disposal of carcasses and other relevant animal by-products**

#### *Availability*

### Parameter 1 – Available disposal option

#### ***M. gallisepticum***

Since *M. gallisepticum* is not a zoonotic and highly contagious agent, adult infected birds can be killed in slaughterhouses and carcasses of birds without clinical signs or lesions can enter human consumption.

Infected eggs and day-old birds should be destroyed.

#### ***M. meleagridis***

Since *M. meleagridis* is not a zoonotic and highly contagious agent, adult infected turkeys can be killed in slaughterhouses and carcasses can enter human consumption if birds do not harbour clinical signs or lesions.

Infected eggs and poults should be destroyed.

#### *Effectiveness*

### Parameter 2 – Effectiveness of disposal option

#### ***M. gallisepticum*/*M. meleagridis***

These disposal options are effective in eliminating the risk of spread of the disease to other flocks by removing one of the major sources of transmission (egg-borne and airborne, or by artificial insemination).

#### *Feasibility*

### Parameter 3 – Feasibility of disposal option

#### ***M. gallisepticum***

These disposal options are already applied in field conditions in case of a *M. gallisepticum* outbreak in breeder flocks (chicken and turkeys) in EU.



## ***M. meleagridis***

These disposal options are already applied in field conditions in case of a *M. meleagridis* outbreak in EU.

### **3.1.5. Article 7(e) The impact of disease prevention and control measures**

#### **3.1.5.1. Article 7(e)(i) The direct and indirect costs for the affected sectors and the economy as a whole**

Parameter 1 – Cost of control (e.g. treatment/vaccine, biosecurity)

#### ***M. gallisepticum***

Most of biosecurity measures are already implemented in breeder farms, even in production farms (broilers, meat turkeys, laying hens).

Vaccination cannot be applied for broilers (short life) and turkeys (no commercial vaccine available) as already seen in Section 3.1.4.2. Vaccination is not performed in breeder flocks to avoid RSA and ELISA positive reaction (due immunological response to the vaccine strain) during surveillance programmes. Vaccination is performed in commercial layer flocks, especially with multiage systems. The MG 6/85 vaccine strain is at an average of €100 for 1,000 doses.

Treatments with antibiotics do not seem to be the best option for this disease as it will not ensure the non-transmission of the infection by the vertical way (even if antibiotic treatment will lower clinical signs and improve flock's performance, it is very unlikely that adult birds will not remain carriers and sources of infection). Medication is just a method for short-term amelioration of signs and economic effect in poultry flocks. Commercial layer flocks can only be treated with oxytetracycline or tylosine: a treatment with oxytetracyclin is less expensive than with Tylan (5–10€ vs 50–60€ for 200 L of drinking water).

#### ***M. meleagridis***

Most of biosecurity measures are already implemented in farms, even in production farms.

As already seen in Sections 3.1.4.2 and 3.1.4.3, there are no vaccine available and treatments with antibiotics do not seem to be the best option for this disease as it will not ensure the non-transmission of the infection by the vertical way (even if antibiotic treatment will lower clinical signs, it is very unlikely that adult birds will not remain carriers and sources of infection).

Parameter 2 – Cost of eradication (culling, compensation)

#### ***M. gallisepticum***

Eradication programmes were applied more than 30 years ago when *M. gallisepticum* infections occurred in many chicken and turkey flocks. Culling is now just implemented in *M. gallisepticum* positive breeder farms to avoid disease spread to production farms and other breeder farms and cost will be therefore limited.

If a *M. gallisepticum* infection is detected in adult chickens or turkeys, these birds can be sent to slaughterhouses and their carcasses can enter human consumption (if no clinical signs or lesions). The cost of eradication in this case should mainly take into account the losses due to the non-production of hatching eggs by the killed adults. To give an idea of compensations that could be paid to farmers, the example of *Salmonella* infections can be taken: each European country fixed the amount of compensation depending on the age of the animals at the date of disposal and type of animal (future breeding or breeding chickens or turkeys, males or females, for meat or commercial egg production). For France, see the NOR AGRG0927983A (turkeys), NOR AGRG0803839A (broilers) and the NOR AGRG0803847A (laying hens) decrees as examples on [www.legifrance.gouv.fr](http://www.legifrance.gouv.fr).

#### ***M. meleagridis***

Eradication programmes were applied more than 30 years ago when *M. meleagridis* infections occurred in almost all turkey flocks. Culling is now just implemented in *M. meleagridis*-positive breeder farms to avoid disease spread to production farms and other breeder farms and cost will be therefore limited.

If a *M. meleagridis* infection is detected in adult turkeys, these birds can be sent to slaughterhouses and their carcasses (without clinical signs or lesions) can enter human consumption.

The cost of eradication in this case should mainly take into account the losses due to the non-production of hatching eggs by the killed adults.

#### Parameter 3 – Cost of surveillance and monitoring

##### ***M. gallisepticum***

The Council Directive 2009/158/EC and the Commission Decision 2011/214/EU fixed the conditions for surveillance and monitoring of *M. gallisepticum* infections in chicken and turkey flocks: 60 samples per flock just before the start of the laying period and every 3 months thereafter, tested by validated serological, bacteriological or molecular tests.

The average costs per sample of the various analyses are: 0.30–1€ for RSA tests, 2–3€ for ELISA, 7–20€ for PCR (depending if samples are pooled per 3 or not) and 30–50€ for isolation by culture and identification by PCR.

##### ***M. meleagridis***

The Council Directive 2009/158/EC and the Commission Decision 2011/214/EU fixed the conditions for surveillance and monitoring of *M. meleagridis* infections in turkey flocks: 60 samples per flock just before the start of the laying period and every 3 months thereafter, tested by validated serological, bacteriological or molecular tests.

The average costs per sample of the various analyses are: 0.75–1€ for RSA tests, 2–3€ for ELISA, 7–20€ for PCR (depending if samples are pooled per 3 or not) and 30–50€ for isolation by culture and identification by PCR.

#### Parameter 4 – Trade loss (bans, embargoes, sanctions) by animal product

##### ***M. gallisepticum*/*M. meleagridis***

As *M. gallisepticum* is not zoonotic and not highly infectious, sanctions are just applied at the farm level (eggs and birds from a *M. gallisepticum*/*M. meleagridis* positive farm cannot be sold and exported) and not at the country level.

#### Parameter 5 – Importance of the disease for the affected sector (% loss or € lost compared to business amount of the sector)

##### ***M. gallisepticum***

*M. gallisepticum* is considered as the most pathogenic and economically significant mycoplasmal pathogen of poultry with condemnation at slaughter, downgrading of carcasses, reduced feed and egg production efficiency, increased medication costs (Stipkovits and Kempf, 1996; Raviv and Ley, 2013). Only few American studies tried to evaluate the real economic impact of this pathogen. The annual economic impact of *M. gallisepticum* infections in the USA was estimated between \$118 and 150 million for the layer industry alone in 1994 (Evans et al., 2005).

Animal products mainly affected by a *M. gallisepticum* outbreak are hatching eggs (in breeder flocks), broilers and meat turkeys and the proportion of commercial egg produced by hens in layer farms. Mohammed et al. in 1987 estimated that an *M. gallisepticum*-infected layer flock without treatment or vaccination produced 12 fewer eggs per hen in the first cycle than an uninfected flock (Mohammed et al., 1987).

With the monitoring and control programmes, losses should be restricted to a few numbers of breeder flocks (outbreak cases mainly due to airborne infection by fomites or contaminated flocks nearby).

##### ***M. meleagridis***

Animal products mainly affected by a *M. meleagridis* outbreak are hatching eggs. But with the surveillance and monitoring programmes, losses should be restricted to a few numbers of flocks.

#### **3.1.5.2. Article 7(e)(ii) The societal acceptance of disease prevention and control measures**

##### ***M. gallisepticum*/*M. meleagridis***

Disease prevention and control measures are already implemented in the EU. Since the impact of a *M. gallisepticum*/*M. meleagridis* outbreak will not have huge consequences as for avian influenza for

example in terms of culling and restriction of animal movements, these measures should not pose a problem of societal acceptance.

### 3.1.5.3. Article 7(e)(iii) The welfare of affected subpopulations of kept and wild animals

#### Parameter 1 – Welfare impact of control measures on domestic animals

##### ***M. gallisepticum***

Control measures are already applied in chicken and turkey breeder flocks and do not have welfare impact on chickens, turkeys or other domestic animals. However, attention should be paid to prevent distress and pain to animals, especially for the manipulation of turkey breeders in case of culling on farm: manipulation of heavy animals by legs or wings can be painful and deleterious.

##### ***M. meleagridis***

Control measures are already applied in turkey breeder flocks and do not have significant welfare impact on turkeys or other domestic animals. However, attention should be paid to prevent distress and pain to animals, especially for the manipulation of turkey breeders in case of culling on farm: manipulation of heavy animals by legs or wings can be painful and deleterious.

#### Parameter 2 – Wildlife depopulation as control measure

##### ***M. gallisepticum*/*M. meleagridis***

Not applicable.

### 3.1.5.4. Article 7(e)(iv) The environment and biodiversity

#### *Environment*

#### Parameter 1 – Use and potential residuals of biocides or medical drugs in environmental compartments (soil, water, feed, manure)

##### ***M. gallisepticum***

Treatments with antibiotics are not the best option for the control of this disease as it will not ensure the non-transmission of the infection by the vertical way and because antibiotic treatment does not completely eliminate *M. gallisepticum* infection in a flock (persistence at low level in the flock and possible re-emergence of the mycoplasma after a stress, a vaccination or a concomitant infection). However, antibiotics are used in production flocks in case of an outbreak with clinical signs and lesions and residuals could therefore be found in environmental compartments (soils and water contaminated by manure from these infected flocks).

##### ***M. meleagridis***

Treatments with antibiotics are not the best option for the control of this disease as it will not ensure the non-transmission of the infection by the vertical way. Possible residuals of antibiotics in environmental compartments would therefore be limited in case of an outbreak.

#### *Biodiversity*

#### Parameter 2 – Mortality in wild species

##### ***M. gallisepticum*/*M. meleagridis***

Not applicable.

## 3.2. Assessment according to Article 5 criteria

This section presents the results of the expert judgement on the criteria of Article 5 of the AHL about avian mycoplasmosis (*M. gallisepticum*, *M. meleagridis*) (Table 1). The expert judgement was based on Individual and Collective Behavioural Aggregation (ICBA) approach described in detail in the opinion on the methodology (EFSA AHAW Panel, 2017). Experts have been provided with information of the disease fact-sheet mapped into Article 5 criteria (see supporting information, Annex A), based on that the experts indicate their Y/N or 'na' judgement on each criterion of Article 5, and the reasoning supporting their judgement.

The minimum number of judges in the judgement was 12. The expert judgement was conducted as described in the methodological opinion (EFSA AHAW Panel, 2017). For details on the interpretation of the questions, see Appendix B of the methodological opinion (EFSA AHAW Panel, 2017).

**Table 1:** Outcome of the expert judgement on the Article 5 criteria for avian mycoplasmosis (*Mycoplasma gallisepticum*, *M. meleagridis*)

<b>Criteria to be met by the disease:</b>		<b>Final outcome</b>
According to AHL, a disease shall be included in the list referred to in point (b) of paragraph 1 of Article 5 if it has been assessed in accordance with Article 7 and meets all of the following criteria		
A(i)	The disease is transmissible	Y
A(ii)	Animal species are either susceptible to the disease or vectors and reservoirs thereof exist in the Union	Y
A(iii)	The disease causes negative effects on animal health or poses a risk to public health due to its zoonotic character	Y
A(iv)	Diagnostic tools are available for the disease	Y
A(v)	Risk-mitigating measures and, where relevant, surveillance of the disease are effective and proportionate to the risks posed by the disease in the Union	Y
<b>At least one criterion to be met by the disease:</b>		
In addition to the criteria set out above at points A(i)–A(v), the disease needs to fulfil at least one of the following criteria		
B(i)	The disease causes or could cause significant negative effects in the Union on animal health, or poses or could pose a significant risk to public health due to its zoonotic character	Y
B(ii)	The disease agent has developed resistance to treatments and poses a significant danger to public and/or animal health in the Union	N
B(iii)	The disease causes or could cause a significant negative economic impact affecting agriculture or aquaculture production in the Union	NC
B(iv)	The disease has the potential to generate a crisis or the disease agent could be used for the purpose of bioterrorism	N
B(v)	The disease has or could have a significant negative impact on the environment, including biodiversity, of the Union	N

Colour code: green = consensus (Yes/No); yellow = no consensus (NC).

### 3.2.1. Non-consensus questions

This section displays the assessment related to each criterion of Article 5 where no consensus was achieved in form of tables (Table 2). The proportion of Y, N or na answers are reported, followed by the list of different supporting views for each answer.

**Table 2:** Outcome of the expert judgement related to criterion 5 B(iii)

Question	Final outcome	Response		
		Y (%)	N (%)	na (%)
B(iii) The disease causes or could cause a significant negative economic impact affecting agriculture or aquaculture production in the Union	NC	83	17	0

NC: non-consensus; number of judges: 12.

#### Reasoning supporting the judgement

##### Supporting Yes:

- Based on the current situation, there is low prevalence, mortality and morbidity, and control measures in place. There may be a potential sporadic occurrence of *M. gallisepticum*.
- It is reported that *M. gallisepticum* infections have a worldwide distribution. They resulted in important flock health problems before implementation of control programmes. Egg-transmission rates may vary from 10% to 60%, with a loss of hatchability of 5–6% of *M. meleagridis*-infected fertile eggs. Air-sacculitis was reported in 10–25% of *M. meleagridis*-infected flocks, and skeletal abnormalities were observed in 5–10% of the chicks hatched from infected eggs.

Supporting No:

- Although theoretically possible, it is unlikely that general biosecurity in the industry would drop to levels to cause a significant negative economic impact in the EU.

### 3.2.2. Outcome of the assessment of avian mycoplasmosis (*M. gallisepticum*, *M. meleagridis*) according to criteria of Article 5(3) of the AHL on its eligibility to be listed

As from the legal text of the AHL, a disease is considered eligible to be listed as laid down in Article 5 if it fulfils all criteria of the first set from A(i) to A(v) and at least one of the second set of criteria from B(i) to B(v). According to the assessment methodology (EFSA AHAW Panel, 2017), a criterion is considered fulfilled when the outcome is 'Yes'. According to the results shown in Table 1, avian mycoplasmosis (*M. gallisepticum*, *M. meleagridis*) complies with all criteria of the first set and with one criterion of the second set, therefore it is considered eligible to be listed as laid down in Article 5 of the AHL.

### 3.3. Assessment according to Article 9 criteria

This section presents the results of the expert judgement on the criteria of Annex IV referring to categories as in Article 9 of the AHL about avian mycoplasmosis (*M. gallisepticum*, *M. meleagridis*) (Tables 3–7). The expert judgement was based on ICBA approach described in detail in the opinion on the methodology. Experts have been provided with information of the disease fact-sheet mapped into Article 9 criteria (see supporting information, Annex A), based on that the experts indicate their Y/N or 'na' judgement on each criterion of Article 9, and the reasoning supporting their judgement. The experts decided to assess some Article 9 criteria separately for the *Mycoplasma* pathogens, on the basis of the evidence available. In this case, in Tables 3, 4 and 5, the outcome of the assessment is reported by pathogen. The minimum number of judges in the judgement was 9. The expert judgement was conducted as described in the methodological opinion (EFSA AHAW Panel, 2017). For details on the interpretation of the questions, see Appendix B of the methodological opinion (EFSA AHAW Panel, 2017).

**Table 3:** Outcome of the expert judgement related to the criteria of Section 1 of Annex IV (category A of Article 9) for avian mycoplasmosis (*Mycoplasma gallisepticum*, *M. meleagridis*) (CI = current impact; PI = potential impact)

Criteria to be met by the disease: The disease needs to fulfil all of the following criteria		Final outcome	
		<i>M. gallisepticum</i>	<i>M. meleagridis</i>
1	The disease is not present in the territory of the Union OR present only in exceptional cases (irregular introductions) OR present in only in a very limited part of the territory of the Union	NC	NC
2.1	The disease is highly transmissible	N	
2.2	There be possibilities of airborne or waterborne or vector-borne spread	na	Y
2.3	The disease affects multiple species of kept and wild animals OR single species of kept animals of economic importance	Y	
2.4	The disease may result in high morbidity and significant mortality rates	N	
<b>At least one criterion to be met by the disease:</b> In addition to the criteria set out above at points 1–2.4, the disease needs to fulfil at least one of the following criteria			
3	The disease has a zoonotic potential with significant consequences on public health, including epidemic or pandemic potential OR possible significant threats to food safety	N	



4 (CI)	The disease has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals	N	
4 (PI)	The disease has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals	Y	NC
5(a) (CI)	The disease has a significant impact on society, with in particular an impact on labour markets	N	
5(a) (PI)	The disease has a significant impact on society, with in particular an impact on labour markets	N	
5(b) (CI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	N	
5(b) (PI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	Y	NC
5(c) (CI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	N	
5(c) (PI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	N	
5(d) (CI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	N	
5(d) (PI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	N	

Colour code: green = consensus (Yes/No); yellow = no consensus (NC); red = not applicable (na), i.e. insufficient evidence or not relevant to judge.

**Table 4:** Outcome of the expert judgement related to the criteria of Section 2 of Annex IV (category B of Article 9) for avian mycoplasmosis (*Mycoplasma gallisepticum*, *M. meleagridis*) (CI = current impact; PI = potential impact)

Criteria to be met by the disease: The disease needs to fulfil all of the following criteria		Final outcome	
		<i>M. gallisepticum</i>	<i>M. meleagridis</i>
1	The disease is present in the whole OR part of the Union territory with an endemic character AND (at the same time) several Member States or zones of the Union are free of the disease	N	
2.1	The disease is moderately to highly transmissible	Y	
2.2	There be possibilities of airborne or waterborne or vector-borne spread	na	Y
2.3	The disease affects single or multiple species	Y	
2.4	The disease may result in high morbidity with in general low mortality	Y	Y*

**At least one criterion to be met by the disease:**

In addition to the criteria set out above at points 1–2.4, the disease needs to fulfil at least one of the following criteria

3	The disease has a zoonotic potential with significant consequences on public health, including epidemic potential OR possible significant threats to food safety	N	
4 (CI)	The disease has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals	N	

4 (PI)	The disease has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals	Y	NC
5(a) (CI)	The disease has a significant impact on society, with in particular an impact on labour markets	N	
5(a) (PI)	The disease has a significant impact on society, with in particular an impact on labour markets	N	
5(b) (CI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	N	
5(b) (PI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	Y	NC
5(c) (CI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	N	
5(c) (PI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	N	
5(d) (CI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	N	
5(d) (PI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	N	

Colour code: green = consensus (Yes/No); yellow = no consensus (NC); red = not applicable (na), i.e. insufficient evidence or not relevant to judge.

\*: Pre-hatch mortality was assessed as production loss.

**Table 5:** Outcome of the expert judgement related to the criteria of Section 3 of Annex IV (category C of Article 9) for avian mycoplasmosis (*Mycoplasma gallisepticum*, *M. meleagridis*) (CI = current impact; PI = potential impact)

Criteria to be met by the disease: The disease needs to fulfil all of the following criteria		Final outcome	
		<i>M. gallisepticum</i>	<i>M. meleagridis</i>
1	The disease is present in the whole OR part of the Union territory with an endemic character	NC	NC
2.1	The disease is moderately to highly transmissible	Y	
2.2	The disease is transmitted mainly by direct or indirect transmission	Y	
2.3	The disease affects single or multiple species	Y	
2.4	The disease usually does not result in high morbidity and has negligible or no mortality AND often the most observed effect of the disease is production loss	Y*	
<b>At least one criterion to be met by the disease:</b> In addition to the criteria set out above at points 1–2.4, the disease needs to fulfil at least one of the following criteria			
3	The disease has a zoonotic potential with significant consequences on public health, or possible significant threats to food safety	N	
4(CI)	The disease has a significant impact on the economy of parts of the Union, mainly related to its direct impact on certain types of animal production systems	N	
4(PI)	The disease has a significant impact on the economy of parts of the Union, mainly related to its direct impact on certain types of animal production systems	N	

5(a) (CI)	The disease has a significant impact on society, with in particular an impact on labour markets	N	
5(a) (PI)	The disease has a significant impact on society, with in particular an impact on labour markets	N	
5(b) (CI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	N	
5(b) (PI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	Y	NC
5(c) (CI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	N	
5(c) (PI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	N	
5(d) (CI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	N	
5(d) (PI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	N	

Colour code: green = consensus (Yes/No); yellow = no consensus (NC).

\*: Pre-hatch mortality was assessed as production loss.

**Table 6:** Outcome of the expert judgement related to the criteria of Section 4 of Annex IV (category D of Article 9) for avian mycoplasmosis (*Mycoplasma gallisepticum*, *M. meleagridis*)

Criteria to be met by the disease: The disease needs to fulfil all of the following criteria		Final outcome
D	The risk posed by the disease in question can be effectively and proportionately mitigated by measures concerning movements of animals and products in order to prevent or limit its occurrence and spread	Y
The disease fulfils criteria of Sections 1, 2, 3 or 5 of Annex IV of AHL		Y

Colour code: green = consensus (Yes/No).

**Table 7:** Outcome of the expert judgement related to the criteria of Section 5 of Annex IV (category E of Article 9) for avian mycoplasmosis (*Mycoplasma gallisepticum*, *M. meleagridis*)

Diseases in category E need to fulfil criteria of Sections 1, 2 or 3 of Annex IV of AHL and/or the following:		Final outcome
E	Surveillance of the disease is necessary for reasons relating to animal health, animal welfare, human health, the economy, society or the environment (If a disease fulfils the criteria as in Article 5, thus being eligible to be listed, consequently category E would apply)	Y

Colour code: green = consensus (Yes/No).

### 3.3.1. Non-consensus questions

This section displays the assessment related to each criterion of Annex IV referring to the categories of Article 9 of the AHL where no consensus was achieved in form of tables (Tables 8–11). The proportion of Y, N or 'na' answers are reported, followed by the list of different supporting views for each answer.

**Table 8:** Outcome of the expert judgement related to criterion 1 of Article 9 for *M. gallisepticum*

Question		Final outcome	Response		
			Y (%)	N (%)	na (%)
1(cat.A)	The disease is not present in the territory of the Union OR present only in exceptional cases (irregular introductions) OR present in only in a very limited part of the territory of the Union	NC	33	67	0
1(cat.C)	The disease is present in the whole OR part of the Union territory with an endemic character	NC	56	44	0

NC: non-consensus; number of judges: 9.

#### Reasoning supporting the judgement

Supporting Yes for 1 (cat.A):

- Sporadic cases of *M. gallisepticum* are reported.

Supporting Yes for 1 (cat.C):

- The fact sheet reports detections of *M. gallisepticum* in wild birds, also game birds (Galliformes), hobby and backyard flocks in multiple MSs where infections do not always lead to clinical signs. They serve as reservoir. Furthermore, a small number of recent studies have reported a low seroprevalence in multiple MSs (Latvia, France, Germany, Belgium) with suggested other MSs with unreported cases.

**Table 9:** Outcome of the expert judgement related to criterion 1 of Article 9 for *M. meleagridis*

Question		Final outcome	Response		
			Y (%)	N (%)	na (%)
1(cat.A)	The disease is not present in the territory of the Union OR present only in exceptional cases (irregular introductions) OR present in only in a very limited part of the territory of the Union	NC	33	67	0
1(cat.C)	The disease is present in the whole OR part of the Union territory with an endemic character	NC	67	33	0

NC: non-consensus; number of judges: 9.

#### Reasoning supporting the judgement

Supporting Yes for 1 (cat.A):

- There are only sporadic outbreaks of *M. meleagridis*. The disease is absent in most of the EU due to eradication programs.

Supporting Yes for 1 (cat.C):

- *M. meleagridis* is asymptomatic for wild birds and has been isolated from birds of prey (Falconiformes) without clinical signs or histopathological alterations in air sac biopsies in Germany (Lierz et al., 2000). If this *Mycoplasma* species can be isolated or is detected by serology in some asymptomatic wild avian species described in the fact sheet these would serve as a possible, albeit unlikely, route of introduction of the disease for young turkeys (before sexual maturity) by an airborne transmission. There is no reported surveillance in wild species throughout the Union.

**Table 10:** Outcome of the expert judgement related to criterion 4(PI) of Article 9 for *M. meleagridis*

Question	Final outcome	Response		
		Y (%)	N (%)	na (%)
4(cat.A, B) The disease has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals	NC	40	60	0

NC: non-consensus; number of judges: 10.

#### Reasoning supporting the judgement

Supporting Yes for 4 (cat.A,B):

- Both *Mycoplasma* species resulted in significant problems before implementation of control programmes and could thus do so again. *M. meleagridis* primarily affects turkey breeders.

Supporting No for 4 (cat.A,B):

- 5–6% decrease in egg hatchability in turkeys is not considered significant.
- The current position will potentially not change. *M. meleagridis* may be a concern primarily in turkey breeders where high biosecurity practices operate, thus there is low potential to change.

**Table 11:** Outcome of the expert judgement related to criterion 5(b)(PI) of Article 9 for *M. meleagridis*

Question	Final outcome	Response		
		Y (%)	N (%)	na (%)
5(b) The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	NC	60	40	0

NC: non-consensus; number of judges: 10.

#### Reasoning supporting the judgement

Supporting Yes:

- Both pathogens resulted in significant problems before implementation of control programmes and could thus do so again. *M. meleagridis* primarily affects turkey breeders.
- There would be high mortality and morbidity, if no control was in place.
- The infection can cause clinical signs with pain, and with a potentially high prevalence, thus the impact on animal welfare can be significant. There would not only be decreased egg hatchability, but also 10–25% sacculitis and skeleton deformations.

Supporting No:

- *M. meleagridis* may be a concern primarily in turkey breeders where high biosecurity practices operate, thus, there is low potential to change.
- The main symptom is embryo mortality for *M. meleagridis*, and that would not be on a large number of animals, but only in breeders.

### 3.3.2. Outcome of the assessment of criteria in Annex IV for avian mycoplasmosis (*Mycoplasma gallisepticum*, *M. meleagridis*) for the purpose of categorisation as in Article 9 of the AHL

As from the legal text of the AHL, a disease is considered fitting in a certain category (A, B, C, D or E corresponding to point (a) to point (e) of Article 9(1) of the AHL) if it is eligible to be listed for Union intervention as laid down in Article 5(3) and fulfils all criteria of the first set from 1 to 2.4 and at least one of the second set of criteria from 3 to 5(d) as shown in Tables 3–7. According to the assessment methodology (EFSA AHAW Panel, 2017), a criterion is considered fulfilled when the outcome is 'Yes'. With respect to different type of impact where the assessment is divided into current and potential impact, a criterion will be considered fulfilled if at least one of the two outcomes is 'Y' and, in case of no 'Y', the assessment is inconclusive if at least one outcome is 'NC'.



A description of the outcome of the assessment of criteria in Annex IV for avian mycoplasmosis (*M. gallisepticum*, *M. meleagridis*) for the purpose of categorisation as in Article 9 of the AHL is presented in Tables 12 and 13.

**Table 12:** Outcome of the assessment of criteria in Annex IV for *M. gallisepticum* for the purpose of categorisation as in Article 9 of the AHL (CI = current impact; PI = potential impact)

Category	Article 9 criteria										
	1° set of criteria					2° set of criteria					
	1	2.1	2.2	2.3	2.4	3	4	5a	5b	5c	5d
	Geographical distribution	Transmissibility	Routes of transmission	Multiple species	Morbidity and mortality	Zoonotic potential	Impact on economy	Impact on society	Impact on animal welfare	Impact on environment	Impact on biodiversity
A	NC	N	na	Y	N	N	CI: N PI: Y	N	CI: N PI: Y	N	N
B	N	Y	na	Y	Y	N	CI: N PI: Y	N	CI: N PI: Y	N	N
C	NC	Y	Y	Y	Y	N	N	N	CI: N PI: Y	N	N
D						Y					
E						Y					

**Table 13:** Outcome of the assessment of criteria in Annex IV for *M. meleagridis* for the purpose of categorisation as in Article 9 of the AHL (CI = current impact; PI = potential impact)

Category	Article 9 criteria										
	1° set of criteria					2° set of criteria					
	1	2.1	2.2	2.3	2.4	3	4	5a	5b	5c	5d
	Geographical distribution	Transmissibility	Routes of transmission	Multiple species	Morbidity and mortality	Zoonotic potential	Impact on economy	Impact on society	Impact on animal welfare	Impact on environment	Impact on biodiversity
A	NC	N	Y	Y	N	N	CI: N PI: NC	N	CI: N PI: NC	N	N
B	N	Y	Y	Y	Y	N	CI: N PI: NC	N	CI: N PI: NC	N	N
C	NC	Y	Y	Y	Y	N	N	N	CI: N PI: NC	N	N
D						Y					
E						Y					

According to the assessment here performed, avian mycoplasmosis (*M. gallisepticum*, *M. meleagridis*) complies with the following criteria of the Sections 1–5 of Annex IV of the AHL for the application of the disease prevention and control rules referred to in points (a)–(e) of Article 9(1):

- 1) To be assigned to category A, a disease needs to comply with all criteria of the first set (1, 2.1–2.4) and according to the assessment *M. gallisepticum* complies with criterion 2.3, but not with 2.1 and 2.4. The assessment is not applicable on criterion 2.2 and inconclusive on compliance with criterion 1. *M. meleagridis* complies with criteria 2.2 and 2.3, but not with 2.1 and 2.4 and the assessment is inconclusive on compliance with criterion 1. To be eligible for category A, a disease needs to comply additionally with one of the criteria of the second set (3, 4, 5a–d) and *M. gallisepticum* complies with criteria 4 and 5b, but not with 3, 5a, 5c and 5d. *M. meleagridis* does not comply with criteria 3, 5a, 5c and 5d and the assessment is inconclusive on compliance with criteria 4 and 5b.
- 2) To be assigned to category B, a disease needs to comply with all criteria of the first set (1, 2.1–2.4) and according to the assessment *M. gallisepticum* complies with criteria 2.1, 2.3 and 2.4, but not with 1 and the assessment is not applicable on criterion 2.2. *M. meleagridis* complies with criteria 2.1, 2.2, 2.3 and 2.4, but not with 1. To be eligible for category B, a disease needs to comply additionally with one of the criteria of the second set (3, 4, 5a–d) and *M. gallisepticum* complies with criteria 4 and 5b, but not with 3, 5a, 5c and 5d. *M. meleagridis* does not comply with criteria 3, 5a, 5c and 5d and the assessment is inconclusive on compliance with criteria 4 and 5b.
- 3) To be assigned to category C, a disease needs to comply with all criteria of the first set (1, 2.1–2.4) and according to the assessment *M. gallisepticum* and *M. meleagridis* comply with criteria 2.1, 2.2, 2.3 and 2.4 and the assessment is inconclusive on compliance with criterion 1. To be eligible for category C, a disease needs to comply additionally with one of the criteria of the second set (3, 4, 5a–d) and *M. gallisepticum* complies only with criterion 5b. *M. meleagridis* does not comply with any of the criteria and the assessment is inconclusive on compliance with criterion 5b.
- 4) To be assigned to category D, a disease needs to comply with criteria of Sections 1, 2, 3 or 5 of Annex IV of the AHL and with the specific criterion D of Section 4, with which avian mycoplasmosis (*M. gallisepticum*, *M. meleagridis*) complies.
- 5) To be assigned to category E, a disease needs to comply with criteria of Sections 1, 2 or 3 of Annex IV of the AHL and/or the surveillance of the disease is necessary for reasons relating to animal health, animal welfare, human health, the economy, society or the environment. The latter is applicable if a disease fulfils the criteria as in Article 5, with which avian mycoplasmosis (*M. gallisepticum*, *M. meleagridis*) complies.

### 3.4. Assessment of Article 8

This section presents the results of the assessment on the criteria of Article 8(3) of the AHL about avian mycoplasmosis (*M. gallisepticum*, *M. meleagridis*). The Article 8(3) criteria are about animal species to be listed, as it reads below:

'3. Animal species or groups of animal species shall be added to this list if they are affected or if they pose a risk for the spread of a specific listed disease because:

- a) they are susceptible for a specific listed disease or scientific evidence indicates that such susceptibility is likely; or
- b) they are vector species or reservoirs for that disease, or scientific evidence indicates that such role is likely'.

For this reason, the assessment on Article 8 criteria is based on the evidence as extrapolated from the relevant criteria of Article 7, i.e. the ones related to susceptible and reservoir species or routes of transmission, which cover also possible role of biological or mechanical vectors.<sup>4</sup> According to the mapping, as presented in Table 5, Section 3.2 of the scientific opinion on the *ad hoc* methodology (EFSA AHAW Panel, 2017), the main animal species to be listed for avian mycoplasmosis

<sup>4</sup> A vector is a living organism that transmits an infectious agent from an infected animal to a human or another animal. Vectors are frequently arthropods. Biological vectors may carry pathogens that can multiply within their bodies and be delivered to new hosts, usually by biting. In mechanical vectors, the pathogens do not multiply within the vector, which usually remains infected for shorter time than in biological vectors.

(*M. gallisepticum*, *M. meleagridis*) according to the criteria of Article 8(3) of the AHL are as displayed in Tables 14 and 15.

**Table 14:** Main animal species to be listed for *Mycoplasma gallisepticum* according to criteria of Article 8 (source: data reported in Section 3.1.1.1)

	Order	Family	Genus/Species
Susceptible and reservoir	Galliformes	Phasianidae	<i>Gallus</i> spp., <i>Meleagris</i> spp., pheasants (Phasianinae), <i>Perdix perdix</i> , <i>Alectoris chukar</i> , <i>Coturnix japonica</i>
		Odontophoridae	<i>Colinus virginianus</i>
	Columbiformes	Columbidae	<i>Columba palumbus</i>
	Pelecaniformes	Ardeidae	<i>Ardea cinerea</i>
	Anseriformes	Anatidae	Not specified
	Passeriformes	Corvidae	<i>Pica pica</i>
		Fringillidae	<i>Haemorhous mexicanus</i> , <i>Spinus tristis</i> , <i>Pinicola enucleator</i> , <i>Coccothraustes vespertinus</i> , <i>Haemorhous purpureus</i> , <i>Serinus canaria domestica</i>
		Corvidae	<i>Corvus frugilegus</i> , <i>Cyanocitta cristata</i>
		Passeridae	Not specified
Psittaciformes	Not specified	Not specified	
Vectors	None		

**Table 15:** Main animal species to be listed for *Mycoplasma meleagridis* according to criteria of Article 8 (source: data reported in Section 3.1.1.1)

	Order	Family	Genus/Species
Susceptible	Galliformes	Phasianidae	<i>Meleagris</i> spp. pheasants (Phasianinae) <i>Tympanuchus pallidicinctus</i> <i>Coturnix japonica</i> <i>Gallus gallus</i>
		Odontophoridae	<i>Callipepla squamata</i>
	Columbiformes	Columbidae	Not specified
	Falconiformes	Not specified	Not specified
Reservoir	None		
Vectors	None		

## 4. Conclusions

**TOR 1:** for each of those diseases an assessment, following the criteria laid down in Article 7 of the AHL, on its eligibility of being listed for Union intervention as laid down in Article 5(3) of the AHL;

- According to the assessment here performed, avian mycoplasmosis (*M. gallisepticum*, *M. meleagridis*) complies with all criteria of the first set and with one criterion of the second set and therefore can be considered eligible to be listed for Union intervention as laid down in Article 5(3) of the AHL.

**TOR 2a:** for each of the diseases which was found eligible to be listed for Union intervention, an assessment of its compliance with each of the criteria in Annex IV to the AHL for the purpose of categorisation of diseases in accordance with Article 9 of the AHL;

- According to the assessment here performed, avian mycoplasmosis (*M. gallisepticum*, *M. meleagridis*) meets the criteria as in Sections 4 and 5 of Annex IV of the AHL, for the application of the disease prevention and control rules referred to in points (d) and (e) of Article 9 (1) of the AHL, while it is inconclusive whether avian mycoplasmosis (*Mycoplasma gallisepticum*, *M. meleagridis*) complies with the criteria as in Section 3 of Annex IV of the AHL, for the application of the disease prevention and control rules referred to in point (c) of Article 9(1) of the

AHL. Compliance of avian mycoplasmosis with the criteria as in Section 3 is dependent on a decision on criteria 1 and 5b.

**TOR 2b:** for each of the diseases which was found eligible to be listed for Union intervention, a list of animal species that should be considered candidates for listing in accordance with Article 8 of the AHL.

- According to the assessment here performed, the animal species that can be considered to be listed for avian mycoplasmosis (*M. gallisepticum*, *M. meleagridis*) according to Article 8(3) of the AHL are mainly domestic and wild bird species belonging to the orders of Galliformes, Columbiformes, Pelecaniformes, Anseriformes, Passeriformes, and Psittaciformes for *M. gallisepticum*; and Galliformes, Columbiformes and Falconiformes for *M. meleagridis*, as reported in Tables 14 and 15 in Section 3.4 of the present document.

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## Abbreviations

AHAW	EFSA Panel on Animal Health and Welfare
AHL	Animal Health Law
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
ELISA	enzyme-linked immunosorbent assay
IBD	infectious bursal disease
IBV	Infectious Bronchitis virus
ICBA	Individual and Collective Behavioural Aggregation
Ig	immunoglobulin
IUCN	International Union for Conservation of Nature
MIC	minimum inhibitory concentration
MS	Member State
OIE	World Organisation for Animal Health
PCR	polymerase chain reaction
RSA	rapid serum agglutination
ToR	Terms of Reference