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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): antimicrobial-resistant *Enterococcus faecalis* in poultry

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Abstract

Enterococcus faecalis (*E. faecalis*) was identified among the most relevant antimicrobial-resistant (AMR) bacteria in the EU for poultry in a previous scientific opinion. Thus, it 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 its eligibility to be listed, Annex IV for its categorisation according to disease prevention and control rules as in Article 9 and Article 8 for listing animal species related to the bacterium. The assessment has been performed following a methodology previously published. The outcome is the median of the probability ranges provided by the experts, which indicates whether each criterion is fulfilled (lower bound $\geq$ 66%) or not (upper bound $\leq$ 33%), or whether there is uncertainty about fulfilment. Reasoning points are reported for criteria with uncertain outcome. According to the assessment here performed, it is uncertain whether AMR *E. faecalis* can be considered eligible to be listed for Union intervention according to Article 5 of the AHL (33–66% probability). According to the criteria in Annex IV, for the purpose of categorisation related to the level of prevention and control as in Article 9 of the AHL, the AHAW Panel concluded that the bacterium does not meet the criteria in Sections 1, 2 and 4 (Categories A, B and D; 0–5%, 5–10% and 1–10% probability of meeting the criteria, respectively) and the AHAW Panel is uncertain whether it meets the criteria in Sections 3 and 5 (Categories C and E, 33–66% and 33–66% probability of meeting the criteria, respectively). The animal species to be listed for AMR *E. faecalis* according to Article 8 criteria are mostly birds of the orders Galliformes and Anseriformes, but also mammals and reptiles can serve as reservoirs.

Keywords: antimicrobial resistance, *Enterococcus faecalis*, Animal Health Law, listing, categorisation, impact

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1. Introduction

The European Food Safety Authority (EFSA) received a mandate from the European Commission to investigate the global state of play as regards antimicrobial-resistant (AMR) animal pathogens that cause transmissible animal diseases (Term of Reference (ToR) 1), to identify the most relevant AMR bacteria in the European Union (EU) (first part of ToR 2), to summarise the existing or potential animal health impact of those identified bacteria in the EU (second part of ToR 2) and to perform the assessment of those bacteria to be listed and categorised according to the criteria in Article 5, Annex IV according to Article 9 and Article 8 within the Regulation (EU) No 2016/429[1] on transmissible animal diseases ('Animal Health Law') (ToR 3).

The global state of play for AMR animal pathogens that cause transmissible animal diseases (ToR 1) and the results of the assessment of the most relevant AMR bacteria in the EU (first part of ToR 2) for poultry were published in a separate EFSA scientific opinion (EFSA AHAW Panel, 2021a).

According to the results of the assessment already conducted, *Enterococcus faecalis* (*E. faecalis*) was identified among the most relevant AMR bacteria in the EU for poultry due to its increasing clinical importance in the last decades, problems associated with its treatment (often due to a late aetiological diagnosis) and its wide distribution, along with the high levels of resistance found for certain antimicrobials, which are also widely used for its treatment (lincosamides and spectinomycin) (EFSA AHAW Panel, 2021a).

This scientific opinion presents the results of the assessment on AMR *E. faecalis* in poultry on its eligibility to be listed and categorised within the AHL framework. Special focus is placed on the animal health impact of AMR *E. faecalis* in poultry in the EU, which is also summarised here as part of the assessment conducted according to the profile of the infection and its impact on animal welfare (Article 7).

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

The background and ToRs as provided by the European Commission for the present document are reported in Sections 1.1 and 1.2 of the scientific opinion on the ad hoc method to be followed for the assessment of animal diseases caused by bacteria resistant to antimicrobials within the AHL framework (EFSA AHAW Panel, 2021b).

1.2. Interpretation of the Terms of Reference

The interpretation of the ToRs is as in Sections 1.2.3 and 1.3.3 of the scientific opinion on the ad hoc method to be followed for the assessment of animal diseases caused by bacteria resistant to antimicrobials within the AHL framework (EFSA AHAW Panel, 2021b).

The present document reports the results of the assessment on AMR *E. faecalis* in poultry according to the criteria of the AHL articles as follows:

- Article 7: AMR *E. faecalis* infection profile and impacts;
- Article 5: eligibility of AMR *E. faecalis* infection to be listed;
- Article 9: categorisation of AMR *E. faecalis* infection according to disease prevention and control rules as in Annex IV;
- Article 8: list of animal species (also apart from poultry) related to AMR *E. faecalis* infection.

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 listing and categorisation of animal diseases within the AHL framework (EFSA AHAW Panel, 2017).

In order to take into account the specifics related to animal diseases caused by bacteria resistant to antimicrobials, the term 'disease' as in the AHL was interpreted in a broader sense, referring also to colonisation by commensal and potentially opportunistic bacteria and the general presence of the identified AMR bacteria in the EU, depending on each criterion.

The following assessment was performed by the EFSA Panel on Animal Health and Welfare (AHAW) based on the information collected and compiled in form of a fact sheet as in Section 3.1 of the

[1] Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health ('Animal Health Law'). OJ L 84, 31.3.2016, p. 1–208.

present document. The outcome is the median of the probability ranges provided by the experts, which are accompanied by verbal interpretations as spelled out in Table 1.

Table 1: Approximate probability scale recommended for harmonised use in EFSA (EFSA Scientific Committee, 2018)

Probability term	Subjective probability range
Almost certain	99–100%
Extremely likely	95–99%
Very likely	90–95%
Likely	66–90%
About as likely as not	33–66%
Unlikely	10–33%
Very unlikely	5–10%
Extremely unlikely	1–5%
Almost impossible	0–1%

3. Assessment

3.1. Assessment of AMR *Enterococcus faecalis* according to Article 7 criteria of the AHL

3.1.1. Article 7(a) Disease profile

E. faecalis (formerly known as *Streptococcus faecalis*) is a non-motile, Gram-positive coccoid bacterium found as commensal in the intestine of most mammals, birds, reptiles and insects (Lebreton et al., 2014). *E. faecalis* is generally considered as an opportunistic pathogen, also in birds (Chadfield et al., 2004). Although considered an opportunistic pathogen, strains of *E. faecalis* may display variations in virulence, as shown by layer chick embryo lethality assays (Blanco et al., 2017, 2018).

Clinical conditions observed in poultry include growth depression (Eyssen and De Somer, 1967), pulmonary hypertension syndrome (Tankson et al., 2001), amyloid arthropathy (Landman et al., 1994), valvular endocarditis, septicaemia, salpingitis and peritonitis (Gregersen et al., 2010). Certain pathological manifestations have been linked with specific genetic linages of *E. faecalis*, e.g. amyloid arthropathy in broiler breeders has been closely associated with the sequence type (ST) 82 (Petersen et al., 2009, 2010). Infections with most other *E. faecalis* clones in poultry are less specific and often occur secondarily to other conditions, such as infection with avian pathogenic *Escherichia coli* (APEC) (Olsen et al., 2012b).

Importantly, *E. faecalis* does generally not display the same 'outbreak nature' as e.g. APEC in poultry flocks. That is, if a single or a few birds are diagnosed with *E. faecalis* infection, there is a low risk of transmission to other birds within the flock because it is already present as a commensal in the intestine of other birds. However, in the presence of e.g. another infection or immunosuppression, many different *E. faecalis* clones will be able to give rise to secondary infection. Such an 'outbreak' would then be polyclonal (Gregersen et al., 2010; Olsen et al., 2012b).

In humans, *E. faecalis* has evolved to become a globally disseminated nosocomial (hospital and healthcare-associated) pathogen. Hospital-associated clones are characterised by the acquisition of adaptive genetic elements, such as genes encoding antimicrobial resistance. Hospital-associated clones are however not confined to hospitals, as they can also be found in healthy carriers in the community, and may give rise to community-acquired infections (Guzman Prieto et al., 2016). As highlighted later in this document, there is also speculation that *E. faecalis* can be transmitted to humans from animals via contact and indirectly via food, including poultry meat.

Importantly, *E. faecalis* is intrinsically resistant to different first-line antimicrobial agents (e.g. low-level resistance to β-lactams and aminoglycosides), and it has the capacity to acquire resistance to several other antimicrobial agents, including last-resort antibiotics such as glycopeptides (Stobberingh et al., 1999; van den Bogaard and Stobberingh, 2000; Hammerum et al., 2010). Whereas some genetic variants (e.g. ST6 and ST9) seem to be mainly adapted to human hospitalised patients (Ruiz-Garbajosa et al., 2006; McBride et al., 2007; Freitas et al., 2009; Kuch et al., 2012), other *E. faecalis* clones are commonly shared between hospitalised patients and other reservoirs (including birds),

e.g. ST16 and ST40 (Pöntinen et al., 2021). Some of these less host-specific clones tend to be resistant to more antibiotics than others (Kawalec et al., 2007; McBride et al., 2007; Freitas et al., 2009; Fertner et al., 2011).

This fact sheet does not focus on any particular AMR phenotypes in *E. faecalis*. For more information on antimicrobial resistance in poultry isolates, we refer to the recent EFSA scientific opinion on the most relevant AMR bacteria in the EU for poultry (EFSA AHAW Panel, 2021a), where this has been reviewed with tables and figures showing proportion of resistance in clinical *E. faecalis* isolates from across the world.

Whenever information in this fact sheet on carriage rate (i.e. proportion of a population colonised or carrying the bacterium somewhere in the body) is not further elaborated in terms of antimicrobial resistance, it is because the information available on carriage does not specify antimicrobial resistance.

For clarification, the term 'layer' refers to hens laying eggs for human consumption, and 'broiler breeder' refers to hens laying eggs for hatching of broilers. As *E. faecalis* does not cause gastrointestinal disease in poultry, the term 'faecal samples' refers to samples from birds that are not clinically affected by the bacterium unless otherwise stated.

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

Susceptible animal species

E. faecalis appears to be the most widespread and abundant species of the *Enterococcus* genus and can be found in the intestines of humans, farmed, companion and wild animals as well as in the environment (Mundt, 1963a,b; Devriese et al., 1992; Tannock and Cook, 2002; Lebreton et al., 2014).

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

There are only limited reports on *E. faecalis* giving rise to disease in wildlife. For example, *E. faecalis* has been reported to cause osteitis deformans in a Golden Lancehead snake (*Bothrops insularis*) (Garcia et al., 2020).

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

Most mammals are susceptible to (opportunistic) infections with *E. faecalis*. In this section, we address only domestic avian species.

In chicken (*Gallus gallus domesticus*), broilers, broiler breeders and layers are susceptible to infection caused by *E. faecalis* (Landman et al., 1994; Gregersen et al., 2010). Information on antimicrobial resistance in chicken isolates is presented in Section 3.1.1.4, whereas information on intestinal carriage rates is presented under Parameter 1 in Section 3.1.1.5.

In farmed duck (Anatidae), Osman et al., (2019) found all 10 investigated faecal *E. faecalis* isolates resistant to ciprofloxacin, clindamycin, erythromycin, oxytetracycline, phenicols and vancomycin, whereas four and nine isolates were resistant to gentamicin and ampicillin, respectively. All isolates were susceptible to linezolid. In 77 *E. faecalis* isolates obtained from faeces of ducks, Na et al. (2019) found the following proportions of resistance: ampicillin (0%), chloramphenicol (21%), ciprofloxacin (31%), daptomycin (1%), erythromycin (27%), florfenicol (21%), kanamycin (13%), streptomycin (27%), tetracycline (79%), tigecycline (23%), tylosin (26%). All isolates were susceptible to ampicillin, gentamicin, salinomycin, linezolid and vancomycin, but the same study identified one of 97 duck carcass isolates as resistant to linezolid (Na et al., 2019).

E. faecalis is the most commonly isolated enterococcal species in turkeys (*Meleagris* sp.). In one study, 80% (n = 50 isolates) of faecal samples from farmed turkey harboured this species (Kacmaz and Aksoy, 2005), of which 26%, 27% and 16% of the isolates were resistant to penicillin, ampicillin and high-level aminoglycoside, respectively. None of the isolates were β-lactamase producing or resistant to glycopeptides.

In pheasant, *E. faecalis* has mainly been associated with decreased hatchability. One study on ring-neck pheasant (*Phasianus colchicus*) reported a drop of more than 80% in hatching of the eggs due to *E. faecalis* infection of embryos (Reynolds and Loy, 2020).

For ostriches (*Struthio camelus*), carriage of (multiresistant) intestinal strains of *E. faecalis* has been reported in at least one study, although the exact prevalence of *E. faecalis*-positive *birds was not stated in that study* (Siwela et al., 2007).

Between 13% and 35% of partridges (*Perdix perdix*) and quails (*Coturnix coturnix*) have been shown to carry intestinal *E. faecalis* (Silvia et al., 2011; Zhang et al., 2017; Saeed and Alkennany, 2018).

Recently, Freitas et al. (2018) identified captive blue-fronted parrot (*Amazona aestiva*) as sources of multidrug-resistant *Enterococcus* spp. in two wild animal screening centres. Levels of resistance in 40 *E. faecalis* isolates were rifampicin (77.5%), ampicillin (2.5%), ciprofloxacin (5.0%), chloramphenicol (5.0%), erythromycin (17.5%), streptomycin (7.5%), norfloxacin (15.0%) and tetracycline (12.5%).

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

No studies on experimentally susceptible wildlife species were found.

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

Salpingitis (infection of the avian reproductive system) has experimentally been produced by Fang et al. (2021) in layers and breeder ducks. For full manifestations of salpingitis, a co-infection including *E. faecalis*, *Escherichia coli* (*E. coli*) and *Chlamydia psittaci* was needed. Likewise, experimental intraperitoneal co-infection with *E. faecalis* and *Ornithobacterium rhinotracheale* resulted in severe haemorrhagic pneumonia, and the authors concluded that co-infections were needed for the full pathological manifestations (Zhao et al., 2015).

In layer birds less than 1 week of age, which is the age group most susceptible to *E. faecalis* infection, single infection with *E. faecalis* has been reproduced experimentally by either aerosol exposure (resulting in bacteraemia), intratracheally (resulting in bacteraemia and arthritis) or intramuscular infection (resulting in polyarthritis) (Landman et al., 2003).

In broilers, the pulmonary hypertension syndrome has been reproduced experimentally after either intra-abdominal or intravenous administration of *E. faecalis* (Tankson et al., 2001).

In Japanese quails (*Coturnix japonica*), experimental intra-arterial (aorta) administration of *E. faecalis* caused lesions very similar to those of atherosclerosis in humans (Saeed and Alkennany, 2018).

Reservoir animal species

Parameter 5 – Wild reservoir species (or family/order)

E. faecalis has frequently been isolated from wild mammals, reptiles, birds and insects that are not clinically affected (Mundt, 1963a,b; Martin and Mundt, 1972).

Oliveira de Araujo et al. (2020) investigated the carriage of intestinal enterococci in Pampas foxes (*Lycalopex gymnocercus*) (n = 5) and Geoffroy's cats (*Leopardus geoffroyi*) (n = 4) in the Brazilian Pampa biome and found that enterococci (including *E. faecalis*) could be detected in 80% of the samples from either animal species. Of the 32 *E. faecalis* isolates further characterised, 65% were multidrug-resistant (resistant to at least three antimicrobials of different families). Resistance was most common to rifampicin, erythromycin and ciprofloxacin/norfloxacin (in 97%, 78% and 47% of isolates, respectively).

In addition to the species mentioned above, *E. faecalis* has been isolated from several other avian wildlife species, including the brown pelican (*Pelecanus occidentalis*), laughing gull (*Larus atricilla*), mourning dove (*Zenaida macroura*), pigeon (*Columba* spp.), American robin (*Turdus migratorius*), wild turkey (*Meleagris gallopavo*), screech owl (*Otus asio*) and great horned owl (*Bubo virginianus*) (Kuntz et al., 2004) as well as from wild European goldfinch (*Carduelis carduelis*), European greenfinch (*Carduelis chloris*), European serin (*Serinus serinus*), African river martin (*Pseudochelidon eurystomina*), herring gull (*Larus argentatus*), common blackbird (*Turdus merula*), grey gull (*Leucophaeus modestus*) and European bee-eater (*Merops apiaster*) (Klibi et al., 2015).

Free-ranging wild birds (rooks (*Corvus frugilegus*) and American crows (*Corvus brachyrhynchos*)) have been identified as possible reservoirs for vancomycin- and gentamicin-resistant *E. faecalis* isolates (Oravcova et al., 2013; Roberts et al., 2016).

A comprehensive study (León-Sampedro et al., 2019) analysing 103 faecal swabs from native wild birds (33 species of 10 orders) retrieved 97 *E. faecalis* isolates, of which 66 (68%) showed resistance to one or more antibiotics, including tetracycline (67%), chloramphenicol (42%), erythromycin (28%) and high-level resistance to different aminoglycosides (5–26%). All isolates were susceptible to ampicillin and vancomycin.

Parameter 6 – Domestic reservoir species (or family/order)

This information is included under Parameter 1 in this section.

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

Morbidity

Parameter 1 – Prevalence/incidence

Prevalence and incidence of disease cannot be accurately measured due to the opportunistic nature of *E. faecalis*, as the immunological competence (and age) of each individual bird must be considered to estimate if it is likely that the bird will develop disease due to *E. faecalis*.

However, although *E. faecalis* is considered an opportunistic pathogen, it can be associated with both high morbidity and mortality, especially in young birds (Olsen et al., 2012b). Already within the eggs, avian embryos are susceptible to *E. faecalis*. Fertner et al. (2011) found a prevalence of 14% of *E. faecalis*-positive *chicks among newly hatched.*

Information on intestinal carriage rates in chicken is presented under Parameter 1 in Section 3.1.1.5 and information for other avian species is available in Section 3.1.1.1.

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

No data are available on case-morbidity rate for *E. faecalis* in poultry.

Mortality

Parameter 3 – Case-fatality rate

The case-fatality rate is difficult to establish, as it will depend on several factors: (1) route of pathogen introduction (intratracheally, orally, etc.), (2) virulence of the individual strains, (3) presence of co-infecting organisms and (4) overall immunocompetence of the birds (Landman et al., 1994, 1999, 2001, 2003; Kandričáková et al., 2015; Naundrup Thøfner et al., 2019). In most cases, due to the intensive production system, an infection with *E. faecalis* in a single animal (not outbreak-related) will proceed until the bird dies from the infection or is culled on humane grounds. Hence, most of the knowledge available on *E. faecalis* infection-related fatalities and culls on farm comes from studies on causes of mortality. In such a study by Olsen et al. (2012b), the authors investigated the cause of mortality in 983 layer chicks, and found that in 23% of the chicks, the mortality was associated with *E. faecalis* infections (8% as single infections, 15% mixed with other pathogens such as *E. coli*). In a study investigating first-week mortality, it was found that approximately 25% of the layer chicks dying within their first week of life had an extra-intestinal *E. faecalis* infection (either as single infection or as co-infection with *E. coli*/*Staphylococcus aureus* (Olsen et al., 2012b). In older birds, the percentage of broiler breeders aged 20–72 weeks dying from (or at least with) an *E. faecalis* infection was 2.9% (Naundrup Thøfner et al., 2019). A study investigating the causes of mortality in Danish broiler breeders estimated that 3% of the mortality in flocks was due to *E. faecalis*, which is considerably less than for e.g. *E. coli* (identified as cause of mortality in 35% of the cases) (Naundrup Thøfner et al., 2019).

E. faecalis infections may result in embryo mortality (Reynolds and Loy, 2020). In that regard, Dolka et al. (2017) found that 15 of 2,828 poultry source enterococci in Polish diagnostic laboratories were *E. faecalis* isolated from hatching eggs and dead-in-shell embryos.

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

Parameter 1 – Report of zoonotic human cases (anywhere)

While numerous studies suggest that poultry could be a reservoir for (multidrug-resistant) *E. faecalis* of human clinical importance (Hammerum, 2012; Olsen et al., 2012c; Stępień-Pyśniak et al., 2018), only few epidemiological studies on transmission of avian *E. faecalis* to humans have been performed. Poulsen et al. (2012) showed by pulsed-field gel electrophoresis (PFGE) that seven *E. faecalis* isolates obtained from 31 cases of urinary tract infections (UTI) were genetically indistinguishable or very closely related to *E. faecalis* isolates of chickens in the household of each patient with UTI. This demonstrates that chicken is a potential source of human *E. faecalis* infections, although the direction and route of transmission was not clear, and a common source of infection could not be ruled out. Importantly, the study by Poulsen et al. (2012) was carried out in Vietnam where people live in close physical contact with their animals. Although the results cannot be directly transferred to the more industrialised European poultry production, the potential, where contact is close, for avian isolates to infect humans, or vice versa, was evident.

In a study by Hasan et al. (2018), genetically comparing 74 *E. faecalis* isolates of poultry or poultry environmental origin with epidemiologically unrelated *E. faecalis* strains from human UTI, the authors found that phenotypic/genetic determinants of virulence and resistance were shared between poultry-associated and human UTI isolates. Agersø et al. (2008) also showed by PFGE identical *E. faecalis* strains obtained from turkey meat, healthy humans and a Danish patient, and isolates were resistant to vancomycin, tetracycline and erythromycin.

It has also been suggested that human infections caused by (multidrug-resistant) enterococci could be due to the consumption of contaminated fresh or processed poultry meats (Hidano et al., 2015; Foulquié et al., 2006). This is exemplified in a study by Manson et al. (2019) who sequenced 32 *E faecalis* isolates from raw chicken products and compared them with whole-genome sequences of 149 *E. faecalis* of human clinical and commensal origin. The authors inferred that both human commensal and clinical enterococcal strains were similar to isolates from chicken meat, including isolates bearing important resistance-conferring elements and virulence factors. The authors finally concluded that, 'The ability of enterococci to persist in the food system positions them as vehicles to move resistance genes from the industrial farm ecosystem into more human-proximal ecologies'.

Human outbreaks caused by the amyloid arthropathy-associated ST82 clone have been documented in Denmark, France, Germany and the USA, and transmission from wild birds has been included among the hypotheses to explain this global clonal expansion (León-Sampedro et al., 2019).

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

E. faecalis is, like other enterococci, intrinsically resistant to several antimicrobial agents (Hollenbeck and Rice, 2012). One example is the low cell wall permeability responsible for intrinsic resistance of *E. faecalis* to aminoglycosides. *E. faecalis* is, however, also highly adapted to the acquisition of mobile genetic elements (Miller et al., 2014).

In a study from Poland, Różańska et al. (2015) tested antimicrobial susceptibility of 24 *E. faecalis* isolates of poultry meat origin and found higher levels of antimicrobial resistance in these compared to *E. faecalis* of bovine or porcine origin. In particular, streptomycin and tylosin resistance was much more frequent in poultry isolates (Table 2). This probably reflects the high use of the two antimicrobials in industrialised poultry production. In a Turkish study of chicken meat, none out of 37 *E. faecalis* isolates were resistant to streptomycin or vancomycin, whereas all isolates were resistant to kanamycin (Sanlibaba et al., 2018). Sanlibaba et al. (2018) also found that 37%, 41% and 3% of the isolates were resistant to erythromycin, ampicillin and gentamicin, respectively.

Table 2: Antimicrobial resistance of *E. faecalis* isolated from meat (Różańska et al. (2015))

	Number/% of resistant strains			
Antimicrobials	**Total (n = 111)**	**Cattle (n = 35)**	**Pigs (n = 52)**	**Poultry (n = 24)**
Chloramphenicol (CHL)	15/13.5	10/28.6	0	5/20.8
Ciprofloxacin (CIP)	2/1.8	0	0	2/8.3
Daptomycin (DAP)	0	0	0	0
Erythromycin (ERY)	40/36.0	10/28.6	23/44.2	7/29.2
Gentamicin (GEN)	3/2.7	0	0	2/8.3
Kanamycin (KAN)	20/18.0	2/5.7	2/3.8	16/66.7
Lincomycin (LIN)	94/84.7	30/85.7	40/76.9	24/100.0
Linezolid (LZD)	8/7.2	4/11.4	2/3.8	2/8.3
Nitrofurantoin (NIT)	0	0	0	0
Penicillin (PEN)	2/1.8	1/2.9	1/1.9	0
Quinupristin/dalfopristin (SYN)	88/79.3	34/97.1	31/59.6	23/95.8
Streptomycin (STR)	20/18.0	2/5.7	2/3.8	16/66.7
Tetracycline (TET)	65/58.6	26/74.3	18/34.6	21/87.5
Tigecycline (TGC)	0	0	0	0
Tylosin (TYLT)	20/18.0	3/8.6	1/1.9	16/66.7
Vancomycin (VAN)	3/2.7	1/2.9	1/1.9	1/4.2

Source: National Veterinary Research Institute in Pulawy, Poland.

In a German study by Maasjost et al. (2015), the authors addressed antimicrobial resistance in 127 clinical *E. faecalis* isolates obtained from extra-intestinal lesions in broilers, layers and turkeys. They found that all the isolates were sensitive to vancomycin and β-lactam antibiotics, including ampicillin, amoxicillin-clavulanic acid and penicillin. In 57 isolates from broilers and 40 isolates from turkeys, approximately half of them were resistant to tylosin (46% and 56%, respectively), while 44% and 56% were resistant to erythromycin. In addition, 51% and 73% of broiler and turkey isolates, respectively, were resistant to gentamicin. In contrast, resistance proportions for 30 isolates in layers were only 27%, 27% and 35% for erythromycin, tylosin and gentamicin, respectively.

In a Portuguese study of free-range broilers, Semedo-Lemsaddek et al. (2021) found that 90%, 50%, 70% and 10% of 10 genetically related commensal *E. faecalis* faecal isolates displayed phenotypic resistance to tetracycline, erythromycin, gentamicin and penicillin, respectively.

In a Polish study by Stępień-Pyśniak et al. (2018), the authors reported that the 27 faecal *E. faecalis* isolates under investigation were obtained from 'injured or weak (wildlife) birds', but it is uncertain if isolates were of clinical origin. The authors found lincomycin resistance in all isolates, whereas ampicillin and vancomycin resistance was detected in only one isolate. Antimicrobial resistance levels for other drugs as well as the multiresistance phenotypes detected are displayed in Table 3.

Table 3: Antimicrobial resistance levels of *E. faecalis* in wild birds (Stępień-Pyśniak et al. (2018))

Isolate	Origin[(a)]	Pulsotype	ST[(b)]	Resistance phenotype[(c)]	Resistance genes
3	White-tailed Eagle	B	290	TE-DO-ERY-LIN	*tet*(L), *erm*(A), *erm*(B)
11	Little Bittern	D	290	TE-DO-LIN	*tet*(M)
12	Eurasian Hoopoe	D	290	TE-DO-LIN	*tet*(M)
48A	Mallard	V	290	ERY-VA-PEN-AMP-LIN	*erm*(B), *msr*(A/B)
20	Mallard	K	374	TE-DO-ERY-STR-KAN-CIP-LIN	*tet*(M), *tet*(L), *erm*(B), *ant(6)-Ia*, *aph(3')-IIIa*
26	Mallard	K	374	TE-DO-ERY-STR-KAN-CIP-LIN	*tet*(M), *tet*(L), *erm*(B), *ant(6)-Ia*, *aph(3')-IIIa*
28	Eurasian Blackbird	K	374	TE-DO-ERY-STR-KAN-CIP-LIN	*tet*(M), *tet*(L), *erm*(B), *ant(6)-Ia*, *aph(3')-IIIa*
32	Tawny Owl	K	374	TE-DO-ERY-STR-KAN-CIP-LIN	*tet*(M), *tet*(L), *erm*(B), *ant(6)-Ia*, *aph(3')-IIIa*
56	Tawny Owl	C	287	LIN	–
25	Mallard	H	287	LIN	–
58	Great Spotted Woodpecker	S	287	LIN	–
30	Eurasian Jay	N	34	LIN	–
31	Short-eared Owl	N	34	LIN	–
46	White-tailed Eagle	F	**752** (CC81)	ERY-LIN	*erm*(B)
61	Grey Heron	R	81 (CC81)	LIN	–
53	Lesser Spotted Woodpecker	P	175 (SLV of ST753)	TE-DO-ERY-STR-CIP-LIN	*tet*(M), *tet*(L), *erm*(B), *ant(6)-Ia*
54	Eurasian Green Woodpecker	Q	753 (SLV of ST175)	TE-DO-LIN	–
8	Tawny Owl	J	**748** (TLV of ST165)	TE-DO-ERY-LIN	*erm*(B)
50	Mallard	O	165 (TLV of ST748)	TE-DO-ERY-LIN	*tet*(M), *tet*(L), *erm*(B)
2B	White-tailed Eagle	A	16	TE-DO-ERY-LIN	*tet*(M), *tet*(L), *erm*(A), *erm*(B), *msr*(A/B)
62	Eurasian Marsh-harrier	G	21	LIN	–
35	Little Bittern	L	35	LIN	–
42	Common Buzzard	I	232	LIN	–

Isolate	Origin[(a)]	Pulsotype	ST[(b)]	Resistance phenotype[(c)]	Resistance genes
33	Mallard	E	**749**	LIN	–
36	Eurasian Sparrowhawk	T	**750**	TE-DO-ERY-LIN	*tet*(M), *tet*(L), *erm*(B)
44	Little Owl	M	**751**	LIN	–
64A	Grey Heron	U	**764**	LIN	–

(a): White-tailed Eagle = *Haliaeetus albicilla*; Little Bittern = *Ixobrychus minutus*; Eurasian Hoopoe = *Upupa epos*; Mallard = *Anas platyrhynchos*; Eurasian Blackbird = *Turdus merula*; Tawny Owl = *Strix aluco*; Great Spotted Woodpecker = *Dendrocopos major*; Eurasian Jay = *Garrulus glandarius*; Short-eared Owl = *Asio flammeus*; Grey Heron = *Ardea cinerea*; Lesser Spotted Woodpecker = *Dendrocopos minor*; Eurasian Green Woodpecker = *Picus viridis*; Eurasian Marsh-harrier = *Circus aeruginosus*; Common Buzzard = *Buteo buteo*; Eurasian Sparrowhawk = *Accipiter nisus*; Little Owl = *Athene noctua*.
(b): ST: sequence type; clonal complex (CC) or lineage to relatives in brackets; SLV: single locus variant; TLV: triple locus variant; boldface numbers indicate new STs found in wild birds.
(c): TE: tetracycline; DO: doxycycline; ERY: erythromycin; LIN: lincomycin; VA: vancomycin; PEN: penicillin; AMP: ampicillin; STR: streptomycin; KAN: kanamycin; CIP: ciprofloxacin.

In another very recent study, Stępień-Pyśniak et al. (2021) assessed antimicrobial resistance in 76 *E. faecalis* isolates (35 Polish isolates and 41 Dutch isolates) from yolk sac infections in broiler chicks. They found the following proportions of resistance: tetracycline (70%), lincomycin (99%), erythromycin (51%), high-level streptomycin (11%), high-level kanamycin (4%), chloramphenicol (8%) and ciprofloxacin (25%, including also intermediate isolates). All isolates were susceptible to penicillin, ampicillin, high-level gentamicin, tigecycline and linezolid. The authors concluded that 'Restrictive programmes for antibiotic use in broiler breeding flocks should be developed to decrease drug resistance in day-old chicks and reduce economic losses during rearing'.

In a large pan-European study by de Jong et al. (2019), 845 commensal isolates of chicken origin collected in the period 2004–2014 were assessed for antimicrobial susceptibility to ampicillin, erythromycin, gentamicin, linezolid, tetracycline, tigecycline and vancomycin (Table 4). Whereas there has been a dramatic decrease in the proportion of resistance to gentamicin and vancomycin over time (from 9% in 2004–2005 to 0–1% in 2013–2014), a concerning rise in the proportion of non-wild-type isolates to tigecycline was reported (from 7% in 2008–2009 to 11% in 2013–2014). During the period, all isolates were susceptible to linezolid, nearly all (> 99%) isolates were wild type to ampicillin, whereas > 50% and > 70% of isolates were resistant to erythromycin and tetracycline, respectively.

Table 4: Antimicrobial susceptibility of *E. faecalis* isolates (n = 1389) from food-producing animals in three time periods (2004–2005, 2008–2009 and 2013–2014) (de Jong et al. (2019))

		Cattle			Pigs			Chickens		
Antimicrobial	**Interpretation[(a)]**	**2004–2005 (n = 34)**	**2008–2009 (n = 56)**	**2013–2014 (n = 115)**	**2004–2005 (n = 74)**	**2008–2009 (n = 89)**	**2013–2014 (n = 176)**	**2004–2005 (n = 11)**	**2008–2009 (n = 346)**	**2013–2014 (n = 448)**
Ampicillin	MIC_{50}	2	1	1	2	0.5	1	0.5	0.5	1
	MIC_{90}	2	1	1	2	1	1	1	0.5	1
	R (≥ 16)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
	NWT (≥ 8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
Erythromycin	MIC_{50}	–	2	1	–	4	2	–	8	16
	MIC_{90}	–	4	> 256	–	> 256	> 256	–	> 256	> 256
	R (≥ 8)	–	**0.0**	**10.4**	–	48.3	47.2	16	50.9	56.6
Gentamicin	MIC_{50}	16	8	8	16	8	8	16	8	8
	MIC_{90}	16	8	16	32	16	128	9.1	16	16
	R (≥ 512)	0.0	0.0	0.9	6.8	2.2	5.1	**9.1**	0.9	0.8
	NWT (≥ 64)	0.0	1.8	2.6	6.8	6.7	12.5	2	**2.6**	**1.0**
Linezolid	MIC_{50}	2	2	2	2	2	2	2	1	2
	MIC_{90}	2	2	4	2	2	2	0.0	2	4
	R (≥ 8)	0.0	0.0	0.0	0.0	0.0	2.3	–	0.0	0.0
Tetracycline	MIC_{50}	–	1	1	–	64	64	–	64	64
	MIC_{90}	–	128	64	–	> 128	256	–	128	128
	R (≥ 16)	–	32.1	30.4	–	**88.8**	**76.1**	–	80.3	78.3

Antimicrobial	Interpretation[a]	Cattle			Pigs			Chickens		
		2004–2005 (n = 34)	**2008–2009 (n = 56)**	**2013–2014 (n = 115)**	**2004–2005 (n = 74)**	**2008–2009 (n = 89)**	**2013–2014 (n = 176)**	**2004–2005 (n = 11)**	**2008–2009 (n = 346)**	**2013–2014 (n = 448)**
	NWT (≥ 8)	–	32.1	30.4	–	**88.8**	**76.1**	–	80.6	78.5
Tigecycline	MIC_{50}	–	0.25	0.25	–	0.25	0.25	–	0.25	0.25
	MIC_{90}	–	0.25	0.25	–	0.25	0.5	–	0.25	0. 5
	R (≥ 1)	–	0.0	0.0	–	0.0	0.0	–	0.0	0.0
	NWT (≥ 0.5)	–	1.8	7.8	–	5.6	13.6	–	**7.2**	**11.5**
Vancomycin	MIC_{50}	2	2	1	1	1	1	2	1	1
	MIC_{90}	2	2	4	2	2	2	4	2	4
	R (≥ 32)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	NWT (≥ 8)	0.0	3.6	0.9	0.0	0.0	0.0	**9.1**	**0.0**	**0.0**

Data in bold indicate a significant difference ($p < 0.05$) between the time periods for a host animal. A dash indicated no data were available.
(a): $MIC_{50/90}$: minimum inhibitory concentration values (mg/L); R: resistance (%); NWT: non-wild type (%).

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

A healthy bird may live an entire lifespan with *E. faecalis* as an intestinal commensal without developing any *E. faecalis*-associated *pathology. Therefore, this section addresses E. faecalis* intestinal carriage in birds without clinical signs of *E. faecalis* infection. It can be assumed that colonisation with *E. faecalis* occurs regularly in most – if not all – avian species, although this theory is based only on *E. faecalis*-positive faecal samples obtained at certain sampling times in species like duck (Gülhan et al., 2012), goose (Middleton and Ambrose, 2005), turkey (Welton et al., 1998), quail (Al-Hamdany and Al-Kennany, 2014), pheasant (Kandricaková et al., 2015), ostrich (Gonçalves et al., 2010) and partridge (Silva et al., 2018).

In layers, Fertner et al. (2011) found, from culture of cloacal swabs, that 14% of chicks were already colonised with *E. faecalis* at the time of hatch. During the hatching period, chicks were kept closely together in an incubator (a hatcher), and 97% of the chicks were *E. faecalis* positive after 24 h. A similar proportion of *E. faecalis*-colonised *birds was found in a study of broilers, investigating the prevalence of broiler chicks positive for E. faecalis* at hatching and after 24 h in the hatcher (Olsen et al., 2012a). Not much is known about the intestinal persistence of *E. faecalis* strains naturally acquired in early life; however, *E. faecalis* remains a frequent commensal, also in older birds (Kempf et al., 2020). The relatively high prevalence (31–56%) of *E. faecalis* found in various studies of raw chicken and turkey meat (Hayes et al., 2003; Manson et al., 2019) supports long-term intestinal carriage of *E. faecalis* in healthy birds, as contamination of the raw meat occurs due to cross-contamination of the meat with intestinal content during slaughter.

Parameter 2 – Presence and duration of latent infection period

E. faecalis has a broad diversity of pathological manifestations. Therefore, the latent period (which will be from when *E. faecalis* enters the extra-intestinal compartment (e.g. blood, joints, etc.) fully depends on the pathogenicity of the isolate, immunocompetence of the bird and the localisation of the infection, e.g. it could be hours in the case of sepsis development, or weeks for development of chronic endocarditis (Larsen et al., 2008).

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

This information is included under Parameter 1 in this section.

Environment

Parameter 4 – Length of survival of the agent and/or detection of DNA in selected matrices (soil, water, air) from the environment

Enterococci can survive and live in harsh environments (Pinto et al., 1999). The survival of *E. faecalis* in water has been thoroughly investigated, e.g. by Lleò et al. (2005), who found that the

bacterium can survive at least 60 days at room temperature in water if protected from direct illumination, and up to 60 days at 4°C. In a study conducted during the summer time in Israel (temperature not further defined), the authors showed that *E. faecalis* rapidly decreased in number from the surface of soil (from 10,000 to 13 in 38 days using the most probable number (MPN) method to estimate quantity), whereas the storage under 10 cm of soil clearly prolonged survival (MPN decreased from 4,000 to 113 in 38 days) (Bergner-Rabinowitz, 1956).

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)

E. faecalis may be transmitted both horizontally and vertically (Olsen et al., 2012a). Fertner et al. (2011) investigated the extent of vertical transmission. In that study, chicks were sampled instantly after hatch where a sterile or only slightly colonised intestine would be expected. The authors suggested that the chicks heavily colonised with *E. faecalis* at hatch had been subjected to 'true' vertical transfer, in which *E. faecalis* organisms present in the reproductive tract had incorporated into the egg at the time of egg formation. This is in contrast to 'indirect' vertical transmission, in which the embryo becomes infected with one or more bacterial species due to migration of bacteria through the eggshell. In the study by Fertner et al. (2011), the *E. faecalis*-positive *chicks did not display clinical disease. Landman et al. (1999) demonstrated that vertical transmission was likely to have contributed to the major challenges with arthropathic and amyloidogenic E. faecalis* infection in layers in the late 1990s.

Horizontal spread between birds occurs through *E. faecalis* aerosol transmission (Landman et al., 2001), or through ascending infections through the cloaca (primarily in older, egg-laying birds) (Naundrup Thøfner et al., 2019).

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

Food-borne transmission (indirect transmission) through *E. faecalis*-contaminated *food products is considered a risk for transmission between animals and humans (Bortolaia et al., 2016). For E. faecalis*, this is supported by studies showing indistinguishable clones in poultry meat and human clinical infections (see Section 3.1.1.3). More direct evidence of poultry-to-human transmission of enterococci comes from an experimental study where human volunteers ingested a chicken *E. faecium* strain. This strain could colonise the digestive tract of volunteers for up to 14 days after ingestion, and it was shown to exchange genes encoding vancomycin and quinupristin–dalfopristin resistance to a human *Enterococcus faecium* strain co-administered to the volunteers (Lester et al., 2006).

As described in Section 3.1.1.3, Poulsen et al. (2012) found several cases of *E. faecalis* UTI isolates being indistinguishable from *E. faecalis* of the chickens in patients' households. Whereas it is safe to conclude that humans can get infections from the avian *E. faecalis*, it is uncertain how (and if) the strain causing UTI was transmitted from the household chickens, as it could be indirectly through contaminated meat or dirt, or it could be directly through close contact with the birds. In addition, a common source of infection could not be ruled out.

Speed of transmission

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

The best estimate of *E. faecalis* transmission comes from studies in the hatcher (Fertner et al., 2011; Olsen et al., 2012a). In those studies, the proportion of chicks sampling positive for *E. faecalis* using cloacal swabs went from ~ 15% to more than 90% within 24 h. At the time around hatch, chicks perform 'cloacal drinking' (ingesting bacteria through retrograd movement of bacteria from the cloacal environment up through the intestinal system), which may contribute to a faster transmission than in older birds in which transmission from one bird's intestine to another occurs through faecal–oral transmission.

High faecal carriage of *E. faecalis* increases the risk of aerosol exposure of *E. faecalis*, which subsequently increases the risk of (extra-intestinal) *E. faecalis* infection. Under experimental settings, 80% of *E. faecalis* aerosol-exposed day-old chicks developed clinical disease (bacteraemia) within 24 h (Landman et al., 2001), whereas a much lower proportion (3/10) of 4-day-old birds developed

bacteraemia following aerosol exposure (even though the 4-day-old birds had been treated with an immunosuppressive drug, namely methylprednisolone). In another group of 4-day-old chicks, the chicks had been pre-exposed to Newcastle disease virus and subsequently exposed to *E. faecalis* aerosols. In this group, only 1/10 developed bacteraemia, and not until 66 days after exposure. Hence, the susceptibility and speed of transmission seem to depend on the age of the birds, with the young chicks being most susceptible to infection-associated disease.

Importantly, *E. faecalis* does generally not display the same 'outbreak nature' as e.g. APEC in poultry flocks. That is, if a single or a few birds are diagnosed with *E. faecalis* infection, there is a low risk of transmission to other birds within the flock because it is already present as a commensal in the intestine of other birds. However, in the presence of e.g. another infection or immunosuppression, many different *E. faecalis* clones will be able to give rise to secondary infection. Such an 'outbreak' would then be polyclonal (Gregersen et al., 2010; Olsen et al., 2012b).

The incidence between animals and humans is unknown.

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

Unknown.

3.1.1.7. Article 7(a)(vii) The absence or presence and distribution of the disease in the Union and, 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

E. faecalis constitutes a normal part of the avian gastrointestinal microbiota. Disease in avian species occurs sporadically.

Risk of introduction

This section is not relevant due to the ubiquitous occurrence of this bacterial species.

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

Diagnostic tools

Parameter 1 – Existence of diagnostic tools

E. faecalis causes a broad diversity of extra-intestinal disease manifestations (Gregersen et al., 2010), none of which can be easily distinguished macroscopically from other extra-intestinal infections. Lesions can be local (e.g. in the case of chronic endocarditis or arthritis), systemic (sepsis), chronic or acute. The acute infections (followed by mortality) are often observed in the very young chicks (within first week of life) (Olsen et al., 2012b), whereas chronic infections occur more frequently in older birds (broiler breeders/layers above 40 weeks of age) (Naundrup Thøfner et al., 2019).

E. faecalis obtained from sample material grows readily on standard growth media, such as blood-supplemented agar, with 24 h of incubation at 37°C. *E. faecalis* appears as greyish, medium-sized colonies and cannot be distinguished from other enterococci based on colony morphology alone (although *E. faecium* often has a greenish haemolysis). It will also grow on modified MacConkey agar and differential/selective agars such as M-Enterococcus or Slanetz-Bartley that allow the growth of pinkish typical enterococci colonies with *E. faecalis* usually showing colonies more dark/vinous than *E. faecium* ones (light pink). Note that *E. faecalis* isolates obtained from lesions of amyloid arthropathy can have a small colony appearance, in which the colonies are almost pinpoint (Petersen et al., 2008).

After culturing, *E. faecalis* can be identified by standard phenotypic tests and matrix-assisted laser desorption ionisation–time-of-flight mass spectrometry (MALDI-TOF MS). Also, polymerase chain reaction (PCR) can be used for species identification, e.g. a PCR test able to distinguish *E. faecalis* from 18 other enterococcal species (Jackson et al., 2004).

Resistance to antibiotics can be detected in various ways, including by determination of the minimum inhibitory concentration (MIC) using broth or agar dilution, or using agar diffusion, e.g. by E-test. Antimicrobial resistance can also be detected using the disk diffusion method for which zone inhibition diameters are read. Importantly, there are no animal-specific breakpoints for enterococci; hence, definition of antimicrobial resistance in this species has to be done using epidemiological

cut-offs or clinical breakpoints for enterococcal infections in humans. Accordingly, the clinical relevance of susceptibility testing for guiding treatment of *E. faecalis* infections in poultry is questionable.

Parameter 2 – Existence of control tools

There are no official measures to control *E. faecalis* infections. Optimised management and avoiding immunosuppressing viral diseases by vaccination to prevent secondary *E. faecalis* infections is the best 'control tool'. If applied as probiotics, *E. faecalis* from healthy birds may even promote growth performance and immunological status and convey beneficial modulation of the caecal microbiota in broilers (Shehata et al., 2020). Hence, too strict measures to decrease/limit the intestinal amount of *E. faecalis* may not even been desirable.

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

The bacterium is ubiquitous; hence, the disease is endemic and therefore likely present in all Member States. Antimicrobial resistance in indicator *E. faecalis* obtained in slaughterhouses from broilers in eight Member States and two European non-Member States was assessed in 2013 (EFSA and ECDC, 2013). With the exception of erythromycin and gentamicin, Belgium had the highest proportion of antimicrobial resistance for all agents tested, while Finland generally had the lowest resistance levels.

Table 5: Resistance (%) to ampicillin, chloramphenicol, erythromycin, gentamicin, linezolid, streptomycin, tetracyclines and vancomycin among *E. faecalis* from broilers in countries reporting MIC data in 2011 (EFSA and ECDC (2013))

Country	Ampicillin		Chloramphenicol		Erythromycin		Gentamicin		Linezolid		Streptomycin		Tetracyclines		Vancomycin	
	N	% R	N	% R	N	% R	N	% R	N	% R	N	% R	N	% R	N	% R
Austria	101	0	101	7.9	101	58.4	101	1.0	101	0	101	16.8	101	58.4	101	0
Belgium	81	11.1	81	9.9	81	76.5	81	3.7	81	6.2	81	59.3	81	90.1	81	3.7
Denmark	110	0	110	0	110	14.5	110	0	110	0	110	3.6	110	17.3	110	0
Finland	169	0	169	0	169	58.0	169	0	169	0	169	0	169	7.1	169	0
France	112	0	112	5.4	112	66.1	112	0.9	112	0	112	31.3	112	94.6	112	0
Ireland	100	0	100	2.0	100	79.0	100	1.0	–	–	100	47.0	100	84.0	101	2.0
Netherlands	276	0	276	3.3	276	79.0	276	1.8	276	0	276	56.2	276	79.0	276	0
Spain	63	1.6	63	15.9	63	85.7	63	27.0	63	0	63	44.4	63	87.3	63	1.6
Total (8 MSs)	1,012	1.0	1,012	4.2	1,012	65.2	1,012	2.8	912	0.5	1,012	33.0	1,012	61.9	1,013	0.6
Norway	62	0	62	11.3	62	25.8	62	0	62	0	62	16.1	62	45.2	62	0
Switzerland	117	0	117	1.7	117	39.3	–	–	117	0	117	12.8	117	65.0	117	0

MS: Member State.

The loss of production due to the disease

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

Yolk sac infections followed by death are a frequent cause of death in chicks up to 1 week old (Stępień-Pyśniak et al., 2021). In a study by Olsen et al. (2012b), the authors investigated the cause of first-week mortality within 50 layer flocks. A total of 938 chicks underwent post-mortem examination, and 50% of these chicks had died from infectious causes (mostly yolk sac infections). *E. faecalis* was isolated from 50% of these infectious cases.

In older birds, chronic infections often proceed unnoticed (Naundrup Thøfner et al., 2019). It must, however, be assumed that a chronic infection is associated with decreased production. To the authors' knowledge, there has so far not been any investigation correlating the presence of chronic *E. faecalis* infection with economic losses.

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

Transmissibility between animals and humans

Parameter 1 – Types of routes of transmission between animals and humans

It is assumed that food-borne transmission is the most likely for *E. faecalis* (Bortolaia et al., 2016), but other routes of transmission (e.g. faecal–oral or direct contact) cannot be ruled out.

Parameter 2 – Incidence of zoonotic cases

While it is clear that certain genetic types of *E. faecalis* can be found both among human clinical cases and healthy poultry or poultry meat (Olsen et al., 2012c; Section 3.1.1.3), there are no data to establish the incidence of zoonotic cases.

Transmissibility between humans

Parameter 3 – Human-to-human transmission is sufficient to sustain sporadic cases or community-level outbreak

Unknown, but unlikely since enterococci (including *E. faecalis*) are not known to cause transmissible infections in humans, except in hospitals where it may act as a nosocomial pathogen.

Parameter 4 – Sporadic, epidemic or pandemic potential

Transmission from poultry to humans is in most cases unlikely to lead to human infection but rather contamination or colonisation of the human gut. If infection occurs anyway, it will likely be sporadic.

The severity of human forms of the disease

Parameter 5 – Disability-adjusted life year (DALY)

DALY has been estimated for vancomycin-resistant enterococci (including *E. faecalis*) to be 5.49 per 100,000 population, which in % corresponded to one of the greatest burdens of infections, only after carbapenem- and colistin-resistant *Klebsiella pneumoniae* or *E. coli* (Cassini et al., 2019).

The availability of effective prevention or medical treatment in humans

Parameter 6 – Availability of medical treatment and their effectiveness (therapeutic effect and any resistance)

Antimicrobial treatment is widely available, but limited options exist for *E. faecalis* given its intrinsic resistance to several antimicrobial classes. Typically, *E. faecalis* infections in humans are treated with an aminopenicillin and either gentamicin or vancomycin depending on acquired resistance. Therapeutic effect would depend on the clone causing the infection, and in that respect it is evident from previous sections (see Section 3.1.1.4) that most poultry *E. faecalis* isolates are susceptible to these agents.

Parameter 7 – Availability of vaccines and their effectiveness (reduced morbidity)

There are no licensed vaccines available for prevention of *E. faecalis* infections, neither in humans nor in poultry although autogenous vaccines have been used, particularly in breeding poultry.

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

Clinical conditions observed in poultry include growth depression (Eyssen and De Somer, 1967), pulmonary hypertension syndrome (Tankson et al., 2001), amyloid arthropathy (Landman et al., 1994), valvular endocarditis, septicaemia, salpingitis and peritonitis (Gregersen et al., 2010). Certain pathological manifestations have been linked with specific genetic linages of *E. faecalis*, e.g. amyloid arthropathy in broiler breeders has been closely associated with ST82 (Petersen et al., 2009, 2010). Infections with most other *E. faecalis* clones in poultry are less specific and often occur secondarily to other conditions, such as infection with APEC (Olsen et al., 2012b).

E. faecalis infections may give rise to acute mortality (Olsen et al., 2012b) and chronic infections in chicken. A common manifestation of chronic *E. faecalis* infection is arthropathy (with or without amyloidosis), in which deposits of acute phase proteins localise in joints. Amyloidosis is mainly observed during the rearing period from 6 weeks of age and onwards, and is observed in both broiler breeders (Gregersen et al., 2010) and layers (Landman et al., 1994). Amyloidosis affecting the joints is

associated with growth depression and lameness. Lame birds have difficulties accessing food and water, and consequently can become dehydrated and die (Blanco et al., 2016). To the authors' knowledge, antimicrobial susceptibility in *E. faecalis* isolates obtained from classical amyloidosis lesions has not been investigated. However, isolates causing amyloidosis in poultry often belong to the genetic lineage ST82, and this ST has also been found as a cause of yolk sac infections in Poland among chicks (Stępień-Pyśniak et al., 2021). In that study, all ST82 isolates were resistant to tetracycline and lincomycin, while some were in addition resistant to ciprofloxacin and erythromycin.

In most avian species, including chickens and ducks, *E. faecalis*-associated salpingitis is a common clinical manifestation (Bisgaard, 1995; Gregersen et al., 2010; Naundrup Thøfner et al., 2019).

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

Geoffroy's cat (*Leopardus geoffroyi*) (Felidae) and the Pampas fox (*Lycalopex gymnocercus*) (Canidae) are listed as species of 'least concern' in the IUCN Red List of Threatened Species. These species can be healthy carriers of multidrug-resistant *E. faecalis* (Oliveira de Araujo et al., 2020); hence, opportunistic infections may develop, even if yet to be proven.

Parameter 2 – Mortality in wild species

E. faecalis may act as an opportunistic pathogen in wild species, but to the authors' knowledge, there is no published evidence on mortality rates in wild species.

Environment

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

Enterococci (including *E. faecalis*) are able to persist for a long time in the environment (Byappanahalli et al., 2012) (see Parameter 4 in Section 3.1.1.5), but as stated in the prior section, there is no published evidence of *E. faecalis* mortality rates in wildlife.

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

Parameter 1 – Listed in OIE/CFSPH classification of pathogens

Not listed.

Parameter 2 – Listed in the Encyclopaedia of Bioterrorism Defence of Australia Group

Not listed.

Parameter 3 – Included in any other list of potential bio-agro-terrorism agents

None identified.

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

There is no need to prevent the presence of *E. faecalis* in the intestine of poultry. The prevention of extra-intestinal disease is obtained by preventing primary causes of immunosuppression by following relevant vaccine programmes for other diseases, and by ensuring that reconstituted Marek's disease vaccines and injection needles do not become contaminated with *E. faecalis* (as reported by Landman et al., 2000).

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

Availability

Parameter 1 – Officially/internationally recognised diagnostic tools, OIE-certified

There are no officially or internationally recognised diagnostic tools; however, general practice is to evaluate clinical signs, to euthanise a subset of affected animals and to sample different body sites during necropsy for culture-based analysis given the wide variety of organs the bacterium may be

isolated from. Detection of antimicrobial resistance is based on the previously mentioned tools (see Section 3.1.1.8), namely MIC testing or disk diffusion.

Effectiveness

Parameter 2 – Sensitivity and specificity of diagnostic tests

Unknown.

Feasibility

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

During necropsy, samples from different body sites with signs of lesions should be taken (in particular heart, yolk sac and joints).

3.1.4.2. Article 7(d)(ii) Vaccination

There are no registered vaccines available to prevent *E. faecalis* infection in poultry. Autogenous vaccines incorporating isolates confirmed to be the same subtype as those causing problems have been used in breeding chickens.

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

Availability

Parameter 1 – Types of drugs available on the market

Various antimicrobial agents can be used for treatment of *E. faecalis* infections (e.g. penicillins and tetracyclines), but availability of registered products varies between countries. Action should, however, also be directed against a correction of any predisposing condition, as *E. faecalis* infections are considered to be secondary in nature.

Parameter 2 – Availability/production capacity (per year)

Antimicrobial drugs for treatment of poultry infections are widely available on the market worldwide.

Effectiveness

Parameter 3 – Therapeutic effects in the field (effectiveness)

There are no systematic assessments on efficacy of different antimicrobial regimens on *E. faecalis*-associated disease.

Feasibility

Parameter 4 – Way of administration

Antibiotics are mostly administered orally to poultry, e.g. via drinking water.

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

Availability

Parameter 1 – Available biosecurity measures

Biosecurity measures (e.g. all-in–all-out production (broilers), thorough cleaning and disinfection of stables, pest control and personal hygiene precautions like hand washing and change of clothes and boots when entering stables) cannot eradicate *E. faecalis* from farmed poultry, but should be installed to avoid or minimise infections with other pathogens that may predispose for *E. faecalis* infections. Thorough attention to hygiene in the reconstitution of any injectable vaccines intended for young birds, in combination with measures to improve the automated cleaning of injection systems in use have been very effective in reducing risk.

Practices (e.g. related to staff hygiene, equipment maintenance and slaughter procedures) should also be installed and maintained in poultry abattoirs to minimise contamination of meat with faecal bacteria like *E. faecalis*. This would reduce the potential risk of zoonotic transmission through the food chain.

Effectiveness

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

As *E. faecalis* is endemic and a natural part of the intestinal flora, there is no risk of pathogen introduction, hence the effectiveness of biosecurity cannot be measured. One exception could be the previously mentioned 'outbreak clones', but there is very little knowledge concerning the existence of such clones, and to the authors' knowledge, no studies have assessed the effect of biosecurity on preventing transmission of specific clones within or between poultry herds.

Feasibility

Parameter 3 – Feasibility of biosecurity measures

Feasibility of biosecurity measures depends on the skills of farm personnel, farm economy and workflow, and on the design of poultry farms. For example, personal hygiene precautions like hand washing and change of clothes may be simple in some farms with changing facilities and sinks, but more complex in other farms.

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

Availability

Parameter 1 – Available movement restriction measures

Movement restriction measures are not needed to prevent dissemination of *E. faecalis*, which is ubiquitous. In order to prevent spread of other pathogens (that may cause infections predisposing to later *E. faecalis* infections), all-in–all-out production should be considered as mentioned above. This means that broilers, and other poultry species raised for meat production, are not moved between flocks. Instead, a full production cycle takes place in a stable followed by transportation to the slaughterhouse. Empty stables should then be cleaned, disinfected and allowed to dry before chicks for a new production cycle are allowed to enter.

Effectiveness

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

Not applicable due to the ubiquitous presence of *E. faecalis*.

Feasibility

Parameter 3 – Feasibility of restriction of animal movement

Not applicable due to the ubiquitous presence of *E. faecalis*.

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

Availability

Parameter 1 – Available methods for killing animals

Since *E. faecalis* is not regarded a highly contagious agent, infected birds can be killed in slaughterhouses, and killed animals can enter human consumption if they do not have clinical signs or lesions. Apart from standard biosecurity measures in abattoirs to prevent faecal contamination of meat (Section 3.1.4.4), no extra precautions at slaughter are needed for flocks suffering from *E. faecalis* colonisation or infection.

Individual treatment of animals experiencing severe disease caused by *E. faecalis* (e.g. septicaemia or endocarditis) is pointless, as there is no chance of recovery. Hence, such animals should be euthanised on farm (neck dislocation manually or with an approved mechanical device).

Effectiveness

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

Killing animals is effective only for animal welfare reasons, not to prevent disease associated with *E. faecalis*.

Feasibility

Parameter 3 – Feasibility of killing animals

Killing of individual diseased birds is feasible for most farmers.

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

There is no need for special concerns regarding disposal of *E. faecalis*-contaminated *carcasses, since E. faecalis* is not regarded a highly contagious agent.

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)

As stated above (Section 3.1.4.4), biosecurity measures are generally not relevant to prevent spread of ubiquitous bacteria like *E. faecalis*, but would be appropriate to prevent other infections that may predispose to *E. faecalis* infections. It has been estimated in 2012 that the total costs in Finland to keep biosecurity at an appropriate level for a batch of 75,000 broilers would be approximately €2,700 (Siekkinen et al., 2012). Costs for antimicrobial treatment vary depending on the drug used and the length of treatment. Development and use of licensed multivalent vaccines for breeding birds may not be cost-effective given the variety of strains and the usually sporadic nature of problems.

Parameter 2 – Cost of eradication (culling, compensation)

Since *E. faecalis* is a ubiquitous commensal, eradication is not an option.

Parameter 3 – Cost of surveillance and monitoring

To the authors' knowledge, there are no formal surveillance programmes for the occurrence and antimicrobial resistance of clinical *E. faecalis* isolates in poultry. Clinicians carrying out diagnostic work normally carry out disk-diffusion antimicrobial resistance testing, but the results are rarely published in the scientific literature.

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

E. faecalis is not likely to cause any trade loss, as it is already present in all poultry production systems.

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

To the authors' knowledge, there has been no official estimation on the cost of *E. faecalis* infection in poultry.

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

Not applicable, as control and preventive measures are not specific for this bacterium and disease caused by it.

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

Control measures aimed at delaying challenge with *E. faecalis* in early life will reduce occurrence of clinical and subclinical disease and so benefit animal welfare. Medication of affected flocks will have little or no benefit for birds already severely affected but may reduce the progression of disease in as yet subclinically affected birds to the benefit of the birds and the producer. Potentially, antibiotics used to control the disease may be ineffective due to the occurrence of antimicrobial resistance, and this would reduce any animal welfare benefits following treatment failure.

Parameter 2 – Wildlife depopulation as control measure

As *E. faecalis* is ubiquitously present in wildlife and domestic animals, depopulation is not an appropriate control measure option.

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)

The extent of antimicrobial treatment for *E. faecalis*-associated *infections in poultry (and consequently spill-over to the environment) is unknown. The same is true for biocides used for poultry house disinfection, but it is worth noting that E. faecalis* may be found after disinfection of poultry houses using a fogging procedure with hydrogen peroxide (220 g/L) and peroxyacetic acid (55 g/L) (Luyckx et al., 2017). Although not investigated by Luyckx et al. (2017), this could be due to various reasons, including improper prior cleaning leaving organic matter prior to disinfection, and the presence of biocide-resistant *E. faecalis* strains.

Biodiversity

Parameter 1 – Mortality in wild species

Control measures like antimicrobial treatment and keeping biosecurity appropriate are not expected to result in mortality in wild species.

3.2. Assessment of AMR *Enterococcus faecalis* according to Article 5 criteria of the AHL on its eligibility to be listed

3.2.1. Detailed outcome on Article 5 criteria

In Table 6 and Figure 1, the results of the expert judgement on the Article 5 criteria of the AHL for AMR *E. faecalis* in poultry are presented.

The distribution of the individual answers (probability ranges) provided by each expert for each criterion is reported in Sections A.1 and A.2 of Appendix A.

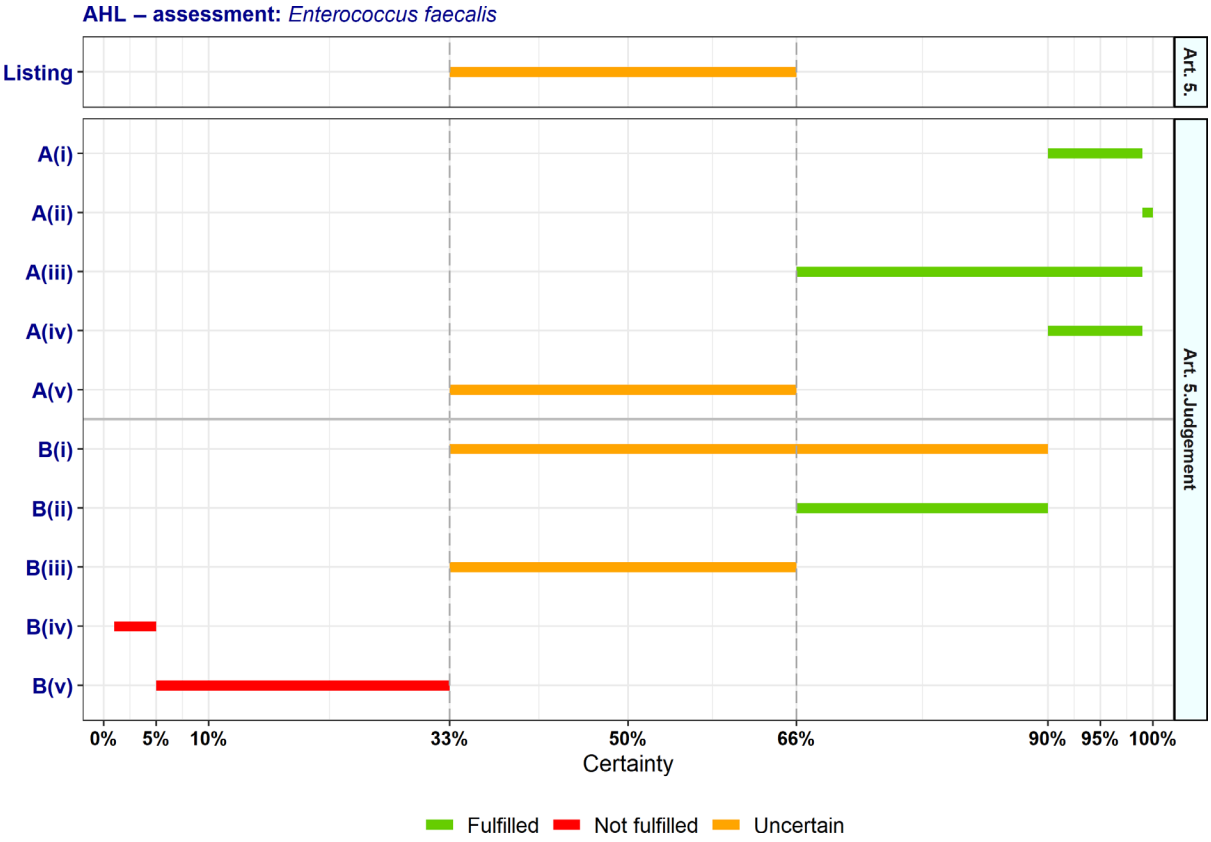
Table 6: Outcome of the expert judgement on Article 5 criteria

Criteria to be met by the disease: According to the 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		**Outcome**			
		Median range (%)	**Criterion fulfilment**	**Number of na**	**Number of experts**
A(i)	The disease is transmissible	90–99	Fulfilled	0	13
A(ii)	Animal species are either susceptible to the disease or vectors and reservoirs thereof exist in the Union	99–100	Fulfilled	0	14
A(iii)	The disease causes negative effects on animal health or poses a risk to public health due to its zoonotic character	66–99	Fulfilled	0	13
A(iv)	Diagnostic tools are available for the disease	90–99	Fulfilled	0	13
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	33–66	Uncertain	0	13
At least one criterion to be met by the disease: 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	33–90	Uncertain	0	13
B(ii)	The disease agent has developed resistance to treatments which poses a significant danger to public and/or animal health in the Union	66–90	Fulfilled	0	13
B(iii)	The disease causes or could cause a significant negative economic impact affecting agriculture or aquaculture production in the Union	33–66	Uncertain	0	13

B(iv)	The disease has the potential to generate a crisis or the disease agent could be used for the purpose of bioterrorism	1–5	Not fulfilled	0	14
B(v)	The disease has or could have a significant negative impact on the environment, including biodiversity, of the Union	5–33	Not fulfilled	0	13

na: not applicable.

In Figure 1, the outcome of the expert judgement is graphically shown together with the estimated overall probability of the AMR bacterium meeting the criteria of Article 5 on its eligibility to be listed.



Listing: The probability of the disease to be listed according to Article 5 criteria of the AHL (overall outcome).

Figure 1: Outcome of the expert judgement on Article 5 criteria and overall probability of AMR *E. faecalis* on its eligibility to be listed

3.2.1.1. Reasoning for uncertain outcome on Article 5 criteria

Criterion 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):

- *E. faecalis* is an opportunistic pathogen and disease is based on host factors.
- There is no structured or harmonised surveillance in the EU.
- Vaccines are not available and no official risk-mitigating measures are in place.
- Treatment options are limited and extensive use of antimicrobials may drive further development of antimicrobial resistance.
- Biosecurity measures may prevent infections with other pathogens predisposing for *E. faecalis* infection.
- Potential risk of zoonotic transmission through the food chain can be reduced by good hygienic slaughter practices.
- AMR clones are widespread in the EU.

Criterion 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):

- Few data are available.
- The pathogen is opportunistic but may be associated with high morbidity and mortality, especially in young birds.
- Increased embryo mortality and a case fatality of 3% have been observed.
- The pathogen is considered relevant by poultry experts.
- There may be a long-term impact on animal health.
- Effects on animal health are sporadic and linked to certain risk factors. The disease is still treatable and manageable.
- There may be a zoonotic role and transmission of AMR clones, but few epidemiological studies are available to evaluate the robustness of this information.

Criterion B(iii) (the disease causes or could cause a significant negative economic impact affecting agriculture or aquaculture production in the Union):

- Few data on economic impact are available. Exact costs are unknown.
- Information on AMR clones is insufficient.
- Case fatality and mortality have not been reported frequently even though *E. faecalis* is ubiquitous.
- Effects on animal health are sporadic and linked to certain risk factors. The disease is still treatable and manageable.
- The pathogen is present in all Member States and multidrug-resistant strains have been reported from several Member States. Therefore, there may be a long-term impact on animal health.
- There could be a significant economic impact on young and adult chickens as well as embryos.

3.2.2. Overall outcome on Article 5 criteria

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, a criterion is considered fulfilled when the lower bound of the median range lays above 66%.

According to the results shown in Table 6, AMR *E. faecalis* complies with four criteria of the first set (A(i)–A(iv)), but there is uncertainty on the assessment on compliance with criterion A(v) (33–66% probability). Therefore, it is uncertain whether AMR *E. faecalis* can be considered eligible to be listed for Union intervention as laid down in Article 5 of the AHL. The estimated overall probability range for the AMR bacterium being eligible to be listed is 33–66% (Figure 1).

3.3. Assessment of AMR *Enterococcus faecalis* according to criteria in Annex IV for the purpose of categorisation as in Article 9 of the AHL

In Tables 7–11 and related graphs (Figures 2–4), the results of the expert judgement on AMR *E. faecalis* in poultry according to the criteria in Annex IV of the AHL, for the purpose of categorisation as in Article 9, are presented.

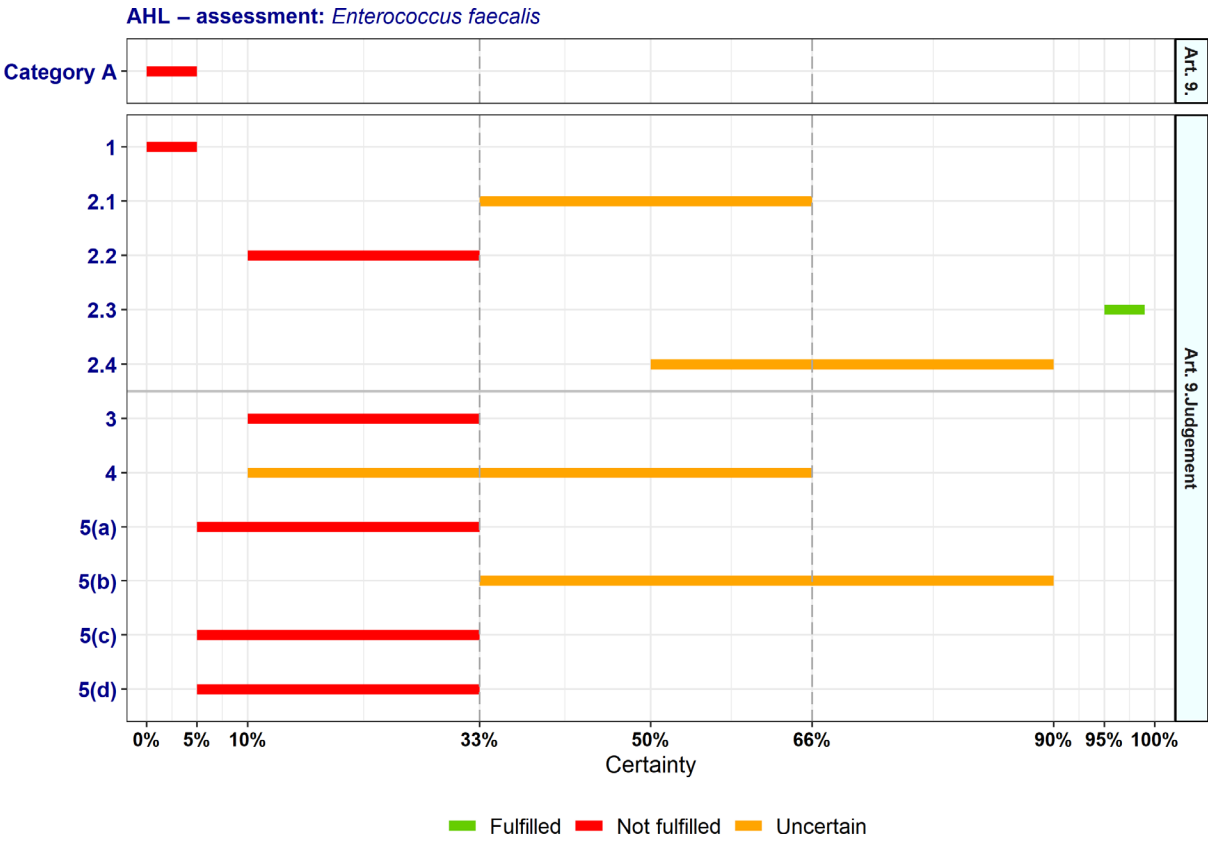
The distribution of the individual answers (probability ranges) provided by each expert for each criterion are reported in Sections B.1 and B.2 of Appendix B.

3.3.1. Detailed outcome on Category A criteria

Table 7: Outcome of the expert judgement related to the criteria of Section 1 of Annex IV (Category A of Article 9)

	Criteria to be met by the disease: The disease needs to fulfil all of the following criteria	**Outcome**			
		Median range (%)	**Criterion fulfilment**	**Number of na**	**Number of experts**
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	0–5	Not fulfilled	0	14
2.1	The disease is highly transmissible	33–66	Uncertain	0	12
2.2	There are possibilities of airborne or waterborne or vector-borne spread	10–33	Not fulfilled	0	12
2.3	The disease affects multiple species of kept and wild animals or single species of kept animals of economic importance	95–99	Fulfilled	0	14
2.4	The disease may result in high morbidity and significant mortality rates	50–90	Uncertain	0	13
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 for public health, including epidemic or pandemic potential or possible significant threats to food safety	10–33	Not fulfilled	0	14
4	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	10–66	Uncertain	0	14
5(a)	The disease has a significant impact on society, with in particular an impact on labour markets	5–33	Not fulfilled	0	14
5(b)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	33–90	Uncertain	0	14
5(c)	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	5–33	Not fulfilled	0	14
5(d)	The disease has a significant impact in the long term on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	5–33	Not fulfilled	0	14

na: not applicable.



Category A: The probability of the disease to be categorised according to Section 1 of Annex IV of the AHL (overall outcome).

Figure 2: Outcome of the expert judgement on criteria of Section 1 of Annex IV and overall probability of the AMR bacterium to be fitting in Category A of Article 9

3.3.1.1. Reasoning for uncertain outcome on Category A criteria

Criterion 2.1 (The disease is highly transmissible):

- *E. faecalis* seems to spread rapidly within flocks.
- It is highly transmissible among young chickens (80% infected in 24 hours under experimental conditions).
- Most animals get exposed early in their life, which supports high transmissibility.
- It can be highly transmissible, but this is not the usual case.
- Antimicrobial resistance genes can be transferred and quickly circulate in a flock.

Criterion 2.4 (The disease may result in high morbidity and significant mortality rates):

- Prevalence and incidence, morbidity and mortality rates are difficult to estimate because AMR *E. faecalis* are virulent only on occasion, and typically cause secondary infections.
- AMR *E. faecalis* may result in high morbidity and significant mortality in certain age groups, i.e. in young birds.
- Case-fatality rates of 23% and 25% in laying hens are significant.
- *E. faecalis* infections may result in embryo mortality.
- *E. faecalis* seems in most cases of mortality to be associated with co-factors, e.g. co-infections.
- Mortality can be reduced by good management practices, which are common in commercial poultry production.
- Mortality rates are not significant at population level.

Criterion 4 (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):

- Few data on economic impact are available. Exact costs are unknown.

- There is a lack of precise estimates on the prevalence of AMR strains.
- Increased mortality in layers and increased embryo mortality can probably cause substantial costs related to AMR strains. These are common now and may be even more common in the future.
- *E. faecalis* may cause high morbidity and mortality in young poultry.
- The pathogen is present in all Member States and multidrug-resistant strains have been reported from several of these. Therefore, there may be a long-term impact on animal health.

Criterion 5(b) (the disease has a significant impact on animal welfare, by causing suffering of large numbers of animals):

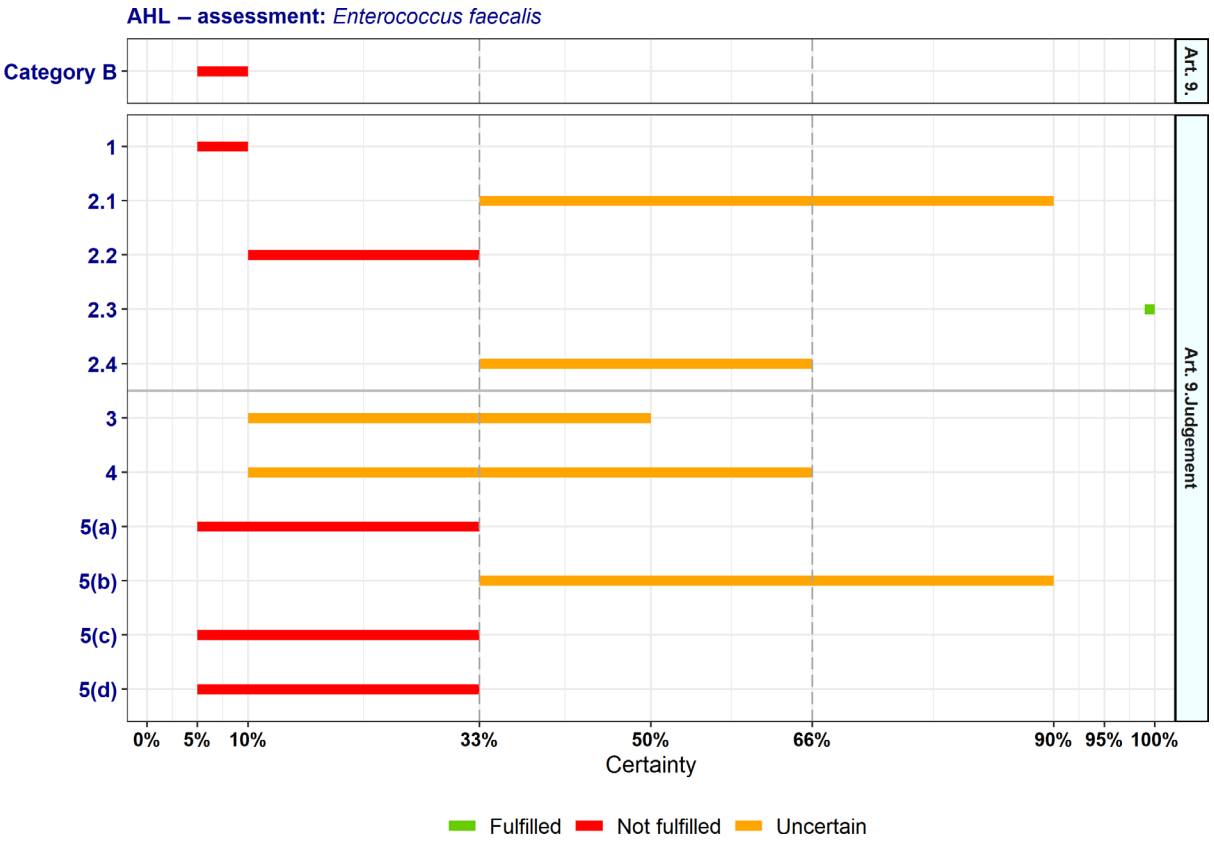
- Clinical conditions can be severe.
- *E. faecalis* can be associated with both high morbidity and mortality, especially in young birds, and also with chronic conditions in adult birds.
- Given that poultry are affected, we are talking about large numbers of animals.
- AMR clones (tetracycline) may increase the impact on animal welfare, as they are linked to pathological manifestations, frequent and hard to treat.
- Morbidity seems to be only slightly above the baseline, compared with other pathogens.

3.3.2. Detailed outcome on Category B criteria

Table 8: Outcome of the expert judgement related to the criteria of Section 2 of Annex IV (Category B of Article 9)

Criteria to be met by the disease: The disease needs to fulfil all of the following criteria		**Outcome**			
		Median range (%)	**Criterion fulfilment**	**Number of na**	**Number of experts**
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	5–10	Not fulfilled	0	13
2.1	The disease is moderately to highly transmissible	33–90	Uncertain	0	12
2.2	There are possibilities of airborne or waterborne or vector-borne spread	10–33	Not fulfilled	0	12
2.3	The disease affects single or multiple species	–	Fulfilled	0	14
2.4	The disease may result in high morbidity with in general low mortality	33–66	Uncertain	0	13
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 for public health, including epidemic potential or possible significant threats to food safety	10–50	Uncertain	0	14
4	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	10–66	Uncertain	0	14
5(a)	The disease has a significant impact on society, with in particular an impact on labour markets	5–33	Not fulfilled	0	14
5(b)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	33–90	Uncertain	0	14
5(c)	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	5–33	Not fulfilled	0	14
5(d)	The disease has a significant impact in the long term on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	5–33	Not fulfilled	0	14

na: not applicable.



Category B: The probability of the disease to be categorised according to Section 2 of Annex IV of the AHL (overall outcome).

Figure 3: Outcome of the expert judgement on criteria of Section 2 of Annex IV and overall probability of the AMR bacterium to be fitting in Category B of Article 9

3.3.2.1. Reasoning for uncertain outcome on Category B criteria

Criterion 2.1 (the disease is moderately to highly transmissible):

- The pathogen is highly transmissible in younger chickens and moderately transmissible in adults. Therefore, there is an age variation.
- *E. faecalis* alone does not display an 'outbreak nature'.
- The transmission rate depends on many factors.
- Many different *E. faecalis* clones are able to give rise to secondary infection. Such an 'outbreak' would then be polyclonal suggesting that host factors are associated with new infections rather than transmission of single strain(s).
- Antimicrobial resistance genes can be transferred.
- There is no evidence suggesting a competitive advantage of AMR strains.

Criterion 2.4 (the disease may result in high morbidity and in general low mortality):

- Prevalence and incidence, morbidity and mortality rates are difficult to calculate.
- High mortality is not commonly reported. It seems to be lower (in general between 2% and 8%) compared to other bacteria.
- Mortality can be reduced by good management practices, which are common in commercial poultry production.

Criterion 3 (the disease has a zoonotic potential with significant consequences for public health, including epidemic potential, or possible significant threats to food safety):

- Few data are available.
- Humans can be affected by *E. faecalis* from poultry and resistance genes can be transferred.

- Food-borne transmission through *E. faecalis*-contaminated *food products is considered a risk for transmission between animals and humans.*
- DALY has been estimated for vancomycin-resistant enterococci (including *E. faecalis*) to be 5.49 per 100,000 population, which in % corresponded to one of the greatest burdens of infection.
- Multidrug-resistant strains are widespread.
- No significant consequences for immunocompetent individuals are expected.
- Hundreds of cases can be considered an epidemic.
- Whereas it is safe to conclude that humans can get infections from avian *E. faecalis*, it is uncertain how (and if) the strain was transmitted from household chickens, as it could be indirectly through contaminated meat or dirt or it could be directly through close contact with the birds. In addition, a common source of infection could not be ruled out. Therefore, a zoonotic potential is not proven.

Criterion 4 (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): See above in Section 3.3.1.1.

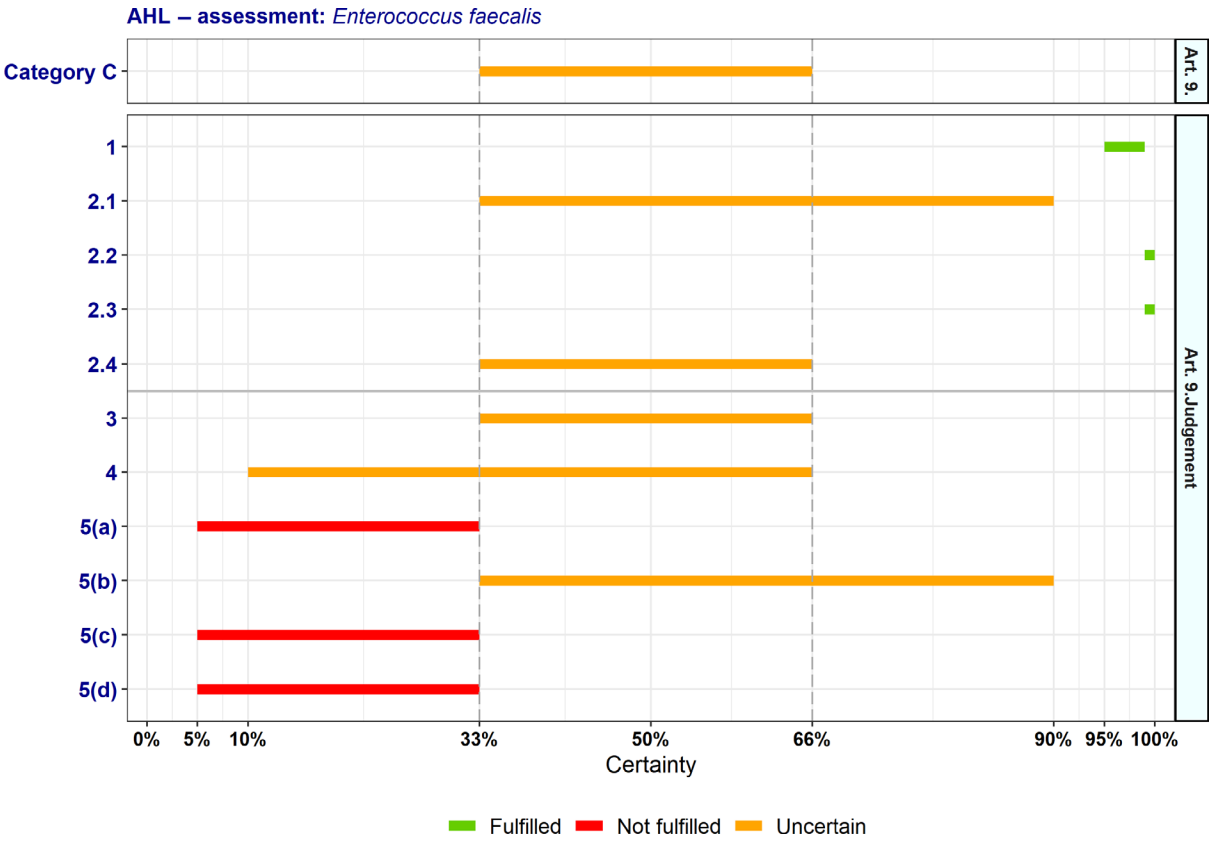
Criterion 5(b) (the disease has a significant impact on animal welfare, by causing suffering of large numbers of animals): See above in Section 3.3.1.1.

3.3.3. Detailed outcome on Category C criteria

Table 9: Outcome of the expert judgement related to the criteria of Section 3 of Annex IV (Category C of Article 9)

Criteria to be met by the disease: The disease needs to fulfil all of the following criteria		**Outcome**			
		Median range (%)	**Criterion fulfilment**	**Number of na**	**Number of experts**
1	The disease is present in the whole or part of the Union territory with an endemic character	95–99	Fulfilled	0	14
2.1	The disease is moderately to highly transmissible	33–90	Uncertain	0	12
2.2	The disease is transmitted mainly by direct or indirect transmission	–	Fulfilled	0	12
2.3	The disease affects single or multiple species	–	Fulfilled	0	14
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	33–66	Uncertain	0	13
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 for public health or possible significant threats to food safety	33–66	Uncertain	0	13
4	The disease has a significant impact on the economy of the Union, mainly related to its direct impact on certain types of animal production systems	10–66	Uncertain	0	14
5(a)	The disease has a significant impact on society, with in particular an impact on labour markets	5–33	Not fulfilled	0	14
5(b)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	33–90	Uncertain	0	14
5(c)	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	5–33	Not fulfilled	0	14
5(d)	The disease has a significant impact in the long term on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	5–33	Not fulfilled	0	14

na: not applicable.



Category C: The probability of the disease to be categorised according to Section 3 of Annex IV of the AHL (overall outcome).

Figure 4: Outcome of the expert judgement on criteria of Section 3 of Annex IV and overall probability of the AMR bacterium to be fitting in Category C of Article 9

3.3.3.1. Reasoning for uncertain outcome on Category C criteria

Criterion 2.1 (The disease is moderately to highly transmissible): See above in Section 3.3.2.1.

Criterion 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):

- Embryo mortality and chronic disease in adult chickens can be considered production loss.

Criterion 3 (The disease has a zoonotic potential with significant consequences for public health OR possible significant threats to food safety):

- Few data are available.
- Humans can be affected by *E. faecalis* from poultry and resistance genes can be transferred.
- Food-borne transmission through *E. faecalis*-contaminated *food products is considered a risk for transmission between animals and humans.*
- DALY has been estimated for vancomycin-resistant enterococci (including *E. faecalis*) to be 5.49 per 100,000 population, which in % corresponded to one of the greatest burdens of infection.
- Multidrug-resistant strains are widespread.
- No significant consequences for immunocompetent individuals are expected.
- Whereas it is safe to conclude that humans can get infections from avian *E. faecalis*, it is uncertain how (and if) the strain was transmitted from household chickens, as it could be indirectly through contaminated meat or dirt or it could be directly through close contact with the birds. In addition, a common source of infection could not be ruled out. Therefore, a zoonotic potential is not proven.

Criterion 4 (The disease has a significant impact on the economy of the Union, mainly related to its direct impact on certain types of animal production systems):

- Few data on economic impact are available. Exact costs are unknown.
- There is a lack of precise estimates on the prevalence of AMR strains.
- Increased mortality in layers and increased embryo mortality can probably cause substantial costs related to AMR strains. These are common now and may be even more common in the future.
- *E. faecalis* may cause high morbidity and mortality in broilers.
- The pathogen is present in all Member States and multidrug-resistant strains have been reported from several. Therefore, there may be a long-term impact on animal health.

Criterion 5(b) (The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals): See above in Section 3.3.1.1.

3.3.4. Detailed outcome on Category D criteria

Table 10: Outcome of the expert judgement related to the criteria of Section 4 of Annex IV (Category D of Article 9)

Diseases in Category D **need to fulfil criteria of Section 1, 2,3 or 5 of Annex IV** of the AHL **and the following**:		Outcome			
		Median range (%)	**Criterion fulfilment**	**Number of na**	**Number of experts**
D	The risk posed by the disease can be effectively and proportionately mitigated by measures concerning movements of animals and products in order to prevent or limit its occurrence and spread	1–10	Not fulfilled	0	14

na: not applicable.

3.3.5. Detailed outcome on Category E criteria

Table 11: Outcome of the expert judgement related to the criteria of Section 5 of Annex IV (Category E of Article 9)

Diseases in Category E **need to fulfil criteria of Section 1, 2or 3 of Annex IV** of the AHL **and/or the following**:		Outcome	
		Median range (%)	**Fulfilment**
E	Surveillance of the disease is necessary for reasons related 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.)	33–66	Uncertain

3.3.6. Overall outcome on criteria in Annex IV for the purpose of categorisation as in Article 9

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 points (a) to (e) of Article 9(1) of the AHL) if it 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 7–11. According to the assessment methodology, a criterion is considered fulfilled when the lower bound of the median range lays above 66%.

The overall outcome of the assessment on criteria in Annex IV of the AHL, for the purpose of categorisation of AMR *E. faecalis* as in Article 9, is presented in Table 12 and Figure 5.

Table 12: Outcome of the assessment on criteria in Annex IV of the AHL for the purpose of categorisation as in Article 9

	Article 9 criteria										
	1° set of criteria					2° set of criteria					
	1	2.1	2.2	2.3	2.4	3	4	5(a)	5(b)	5(c)	5(d)
Category	**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	0–5	33–66	10–33	95–99	50–90	10–33	10–66	5–33	33–90	5–33	5–33
B	5–10	33–90	10–33	–	33–66	10–50	10–66	5–33	33–90	5–33	5–33
C	95–99	33–90	–	–	33–66	33–66	10–66	5–33	33–90	5–33	5–33
D	1–10										
E	33–66										

Probability ranges (% certainty) (green: fulfilled; red: not fulfilled; orange: uncertain).

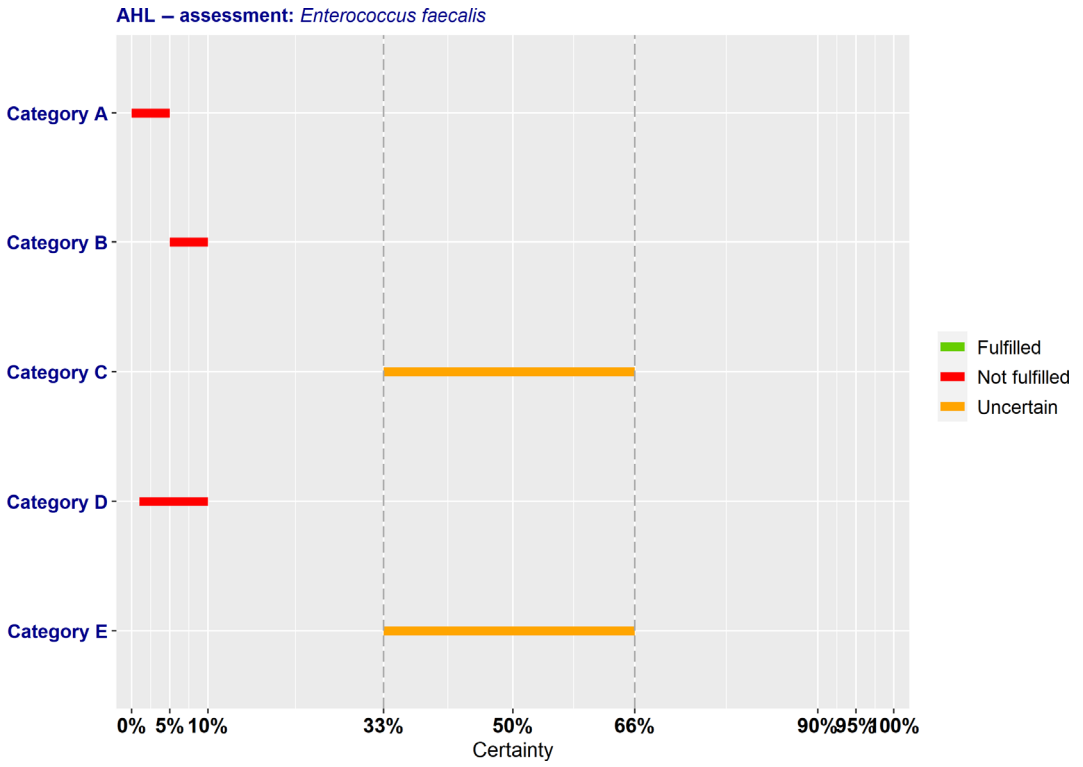


Figure 5: Outcome of the expert judgement on criteria in Annex IV and overall probabilities for categorisation of the AMR bacterium in accordance with Article 9

According to the assessment here performed, AMR *E. faecalis* complies with the following criteria of Section 1–5 of Annex IV of the AHL for the application of the disease prevention and control rules referred to in points (a) to (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, AMR *E. faecalis* complies only with criterion 2.3 (95–99% probability). The assessment was inconclusive on compliance with criteria 2.1 (33–66% probability) and 2.4 (50–90% probability). To be eligible for Category A, a disease needs to comply additionally with one of the criteria of the second set (3, 4, 5(a)–(d)) and AMR *E. faecalis* does not comply with any apart from criteria 4 (10–66% probability) and 5 (b) (33–90% probability), for which the assessment was inconclusive. Overall, it was assessed with 0–5% probability that AMR *E. faecalis* may be assigned to Category A according to criteria in Section 1 of Annex IV for the purpose of categorisation as in Article 9 of the AHL.
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, AMR *E. faecalis* complies only with criterion 2.3. The assessment was inconclusive on compliance with criteria 2.1 (33–90% probability) and 2.4 (33–66% probability). To be eligible for Category B, a disease needs to comply additionally with one of the criteria of the second set (3, 4, 5(a)–(d)) and AMR *E. faecalis* does not comply with any apart from criteria 3, 4 and 5(b), for which the assessment was inconclusive (10–50%, 10–66% and 33–90% probability of meeting the criteria, respectively). Overall, it was assessed with 5–10% probability that AMR *E. faecalis* may be assigned to Category B according to criteria in Section 2 of Annex IV for the purpose of categorisation as in Article 9 of the AHL.
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, AMR *E. faecalis* complies with criteria 1 (95–99% probability), 2.2 and 2.3. The assessment was inconclusive on compliance with criteria 2.1 (33–90% probability) and 2.4 (33–66% probability). To be eligible for Category C, a disease needs to comply additionally with one of the criteria of the second set (3, 4, 5(a)–(d)) and AMR *E. faecalis* does not comply with any apart from criteria 3, 4 and 5(b), for which the assessment was inconclusive (33–66%, 10–66% and 33–90% probability of meeting the criteria, respectively). Overall, it was assessed with 33–66% probability that AMR *E. faecalis* may be assigned to Category C according to criteria in Section 3 of Annex IV for the purpose of categorisation as in Article 9 of the AHL.
4) To be assigned to Category D, a disease needs to comply with criteria of Section 1, 2, 3 or 5 of Annex IV of the AHL and with the specific criterion D of Section 4, with which AMR *E. faecalis* does not comply (1–10% probability).
5) To be assigned to Category E, a disease needs to comply with criteria of Section 1, 2 or 3 of Annex IV of the AHL, and/or the surveillance of the disease is necessary for reasons related 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, for which the assessment is inconclusive (33–66% probability of fulfilling the criteria).

3.4. Assessment of AMR *Enterococcus faecalis* according to Article 8 criteria of the AHL

In this section, the results of the assessment on the criteria of Article 8(3) of the AHL for AMR *E. faecalis* are presented. 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 the list if they are affected or if they pose a risk for the spread of a specific listed disease because:

a) they are susceptible to 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 the possible role of biological or mechanical vectors.[2]

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 animal species to be listed for AMR *E. faecalis* according to the criteria of Article 8(3) of the AHL are as displayed in Table 13 (elaborated from information reported in Section 3.1.1.1 of the present document).

The table contains all animal species in which AMR *E. faecalis* has been described, but also those animal species from which only the bacterium itself has been isolated. The latter makes susceptibility to AMR clones likely.

Table 13: Animal species to be listed for AMR *E. faecalis* according to the criteria of Article 8

	Class/order	Family	Genus/species
Susceptible	Anseriformes	Anatidae	Duck (*Anas platyrhynchos domesticus*)
	Galliformes	Phasianidae	Chicken (*Gallus gallus domesticus*)
			Grey partridge (*Perdix perdix*)
			Ring-necked pheasant (*Phasianus colchicus*)
			Quail (*Coturnix coturnix*)
			Japanese quail (*Coturnix japonica*)
			Turkey (*Meleagris*)
	Psittaciformes	Psittacidae	Blue-fronted parrot (*Amazona aestiva*)
	Struthioniformes	Struthionidae	Ostrich (*Struthio camelus*)
	Mammals		
	Squamata	Viperidae	Golden lancehead (*Bothrops insularis*)
Reservoir	Mammals, reptiles, birds, insects		
	Charadriiformes	Laridae	European herring gull (*Larus argentatus*)
			Grey gull (*Leucophaeus modestus*)
			Laughing gull (*Leucophaeus atricilla*)
	Columbiformes	Columbidae	Pigeon (*Columba*)
			Mourning dove (*Zenaida macroura*)
	Coraciiformes	Meropidae	European bee-eater (*Merops apiaster*)
	Galliformes	Phasianidae	Wild turkey (*Meleagris gallopavo*)
	Passeriformes	Corvidae	American crow (*Corvus brachyrhynchos*)
			Rook (*Corvus frugilegus*)
		Fringillidae	European goldfinch (*Carduelis carduelis*)
			European greenfinch (*Carduelis chloris*)
			European serin (*Serinus serinus*)
		Hirundinidae	African river martin (*Pseudochelidon eurystomina*)
		Turdidae	American robin (*Turdus migratorius*)
			Common blackbird (*Turdus merula*)
	Pelecaniformes	Pelecanidae	Brown pelican (*Pelecanus occidentalis*)
	Strigiformes	Strigidae	Eastern screech owl (*Megascops asio*)
			Great horned owl (*Bubo virginianus*)
	Carnivora	Canidae	Pampas fox (*Lycalopex gymnocercus*)
		Felidae	Geoffroy's cat (*Leopardus geoffroyi*)
Vector	None		

[2] 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.

4. Conclusions

The AHAW Panel emphasises that the assessment of impacts, as well as prevention and control measures, related to AMR bacteria using the criteria as laid down in Articles 5 and 9 of the AHL is particularly challenging for opportunistic pathogens that can also be found as commensal bacteria in healthy animals.

Generally, there is high level of uncertainty around the occurrence, frequency and distribution of antimicrobial resistance in *E. faecalis*. Since there is no structured data collection or surveillance in place in the EU, it is unclear whether the sporadic reports on the detrimental effects of infection due to AMR *E. faecalis* strains may be representative of the full damage caused by this AMR pathogen. Estimates of prevalence, incidence, morbidity and mortality are difficult to interpret due to the opportunistic nature of *E. faecalis* and disease development being multifactorial (i.e. depending on host and other risk factors, co-infections with other pathogens). Furthermore, assessment of the clinical significance of antimicrobial resistance is difficult due to the lack of poultry-specific clinical breakpoints. Clinical importance, economic impact and zoonotic implications of this bacterial species need further investigation. However, AMR *E. faecalis* (and AMR enterococci in general) are recognised as an emerging problem in the poultry industry and their role is yet to be fully understood. Zoonotic implications around *E. faecalis* seem to be the highest among all AMR pathogens discussed within this framework, and, on top of being an indicator for faecal contamination, the bacterium itself could be considered a potential risk for food hygiene in future.

TOR 1: *For each of those identified AMR bacteria considered most relevant in the EU, following the criteria laid down in Article 7 of the AHL, an assessment on its eligibility to be listed for Union intervention as laid down in Article 5(3) of the AHL*;

- It is uncertain (33–66% probability, 'as likely as not') whether AMR *E. faecalis* can be considered eligible to be listed for Union intervention as laid down in Article 5 of the AHL.

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

- The AHAW Panel considered with 0–5% probability (from 'almost impossible' to 'extremely unlikely') that AMR *E. faecalis* meets the criteria as in Section 1 of Annex IV of the AHL, for the application of the disease prevention and control rules referred to in point (a) of Article 9(1) of the AHL.
- The AHAW Panel considered with 5–10% probability ('very unlikely') that AMR *E. faecalis* meets the criteria as in Section 2 of Annex IV of the AHL, for the application of the disease prevention and control rules referred to in point (b) of Article 9(1) of the AHL.
- The AHAW Panel was uncertain (33–66% probability, 'as likely as not') whether AMR *E. faecalis* meets 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.
- The AHAW Panel considered with 1–10% probability (from 'extremely unlikely' to 'very unlikely') that AMR *E. faecalis* meets the criteria as in Section 4 of Annex IV of the AHL, for the application of the disease prevention and control rules referred to in point (d) of Article 9(1) of the AHL.
- The AHAW Panel was uncertain (33–66% probability, 'as likely as not') whether AMR *E. faecalis* meets the criteria as in Section 5 of Annex IV of the AHL, for the application of the disease prevention and control rules referred to in point (e) of Article 9(1) of the AHL.

TOR 3: *For each of the AMR bacteria 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;*

- The animal species that can be considered to be listed for AMR *E. faecalis* according to Article 8(3) of the AHL are mostly birds of the orders Galliformes and Anseriformes, but also mammals and reptiles can serve as reservoirs, as reported in Table 13 in Section 3.4 of the present document.

The AHAW Panel highlights that monitoring of antimicrobial resistance in opportunistic pathogens could help to assess their impacts. Therefore, even though the assessment on AMR *E. faecalis* is

inconclusive on its eligibility to be listed for Union intervention, specific initiatives (e.g. monitoring or applied research) into various aspects of AMR *E. faecalis* can be useful to better understand its distribution and to assess its impact on animal health and welfare in the EU.

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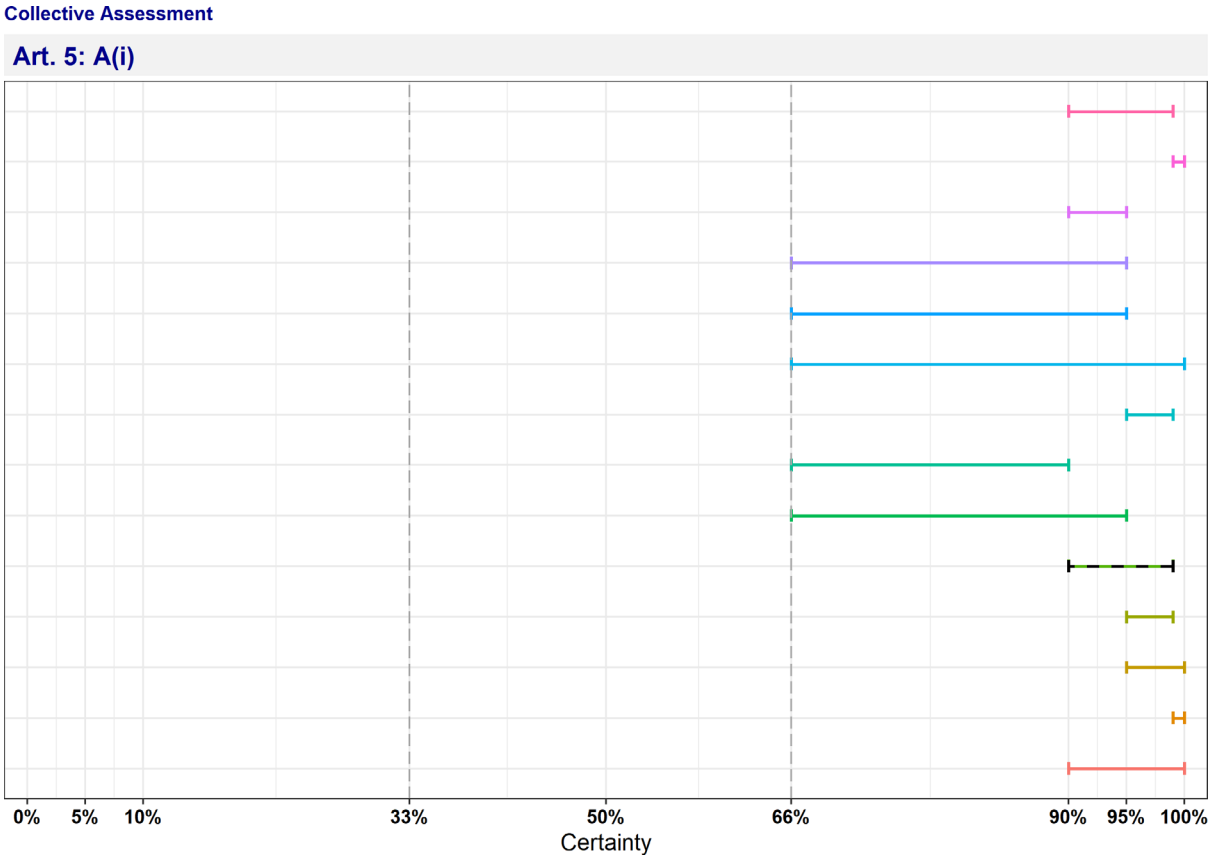
Abbreviations

AHAW	Animal Health and Welfare
AHL	Animal Health Law
AMP	Ampicillin
AMR	Antimicrobial-resistant
APEC	Avian pathogenic *Escherichia coli*
CC	Clonal complex
CFSPH	Center for Food Security and Public Health
CI	Current impact
CITES	Convention on International Trade in Endangered Species
DALY	Disability-adjusted life year
DO	Doxycycline
ERY	Erythromycin
IUCN	International Union for Conservation of Nature
LIN	Lincomycin
MALDI-TOF MS	Matrix-assisted laser desorption ionisation–time-of-flight mass spectrometry
MIC	Minimum inhibitory concentration
MPN	Most probable number
MS	Member State
NWT	Non-wild type
OIE	Office International des Épizooties (World Organisation for Animal Health)
PCR	Polymerase chain reaction
PEN	Penicillin
PI	Potential impact
PFGE	Pulsed-field gel electrophoresis
R	Resistance
SLV	Single locus variant
ST	Sequence type
TLV	Triple locus variant
UTI	Urinary tract infection
TE	Tetracycline
ToR	Term of Reference
VA	Vancomycin

Appendix A – Criteria with certain outcome

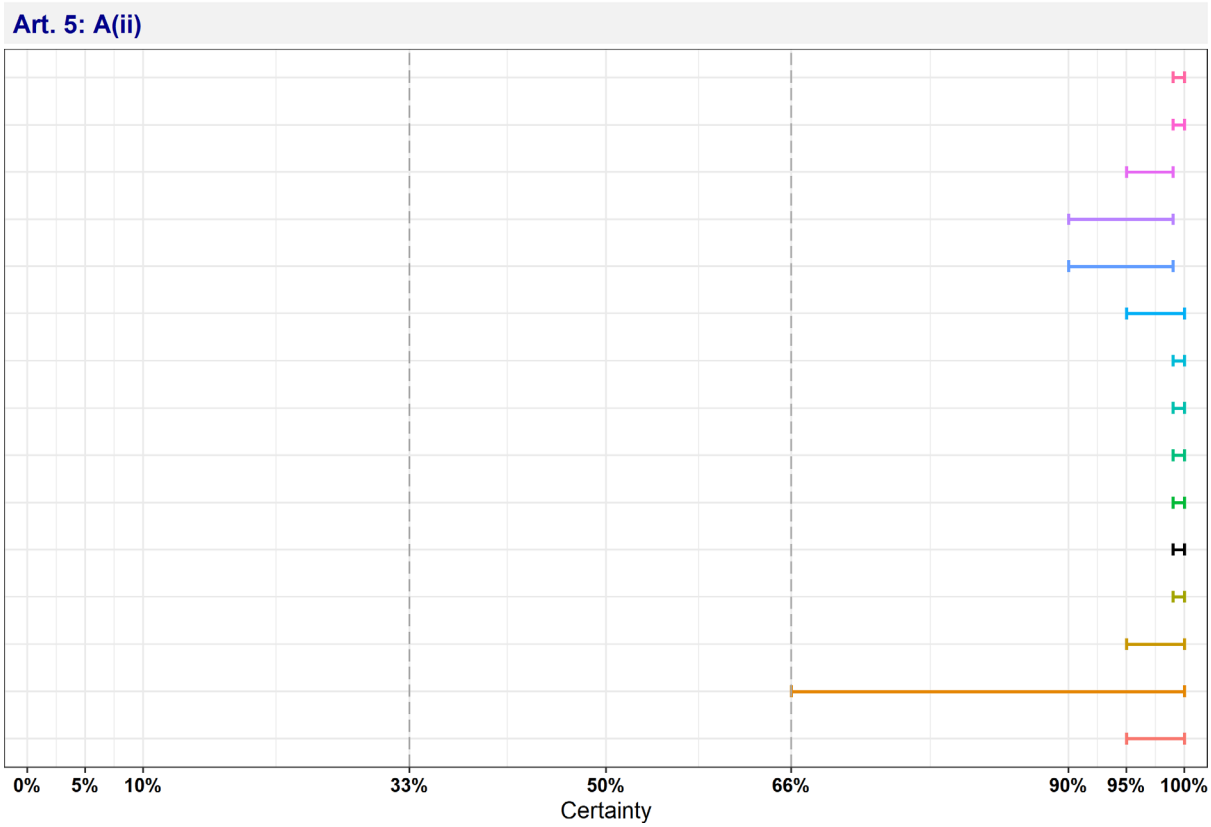
A.1 Article 5 criteria



The median range is displayed as a dashed line.

Figure A.1: Individual probability ranges reflecting fulfilment of criterion A(i) (the disease is transmissible) after the collective judgement

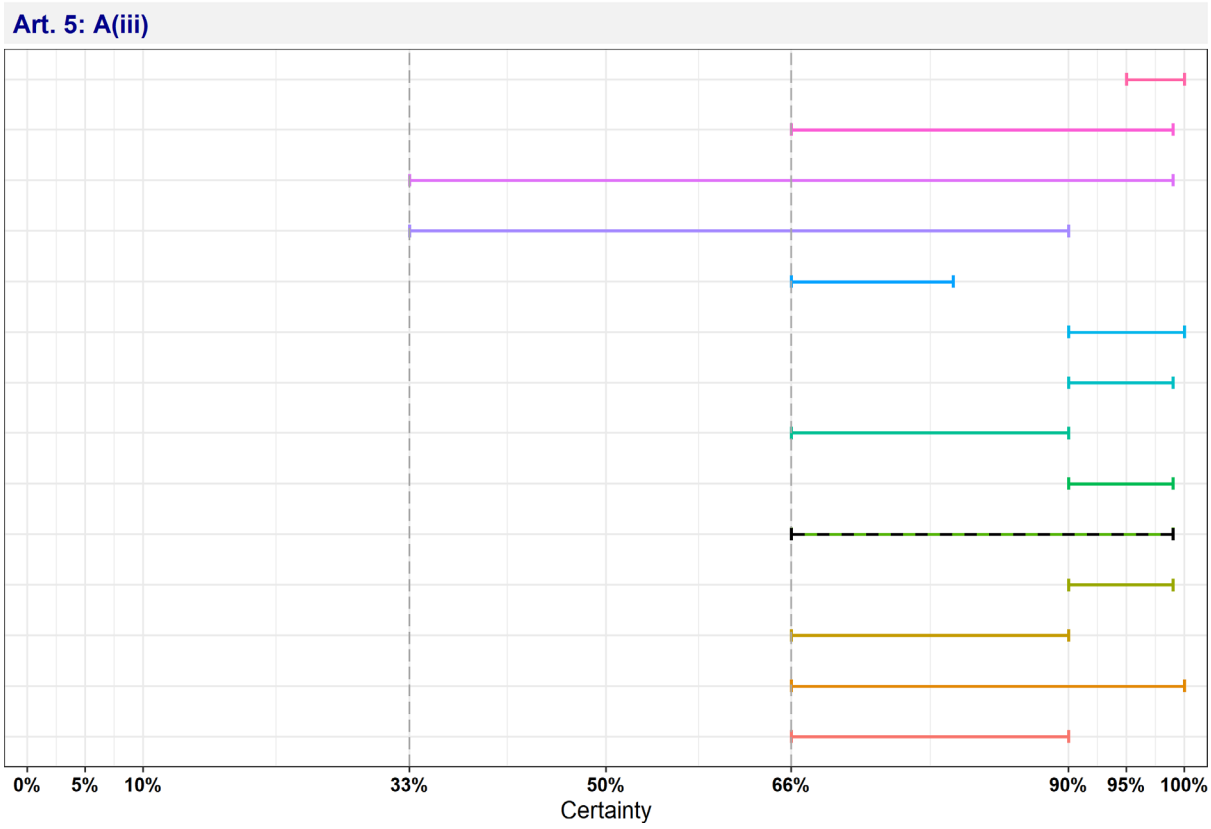
Collective Assessment



The median range is displayed as a dashed line.

Figure A.2: Individual probability ranges reflecting fulfilment of criterion A(ii) (animal species are either susceptible to the disease or vectors and reservoirs thereof exist in the Union) after the collective judgement

Collective Assessment



The median range is displayed as a dashed line.

Figure A.3: Individual probability ranges reflecting fulfilment of criterion A(iii) (the disease causes negative effects on animal health or poses a risk to public health due to its zoonotic character) after the collective judgement

Collective Assessment

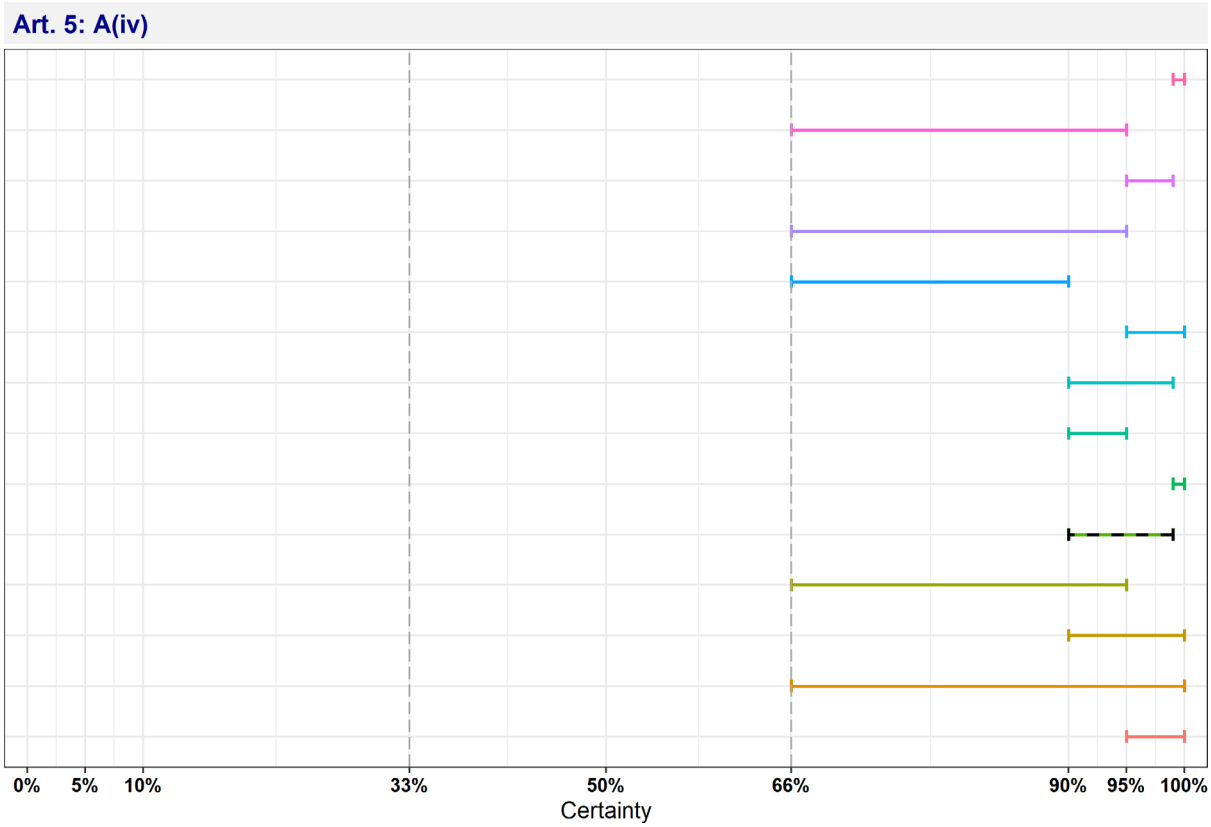


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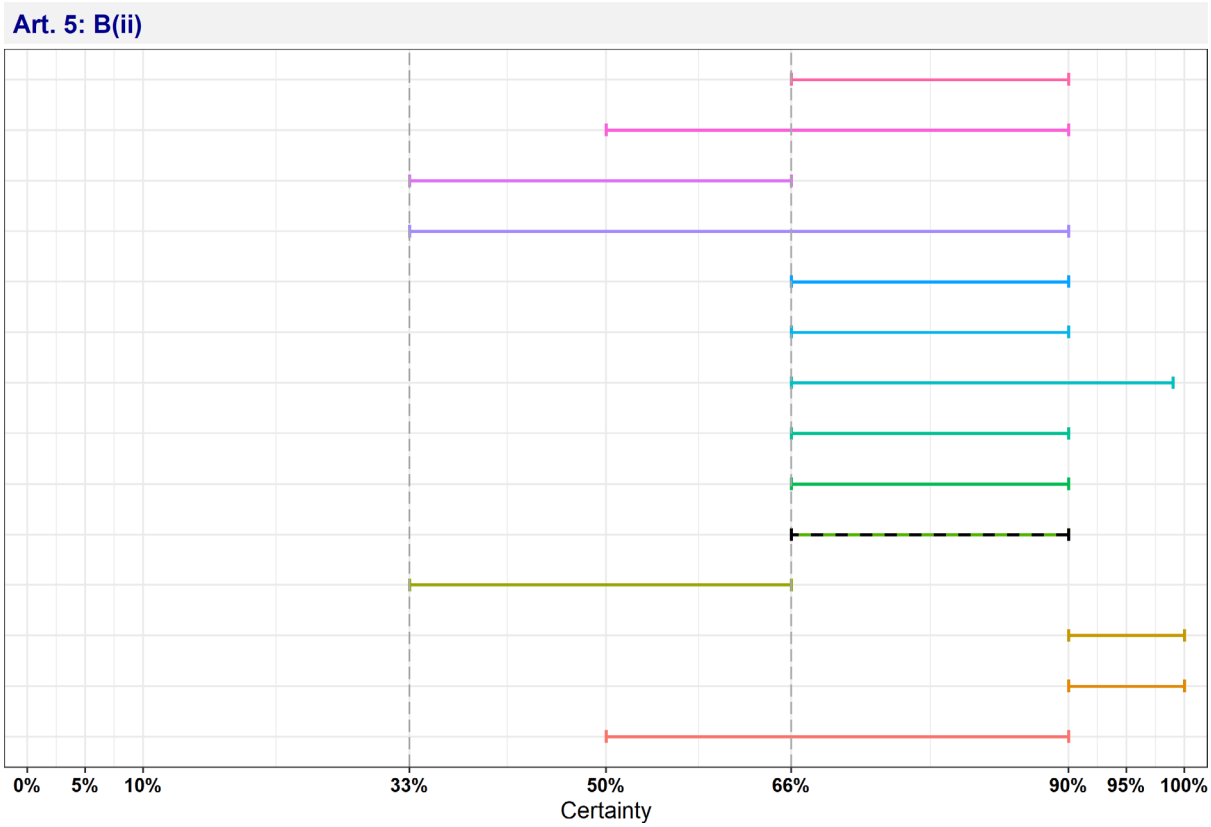
Figure A.4: Individual probability ranges reflecting fulfilment of criterion A(iv) (diagnostic tools are available for the disease) after the collective judgement

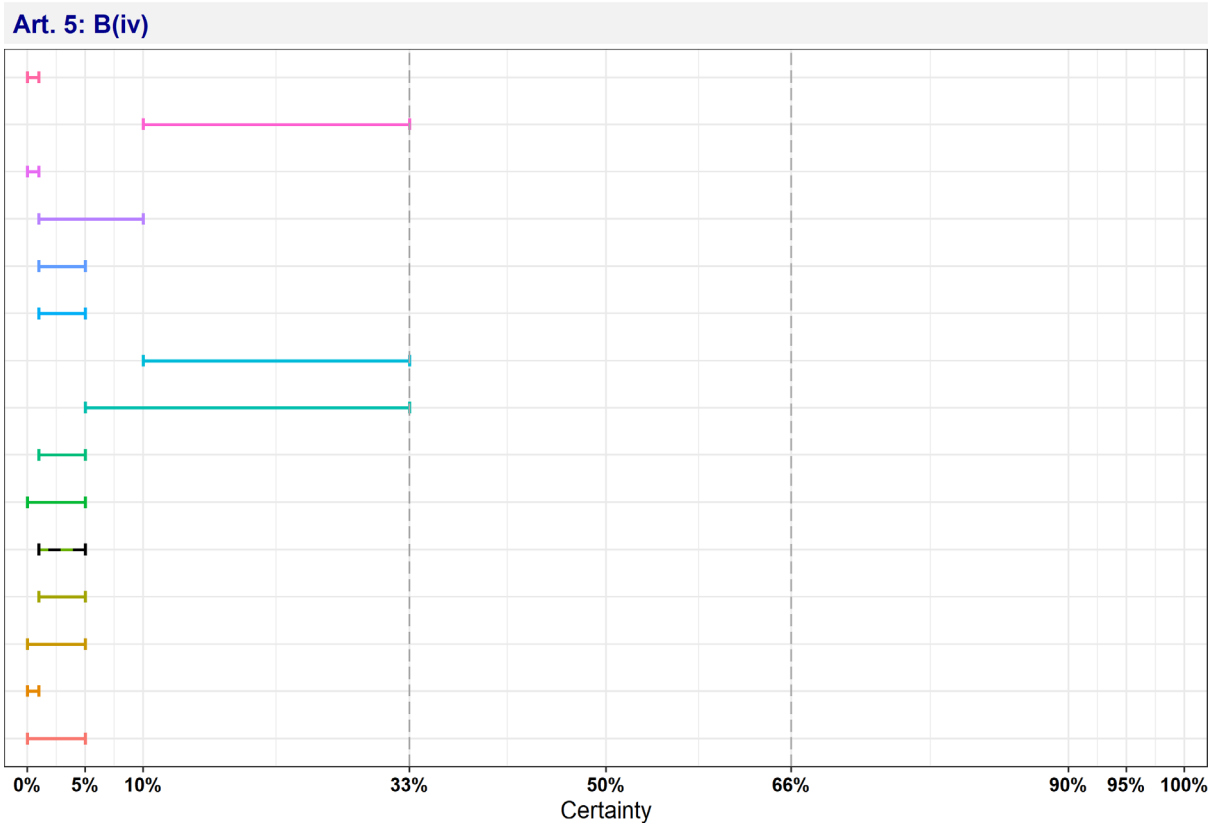
Collective Assessment



The median range is displayed as a dashed line.

Figure A.5: Individual probability ranges reflecting fulfilment of criterion B(ii) (the disease agent has developed resistance to treatments which poses a significant danger to public and/or animal health in the Union) after the collective judgement

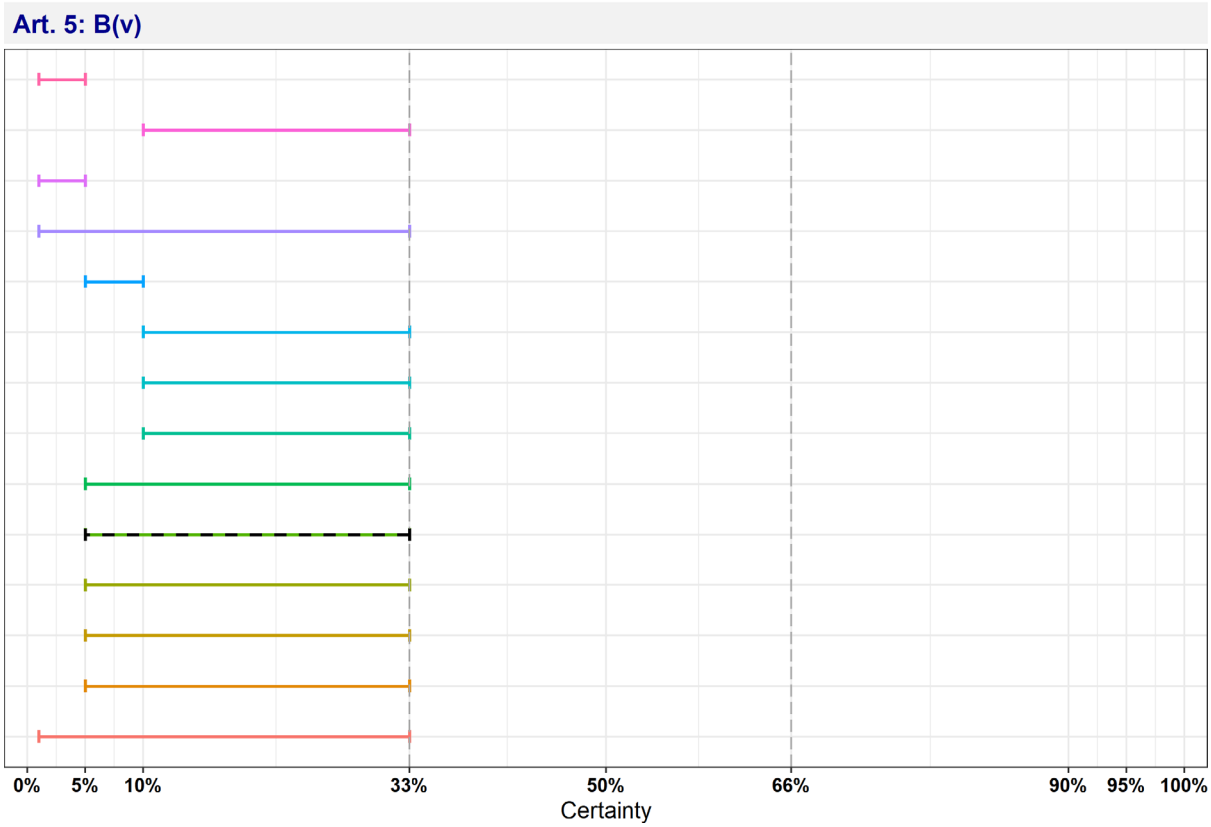
Collective Assessment



The median range is displayed as a dashed line.

Figure A.6: Individual probability ranges reflecting non-fulfilment of criterion B(iv) (the disease has the potential to generate a crisis or the disease agent could be used for the purpose of bioterrorism) after the collective judgement

Collective Assessment



The median range is displayed as a dashed line.

Figure A.7: Individual probability ranges reflecting non-fulfilment of criterion B(v) (the disease has or could have a significant negative impact on the environment, including biodiversity, of the Union) after the collective judgement

A.2. Article 9 criteria

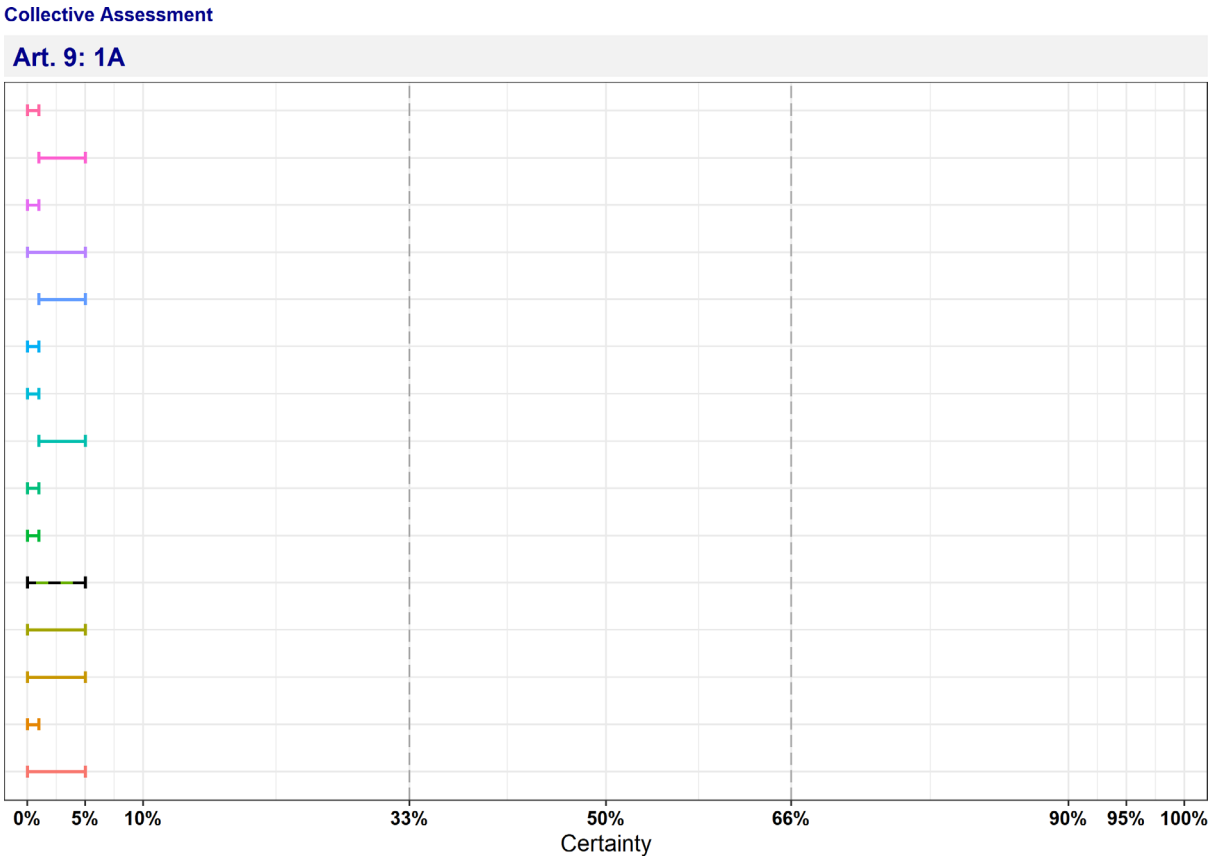
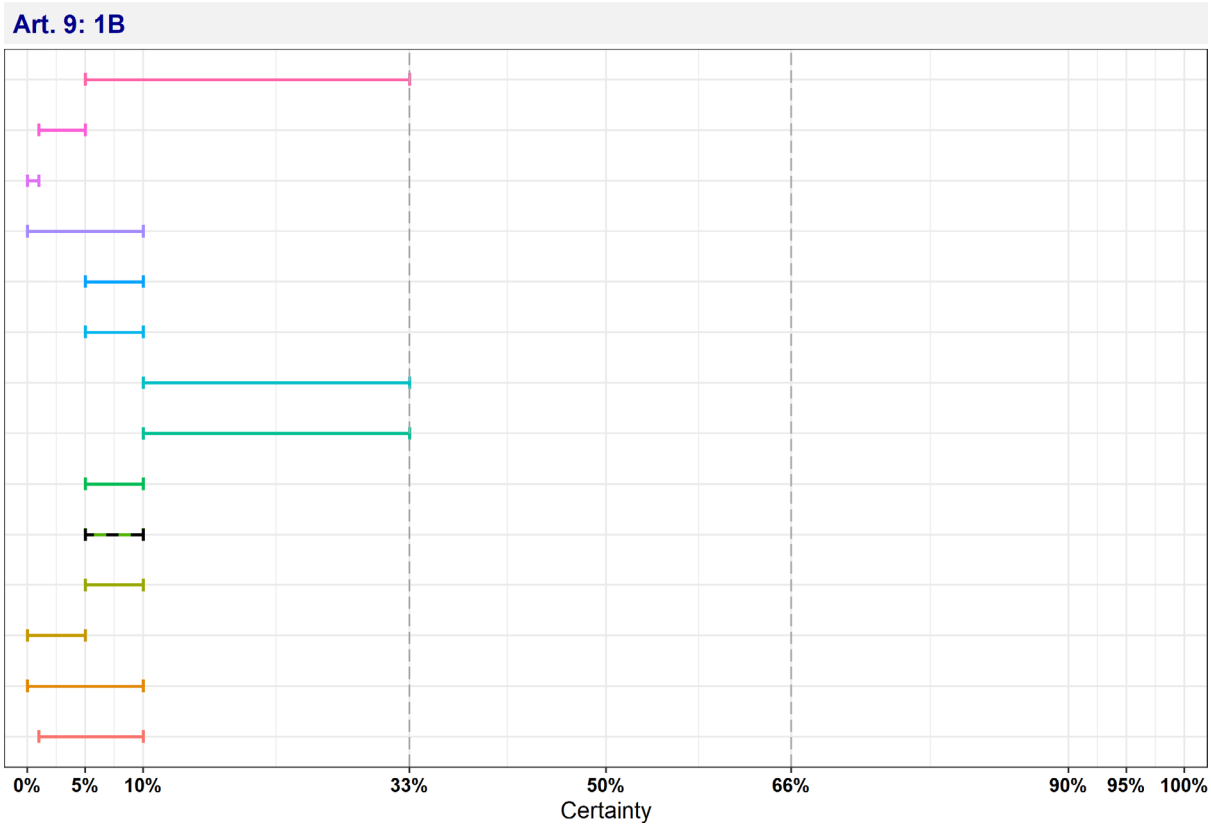


Figure A.8: Individual probability ranges reflecting non-fulfilment of criterion 1A (the disease is not present in the territory of the Union or present only in exceptional cases (irregular introductions) or present in only in a very limited part of the territory of the Union) after the collective judgement

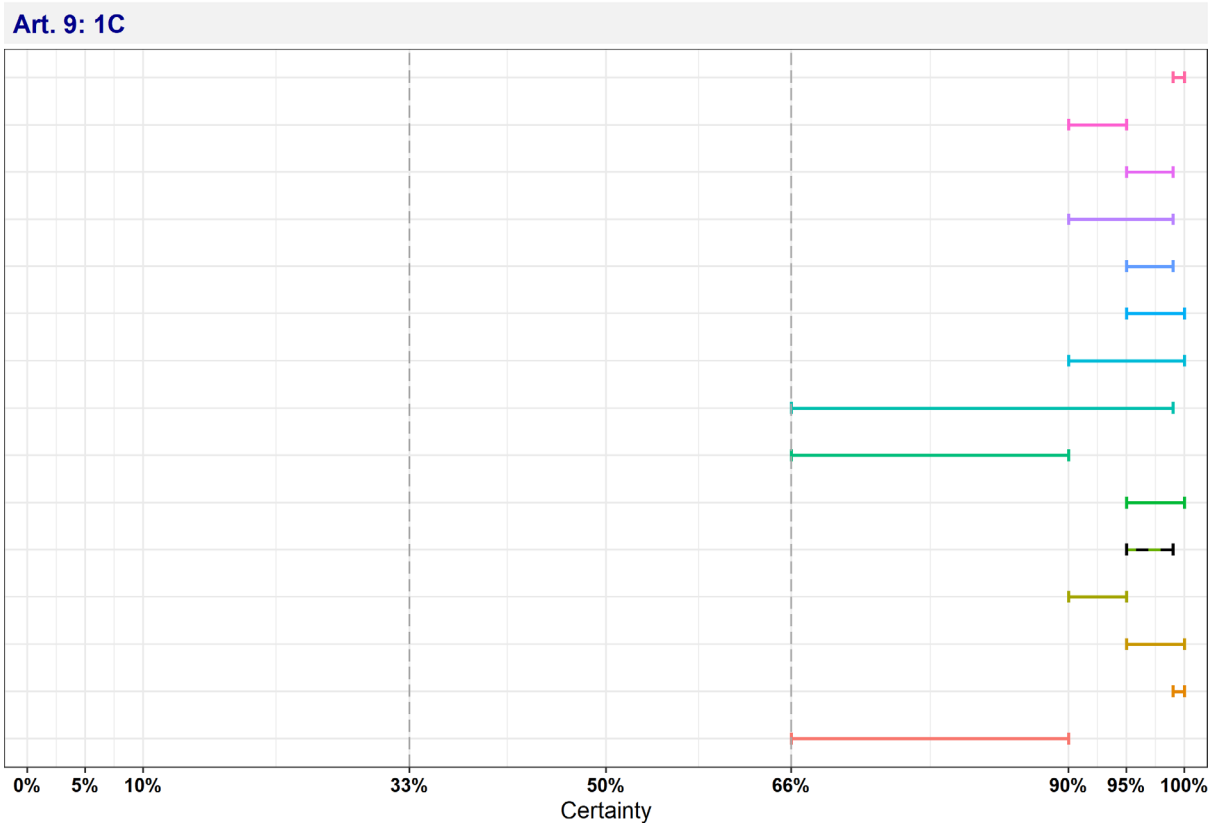
Collective Assessment



The median range is displayed as a dashed line.

Figure A.9: Individual probability ranges reflecting non-fulfilment of criterion 1B (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) after the collective judgement

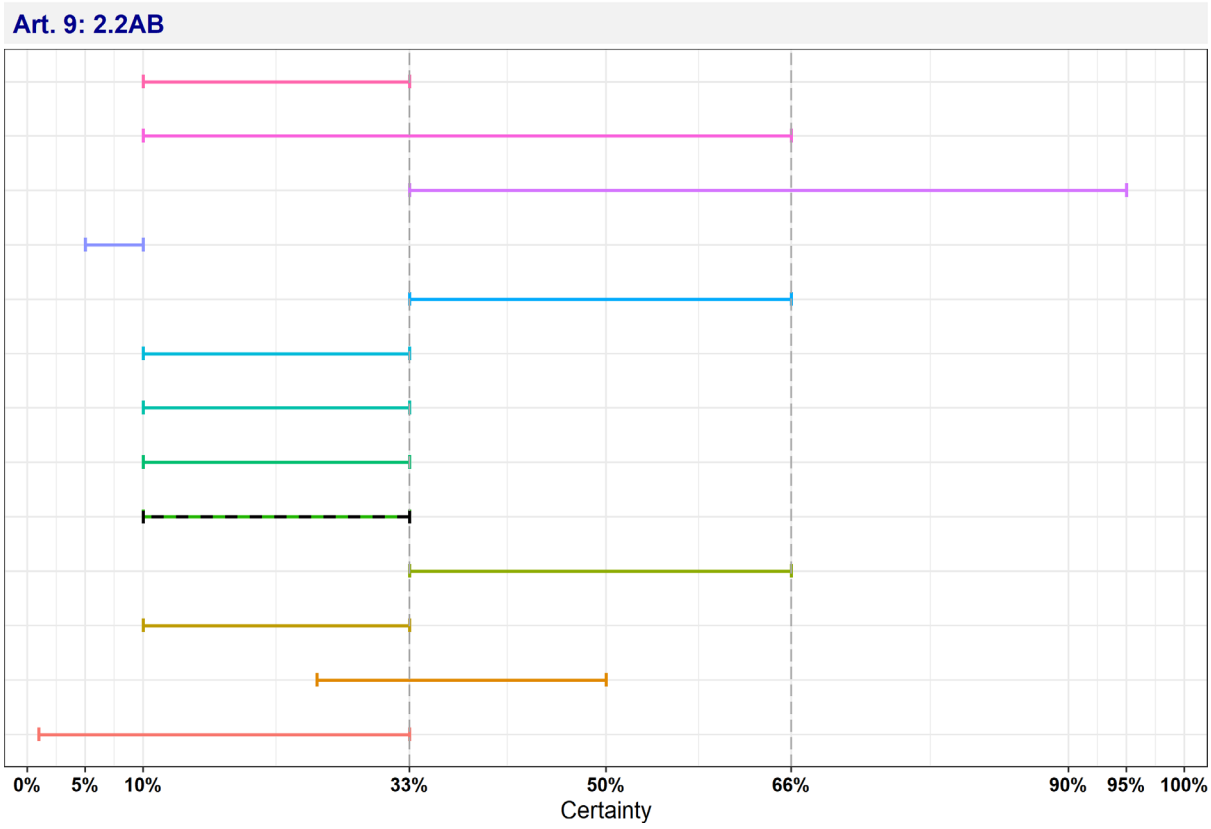
Collective Assessment



The median range is displayed as a dashed line.

Figure A.10: Individual probability ranges reflecting fulfilment of criterion 1C (the disease is present in the whole or part of the Union territory with an endemic character) after the collective judgement

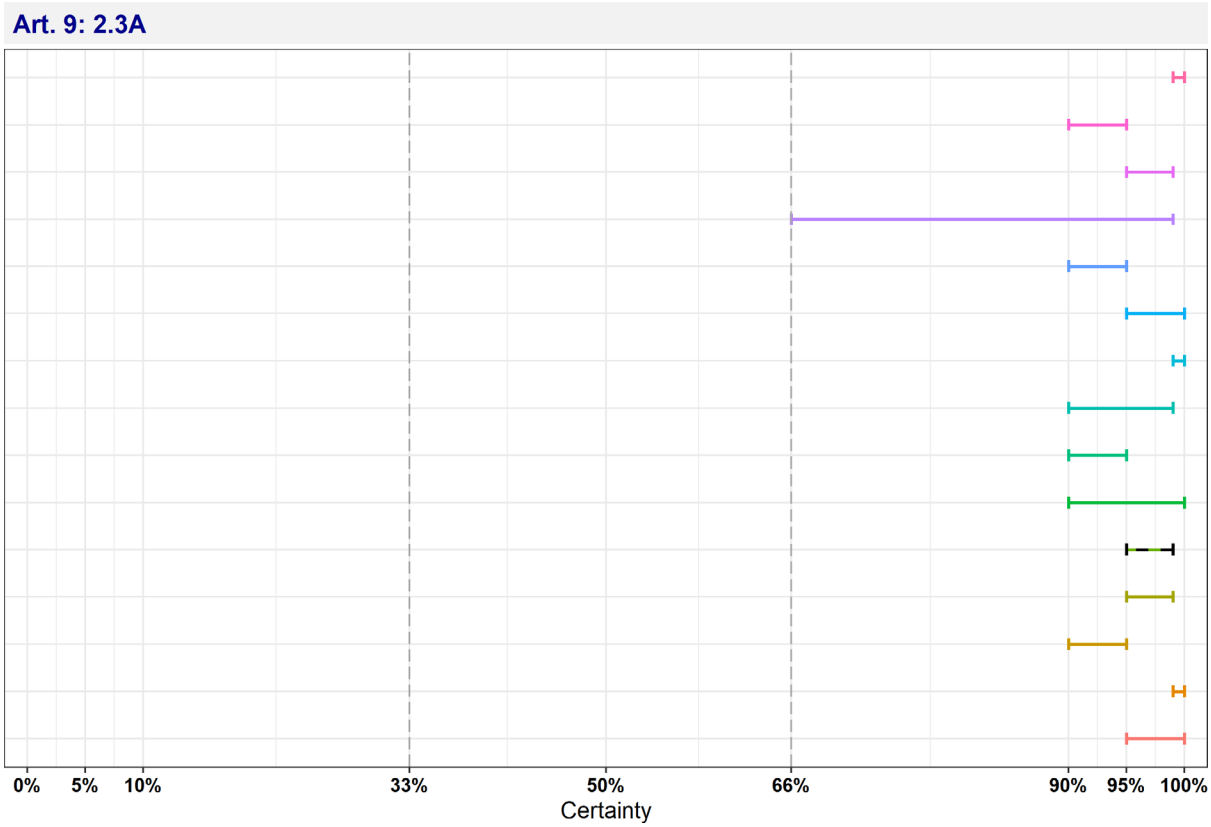
Collective Assessment



The median range is displayed as a dashed line.

Figure A.11: Individual probability ranges reflecting non-fulfilment of criterion 2.2AB (there are possibilities of airborne or waterborne or vector-borne spread) after the collective judgement

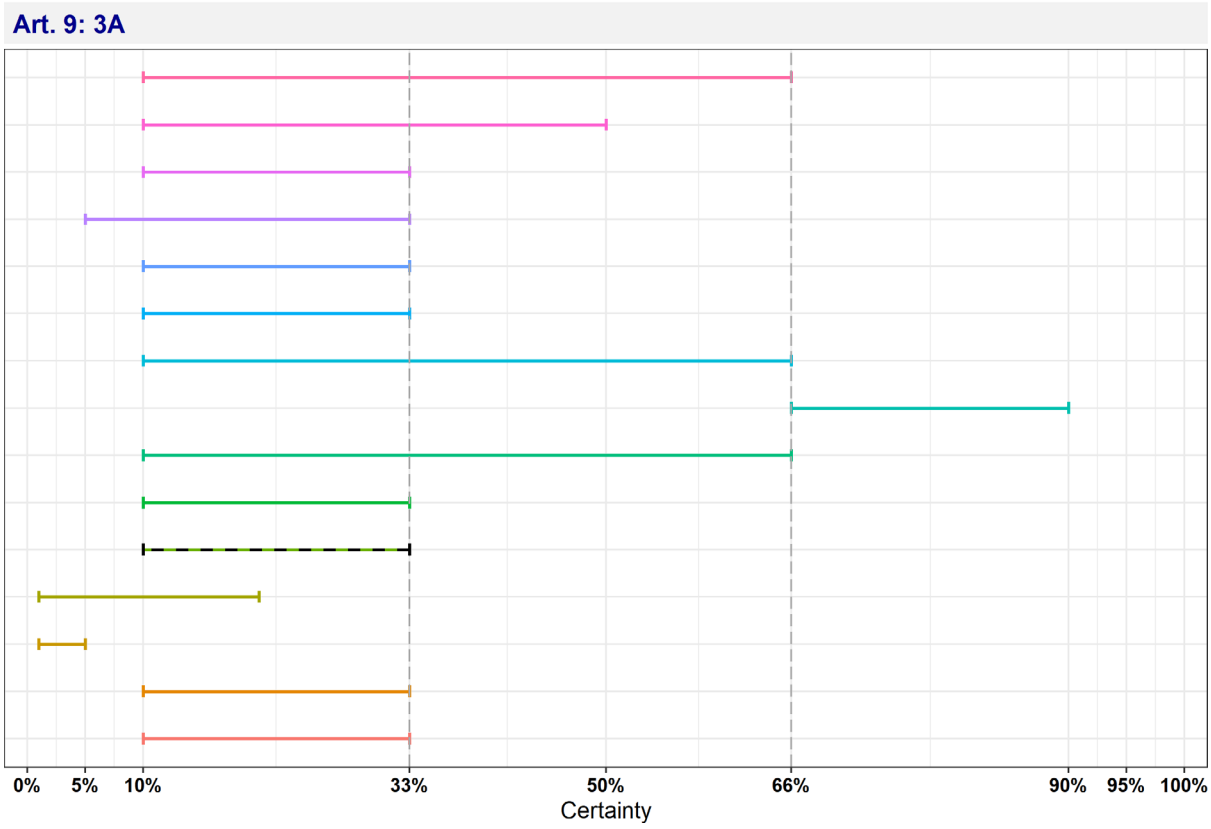
Collective Assessment



The median range is displayed as a dashed line.

Figure A.12: Individual probability ranges reflecting fulfilment of criterion 2.3A (the disease affects multiple species of kept and wild animals or single species of kept animals of economic importance) after the collective judgement

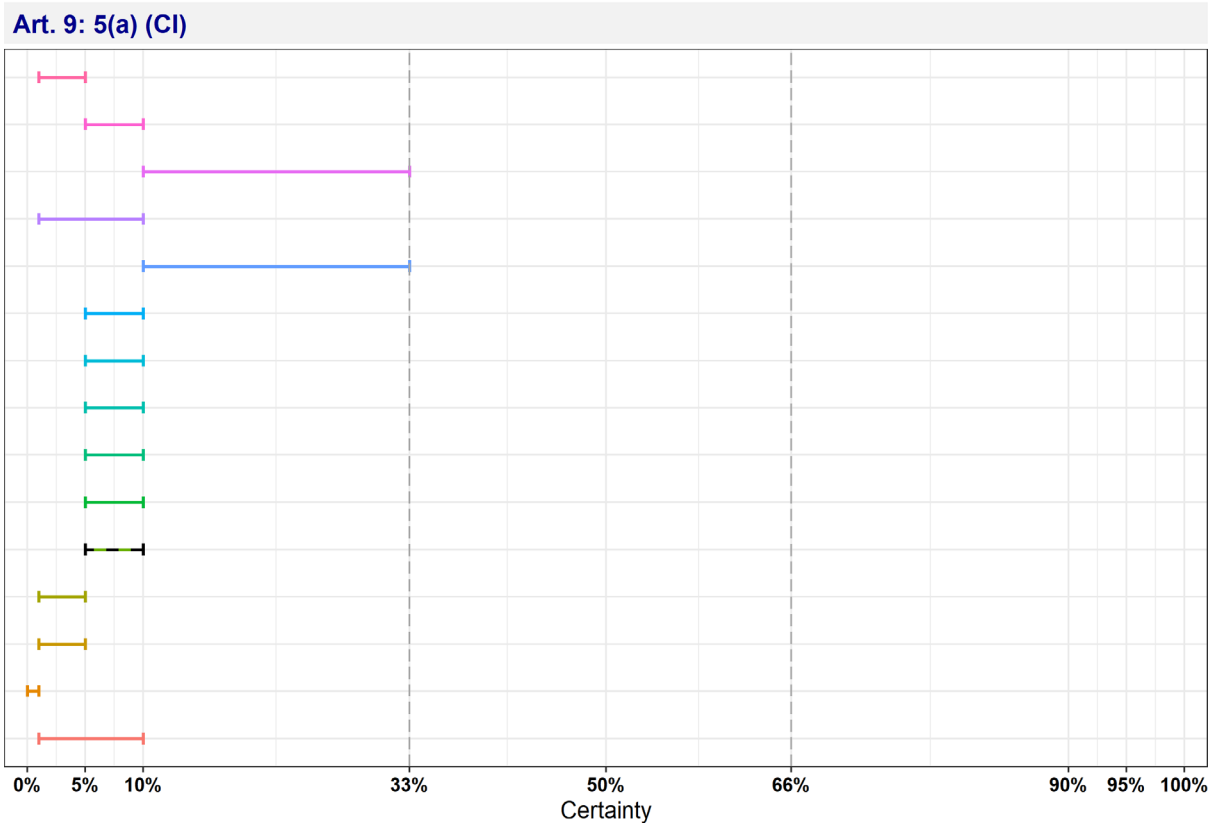
Collective Assessment



The median range is displayed as a dashed line.

Figure A.13: Individual probability ranges reflecting non-fulfilment of criterion 3A (the disease has a zoonotic potential with significant consequences for public health, including epidemic or pandemic potential or possible significant threats to food safety) after the collective judgement

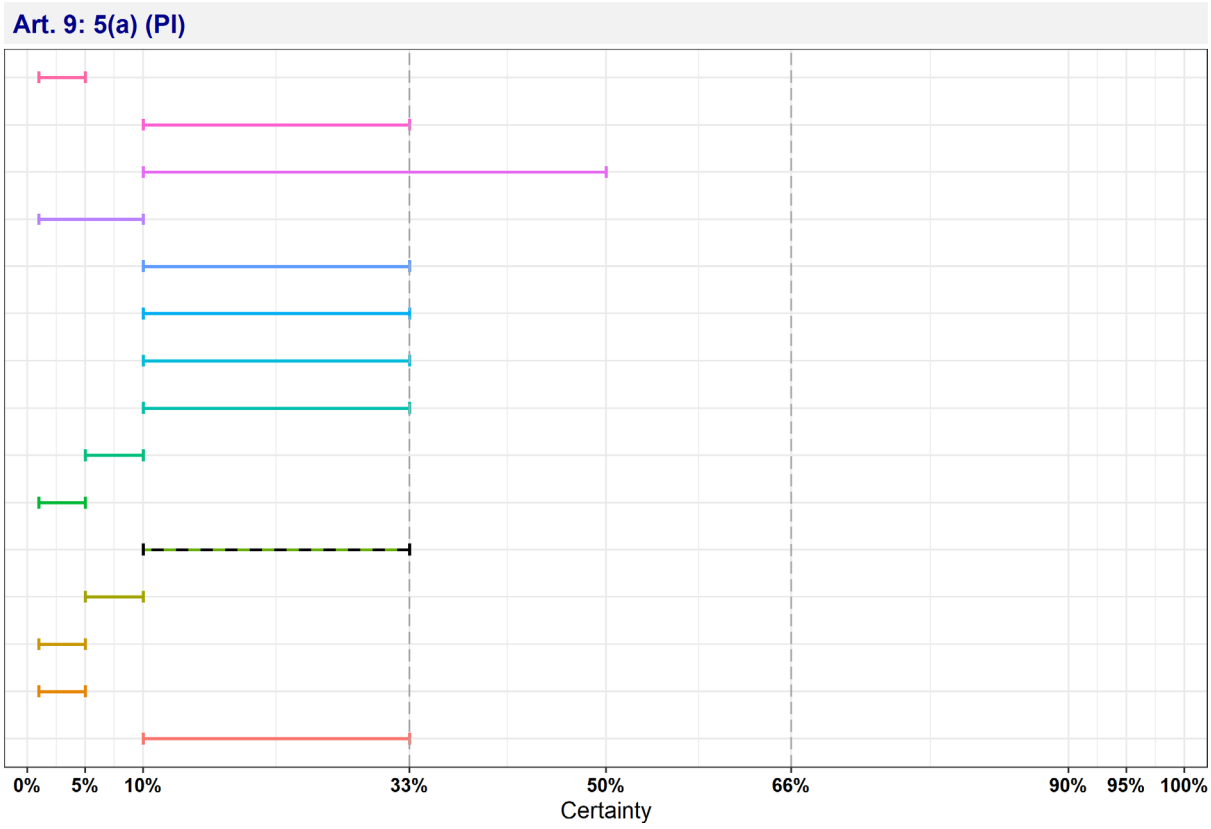
Collective Assessment



CI: current impact.
The median range is displayed as a dashed line.

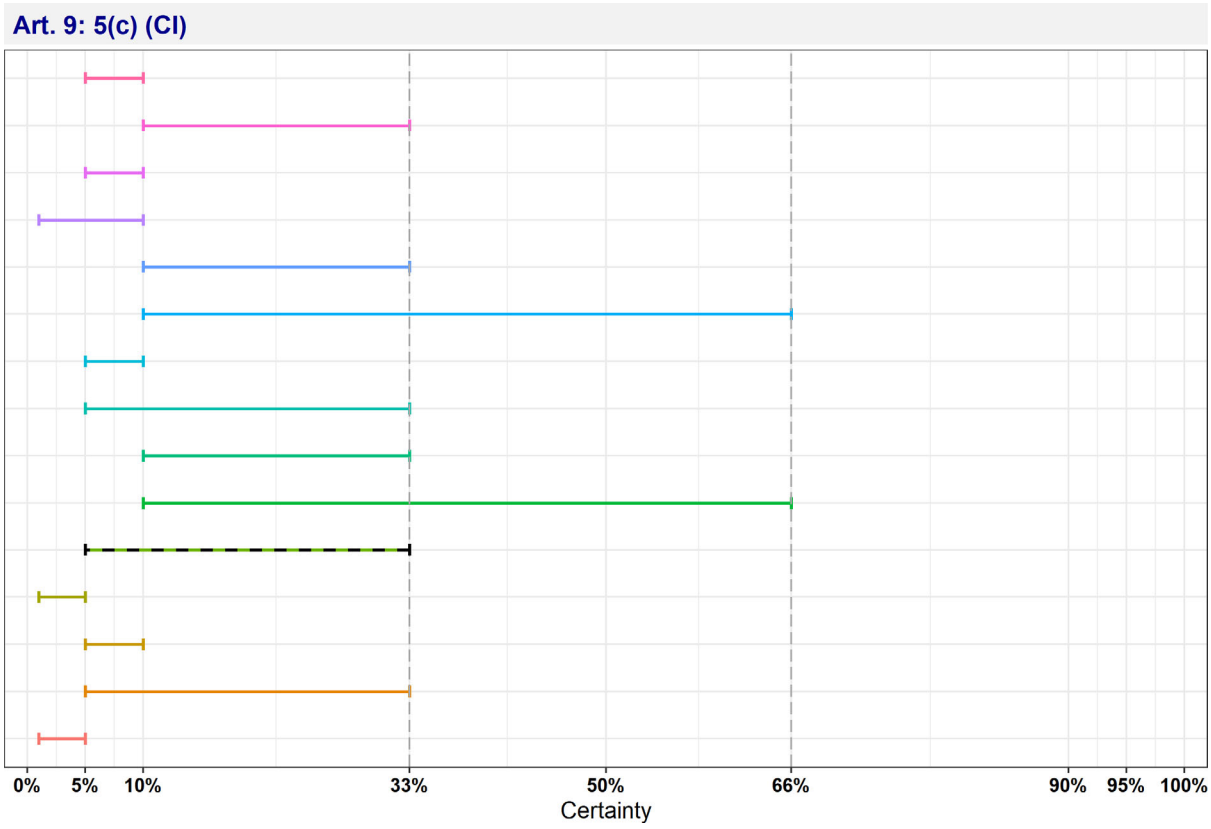
Figure A.14: Individual probability ranges reflecting non-fulfilment of criterion 5(a) (current impact) (the disease has a significant impact on society, with in particular an impact on labour markets) after the collective judgement

Collective Assessment



PI: potential impact.
The median range is displayed as a dashed line.

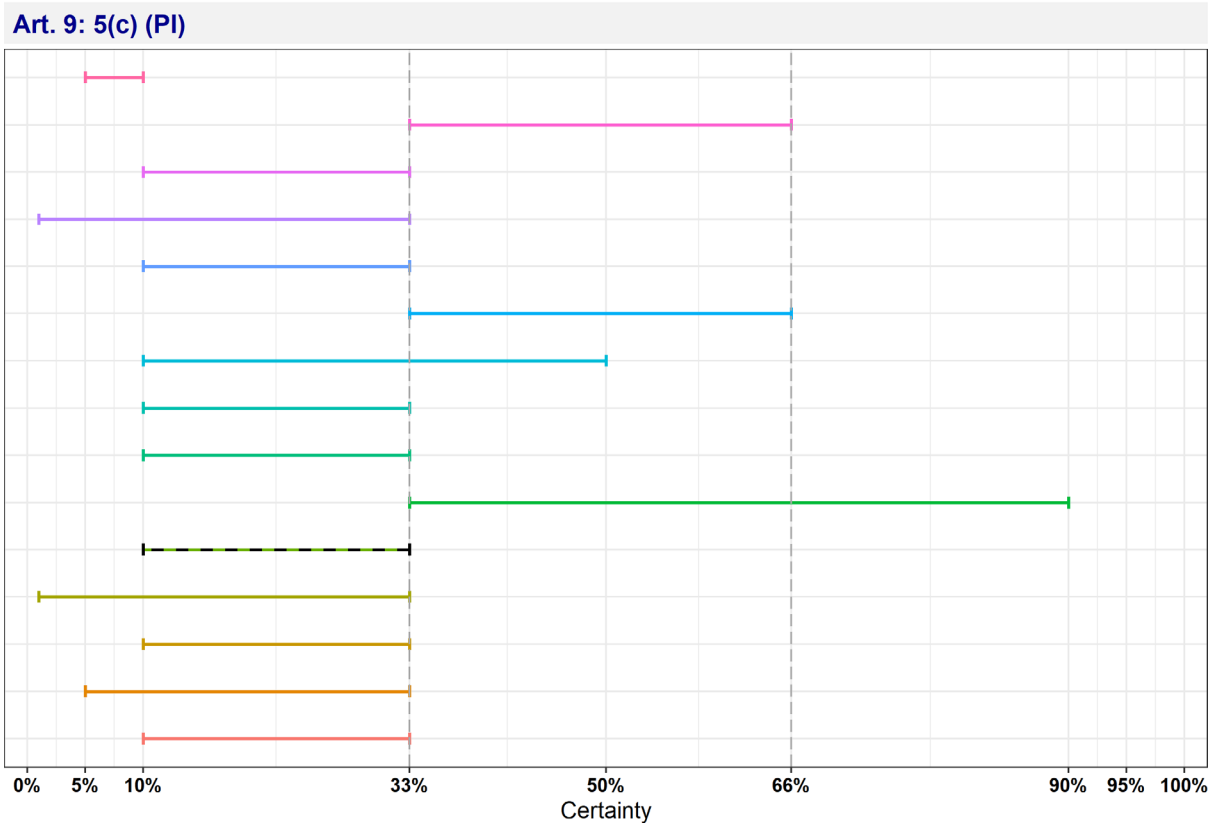
Figure A.15: Individual probability ranges reflecting non-fulfilment of criterion 5(a) (potential impact) (the disease has a significant impact on society, with in particular an impact on labour markets) after the collective judgement



CI: current impact.
The median range is displayed as a dashed line.

Figure A.16: Individual probability ranges reflecting non-fulfilment of criterion 5(c) (current impact) (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) after the collective judgement

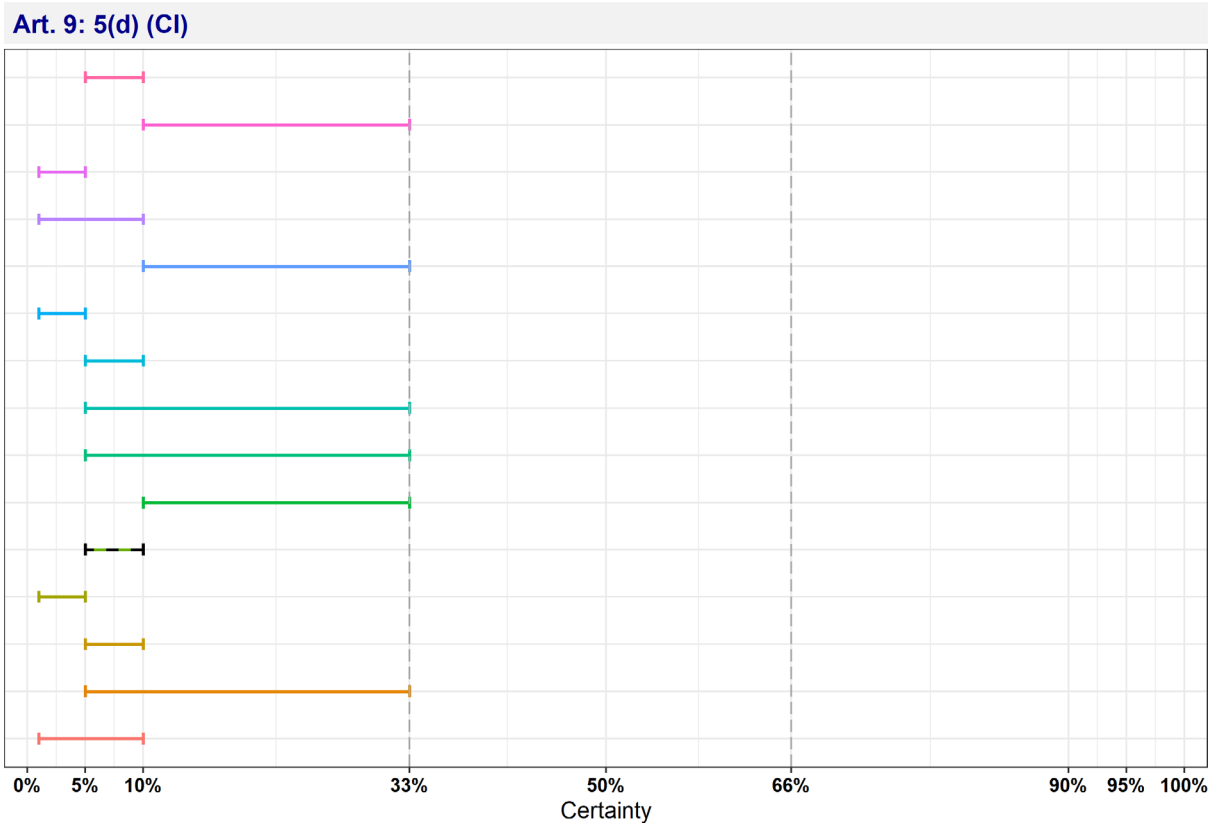
Collective Assessment



PI: potential impact.
The median range is displayed as a dashed line.

Figure A.17: Individual probability ranges reflecting non-fulfilment of criterion 5(c) (potential impact) (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) after the collective judgement

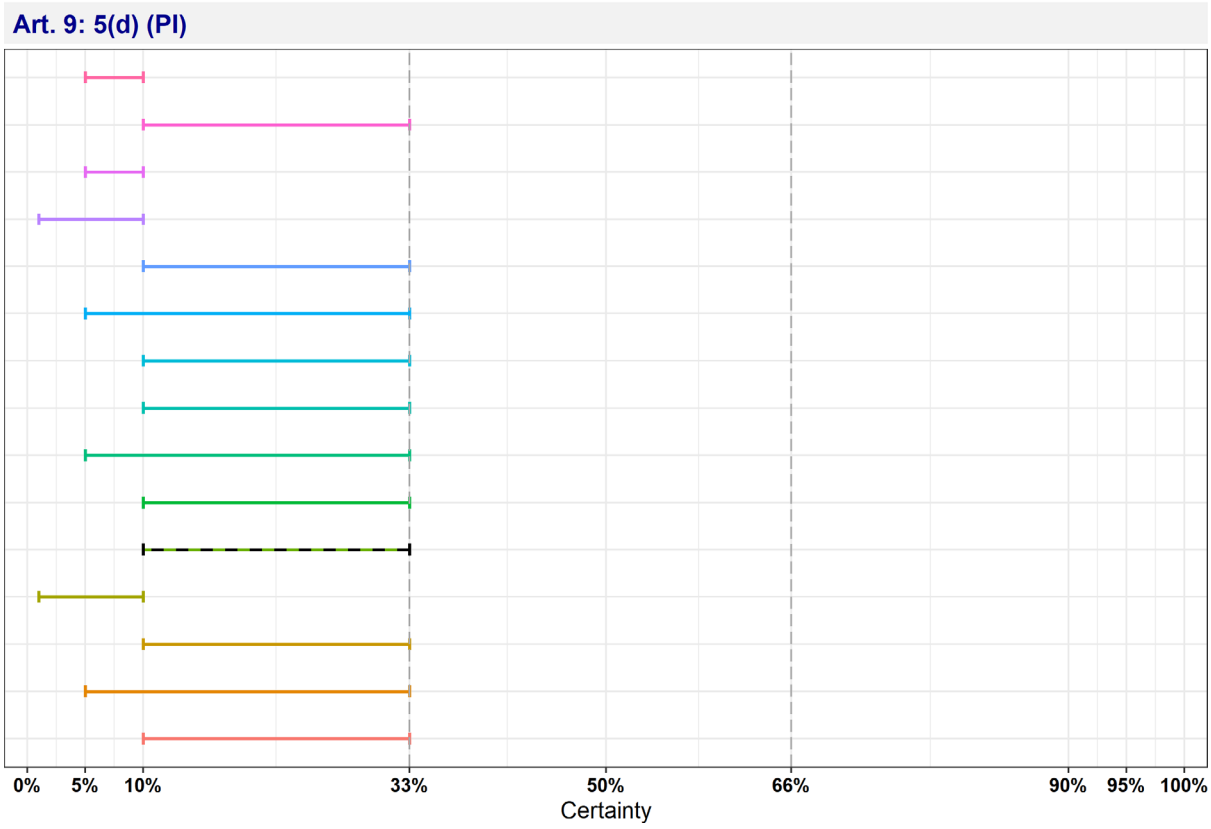
Collective Assessment



CI: current impact.
The median range is displayed as a dashed line.

Figure A.18: Individual probability ranges reflecting non-fulfilment of criterion 5(d) (current impact) (the disease has a significant impact in the long term on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds) after the collective judgement

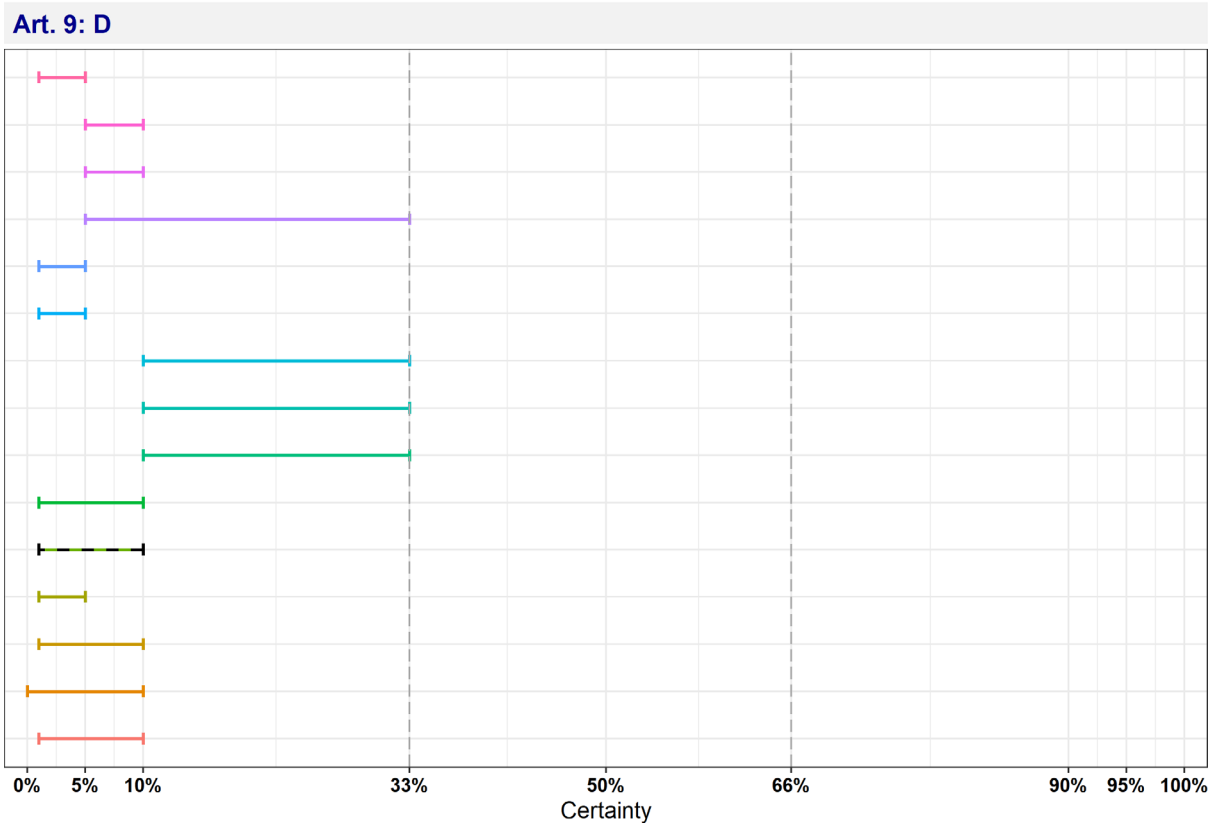
Collective Assessment



PI: potential impact.
The median range is displayed as a dashed line.

Figure A.19: Individual probability ranges reflecting non-fulfilment of criterion 5(d) (potential impact) (the disease has a significant impact in the long term on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds) after the collective judgement

Collective Assessment

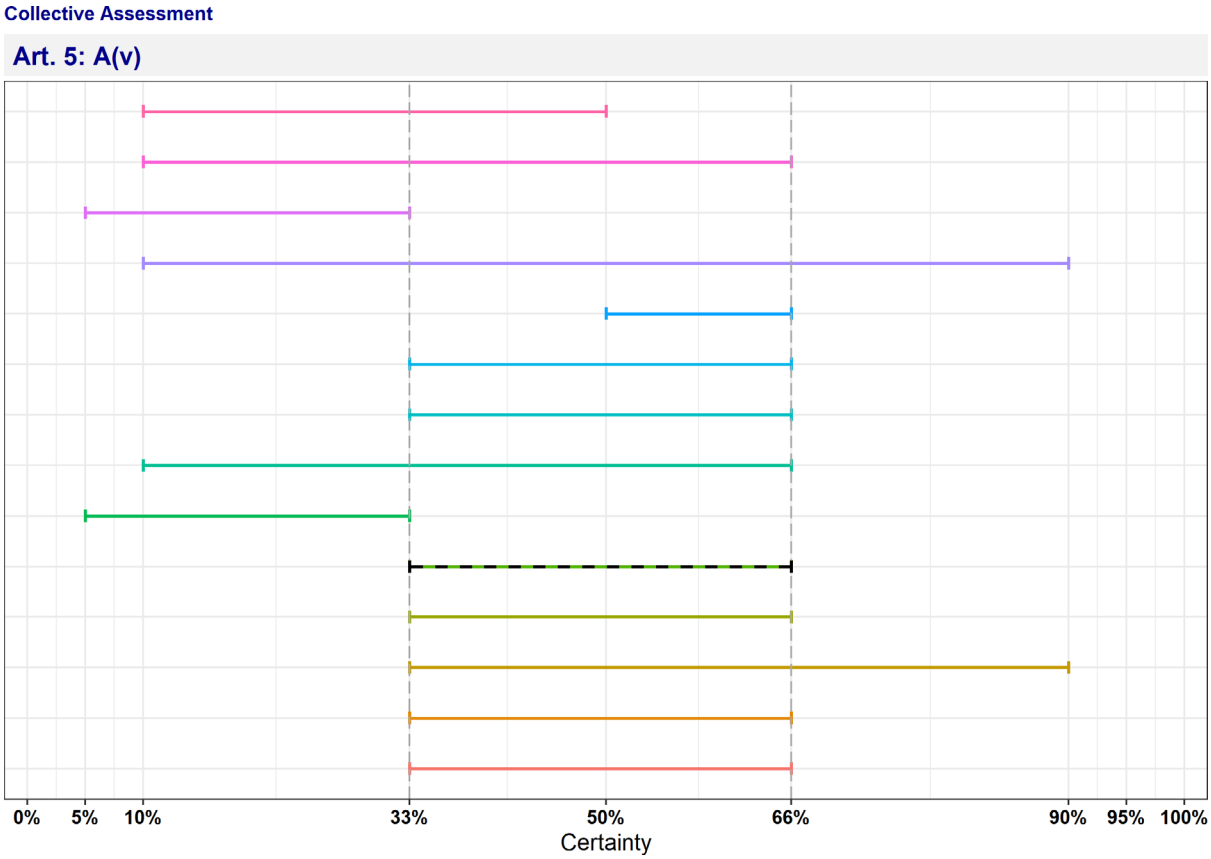


The median range is displayed as a dashed line.

Figure A.20: Individual probability ranges reflecting non-fulfilment of criterion D (the risk posed by the disease can be effectively and proportionately mitigated by measures concerning movements of animals and products in order to prevent or limit its occurrence and spread) after the collective judgement

Appendix B – Criteria with uncertain outcome

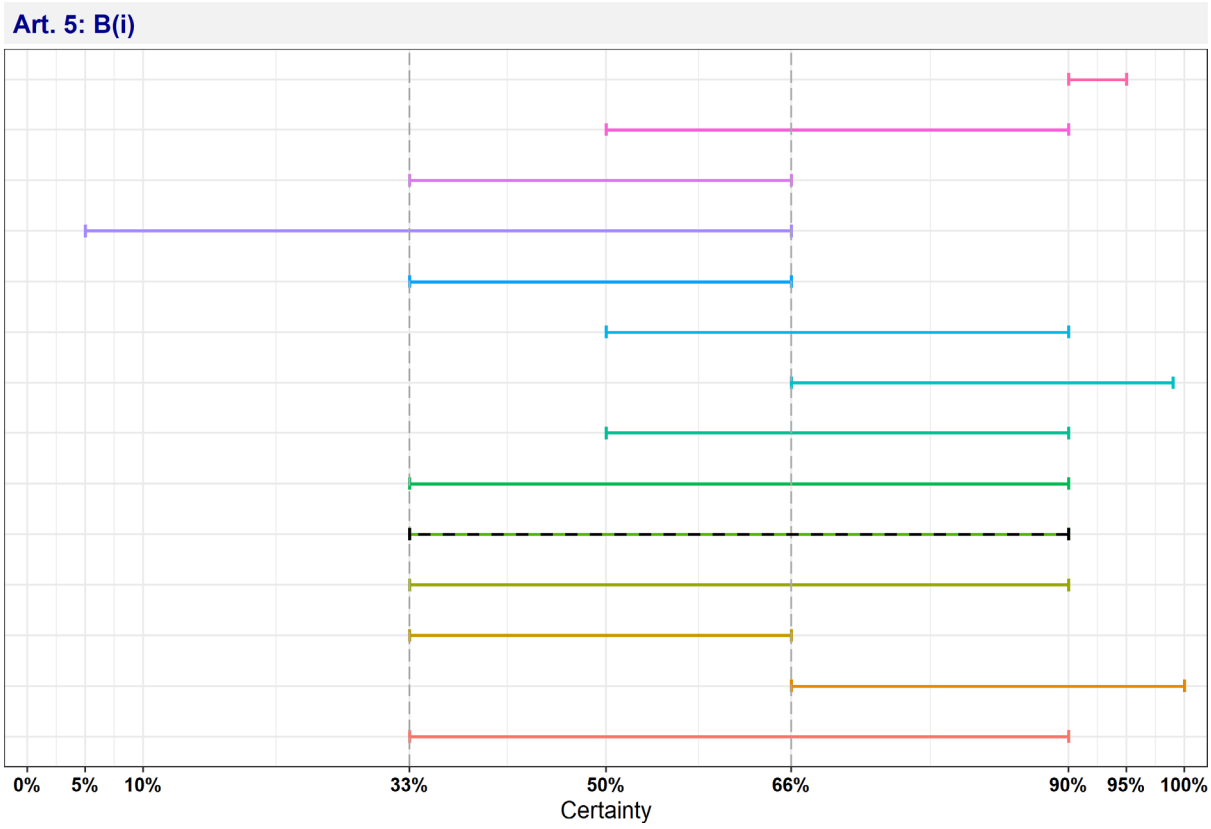
B.1. Article 5 criteria



The median range is displayed as a dashed line.

Figure B.1: Individual probability ranges reflecting uncertain outcome on criterion 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) after the collective judgement

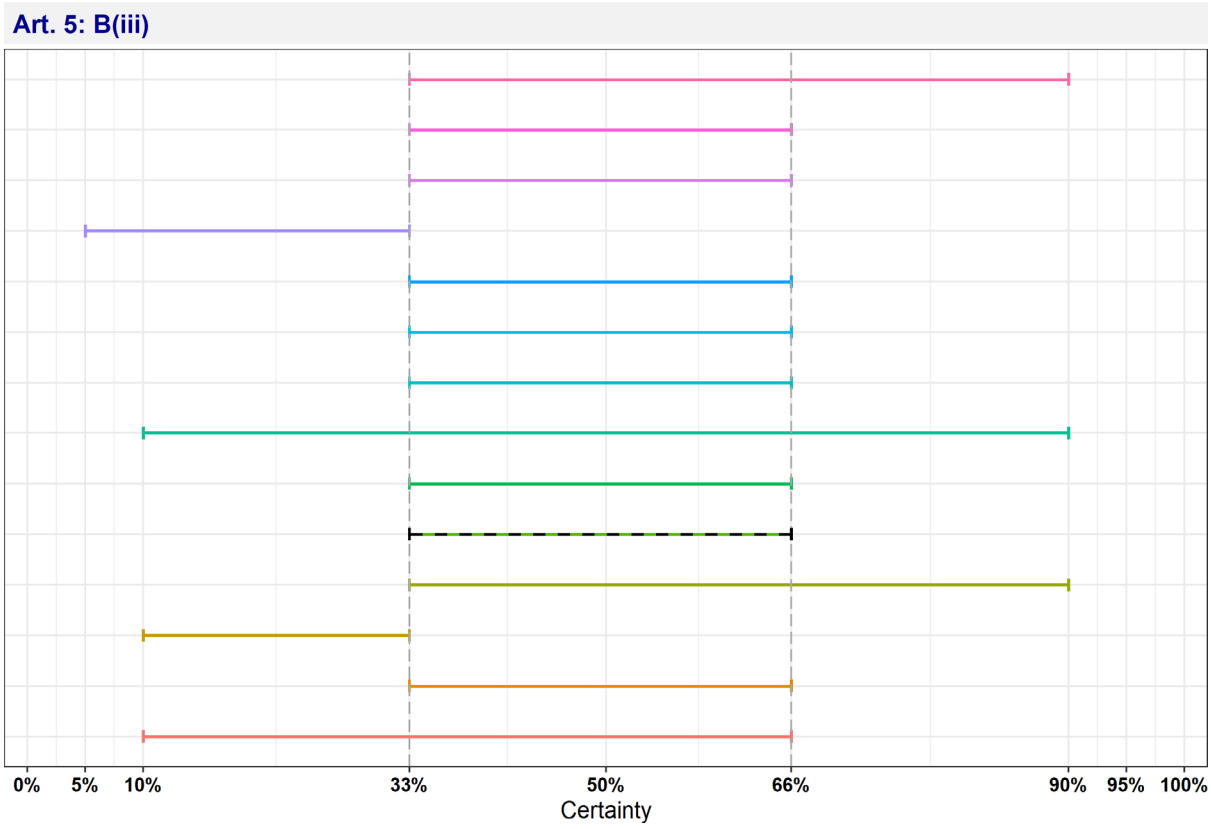
Collective Assessment



The median range is displayed as a dashed line.

Figure B.2: Individual probability ranges reflecting uncertain outcome on criterion 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) after the collective judgement

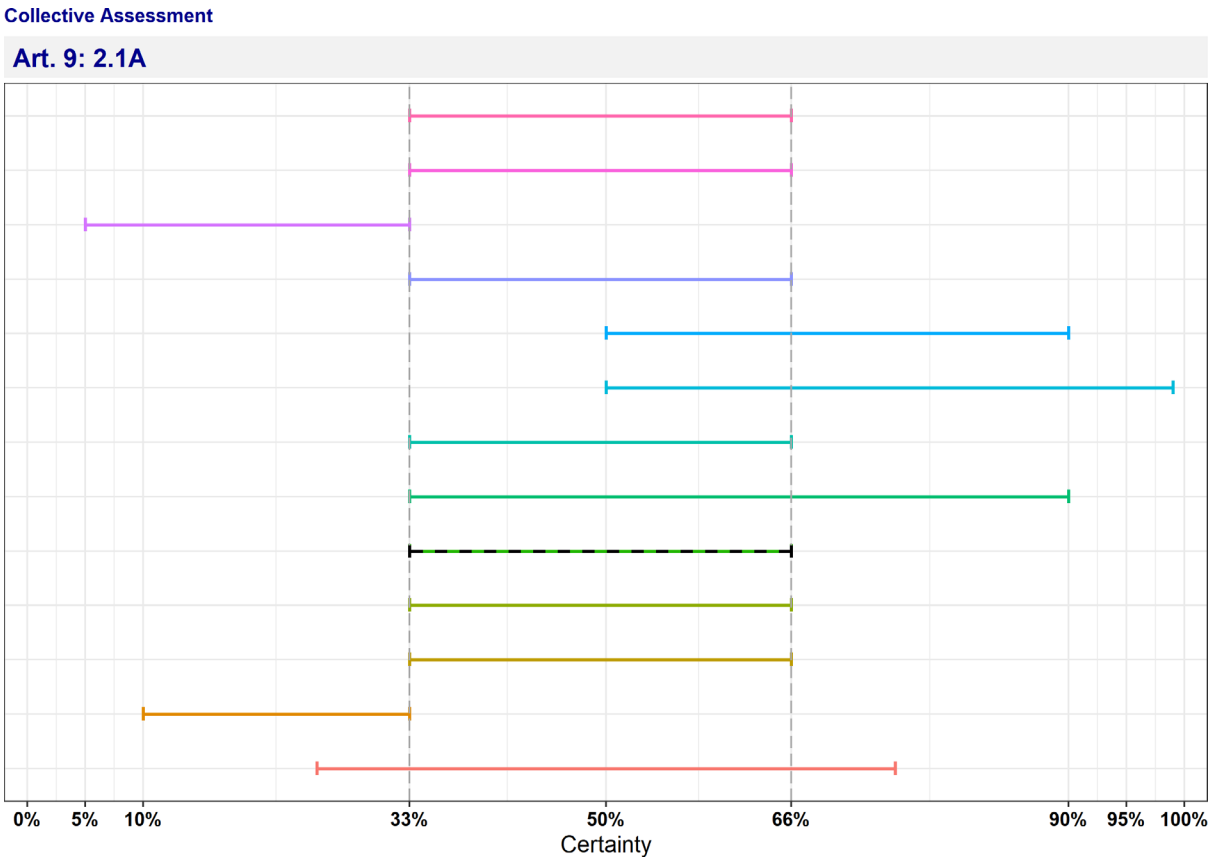
Collective Assessment



The median range is displayed as a dashed line.

Figure B.3: Individual probability ranges reflecting uncertain outcome on criterion B(iii) (the disease causes or could cause a significant negative economic impact affecting agriculture or aquaculture production in the Union) after the collective judgement

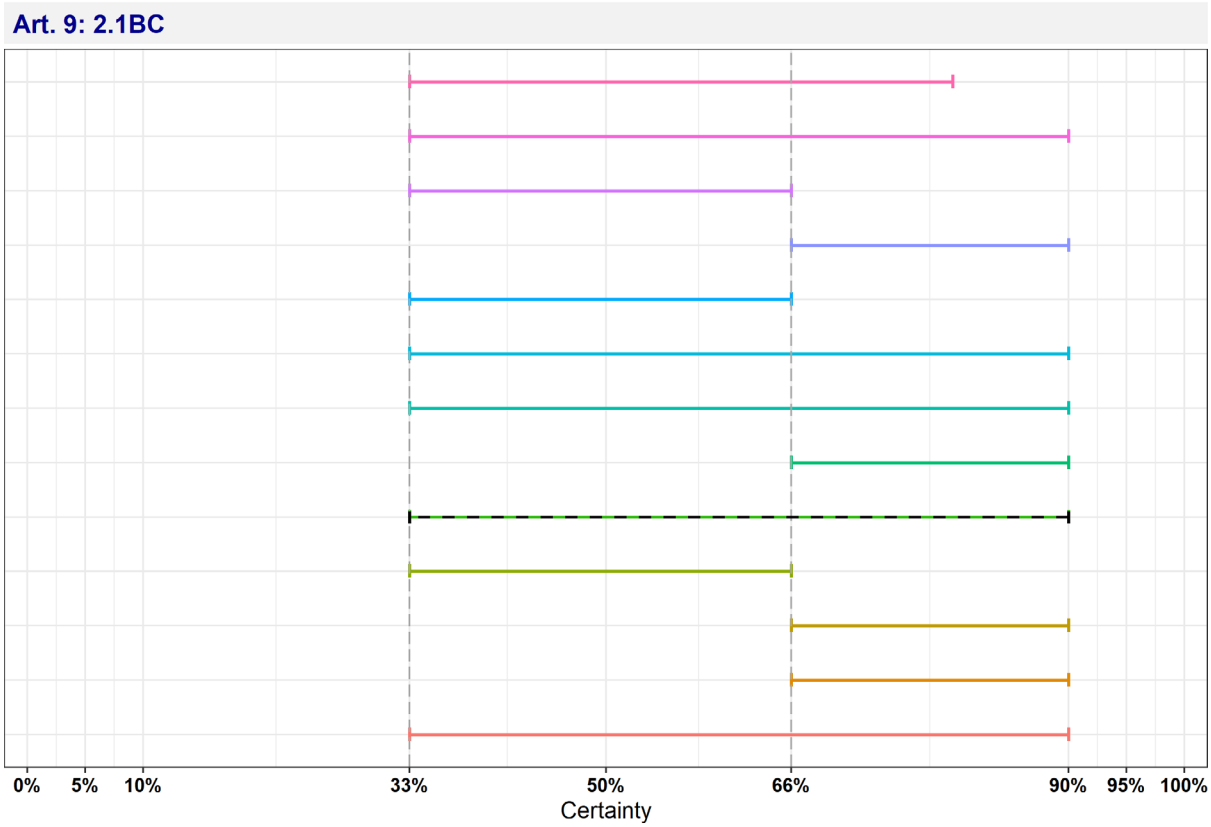
B.2. Article 9 criteria



The median range is displayed as a dashed line.

Figure B.4: Individual probability ranges reflecting uncertain outcome on criterion 2.1A (the disease is highly transmissible) after the collective judgement

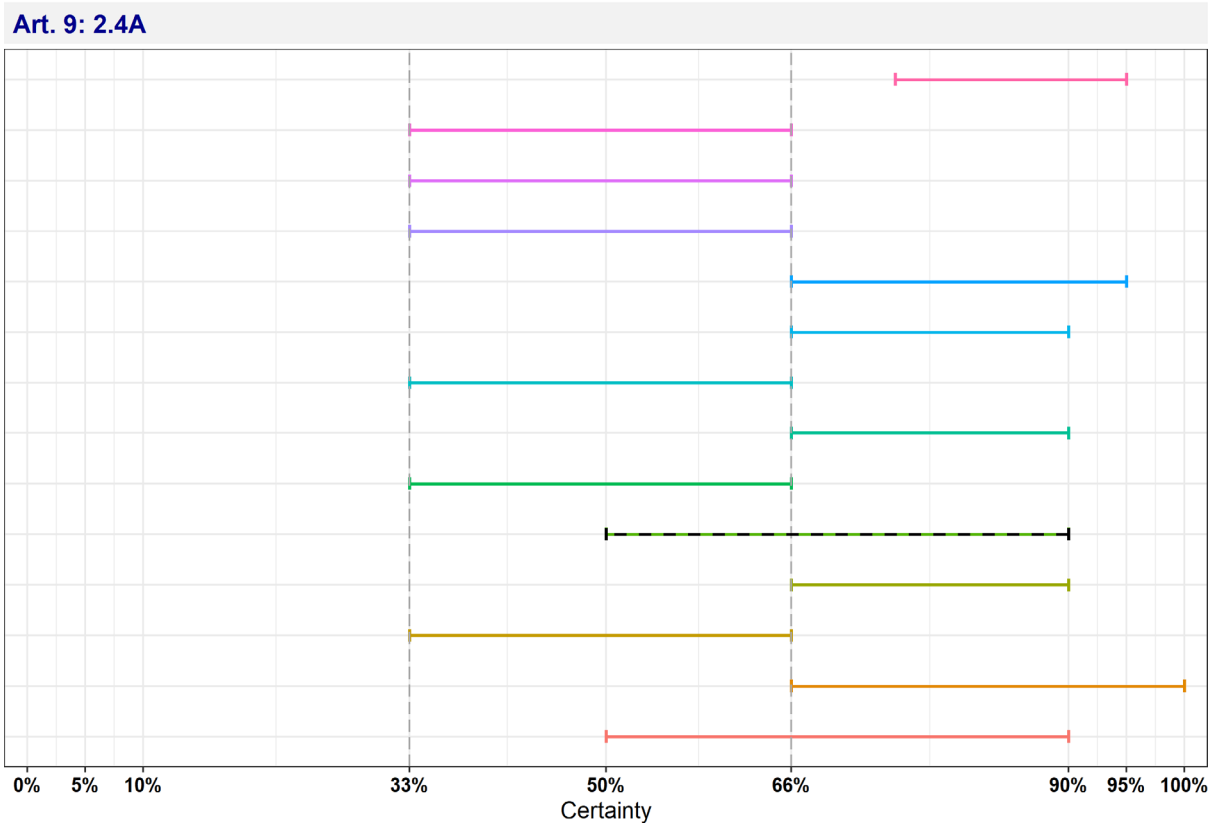
Collective Assessment



The median range is displayed as a dashed line.

Figure B.5: Individual probability ranges reflecting uncertain outcome on criterion 2.1BC (the disease is moderately to highly transmissible) after the collective judgement

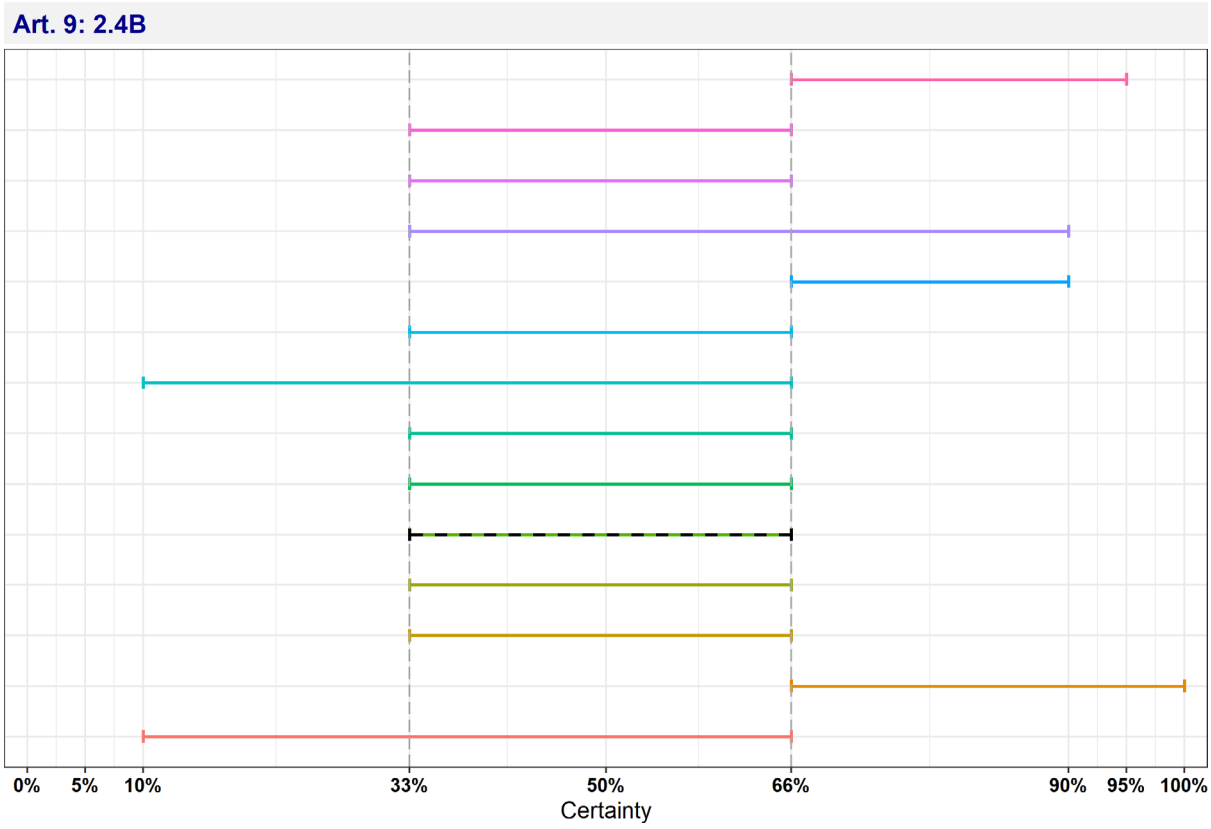
Collective Assessment



The median range is displayed as a dashed line.

Figure B.6: Individual probability ranges reflecting uncertain outcome on criterion 2.4A (the disease may result in high morbidity and significant mortality rates) after the collective judgement

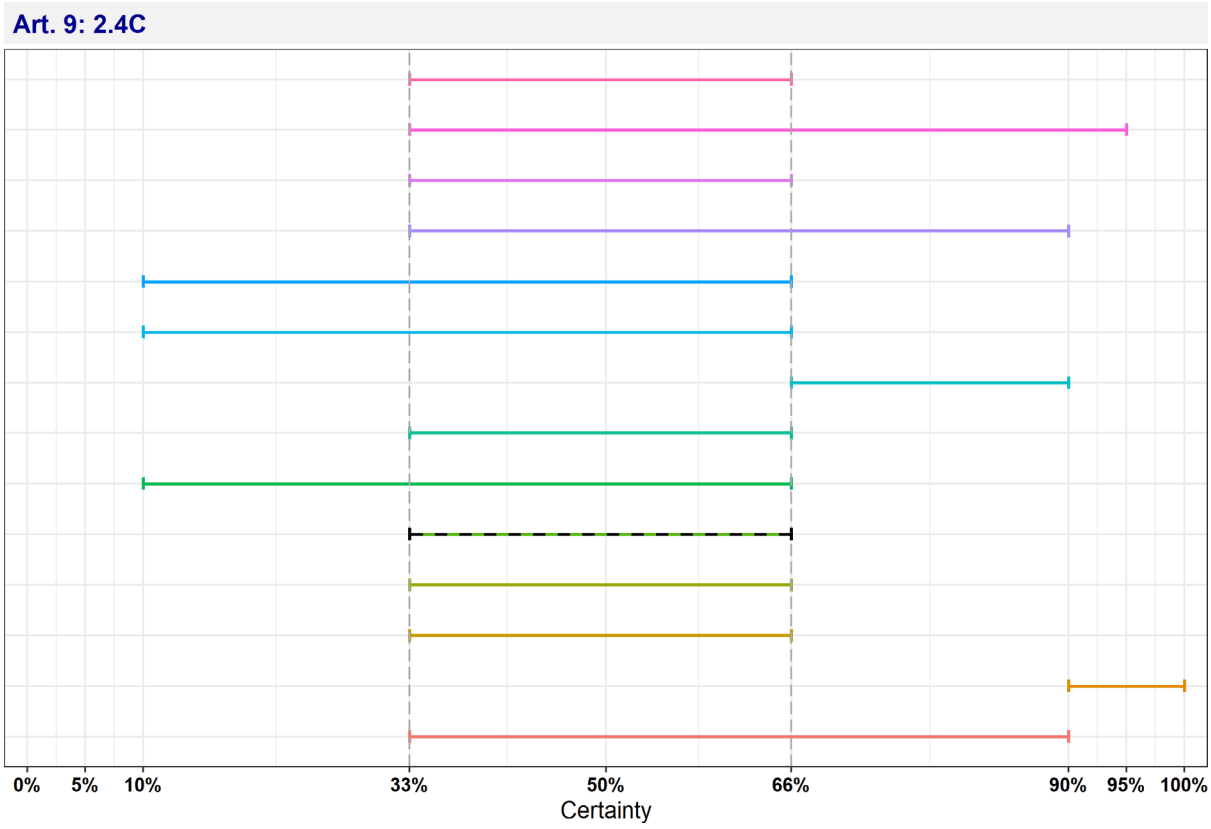
Collective Assessment



The median range is displayed as a dashed line.

Figure B.7: Individual probability ranges reflecting uncertain outcome on criterion 2.4B (the disease may result in high morbidity with in general low mortality) after the collective judgement

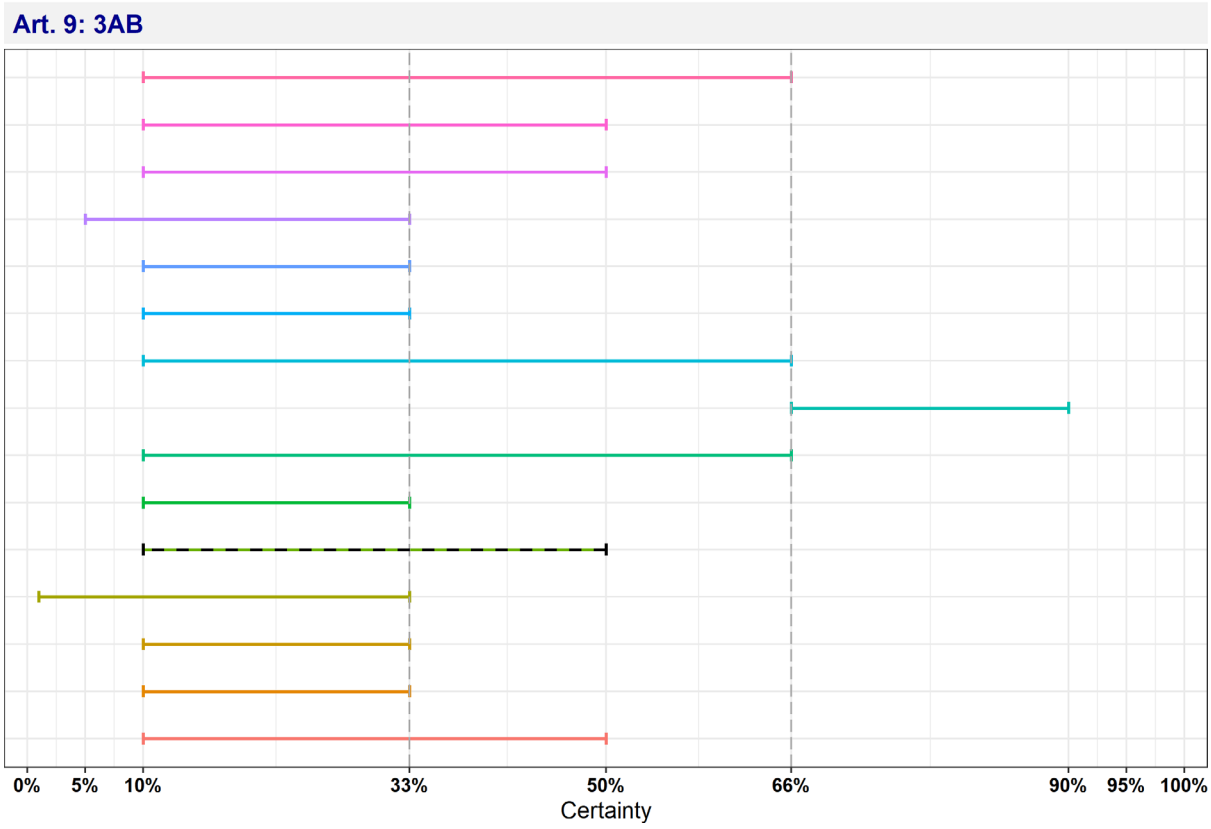
Collective Assessment



The median range is displayed as a dashed line.

Figure B.8: Individual probability ranges reflecting uncertain outcome on criterion 2.4C (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) after the collective judgement

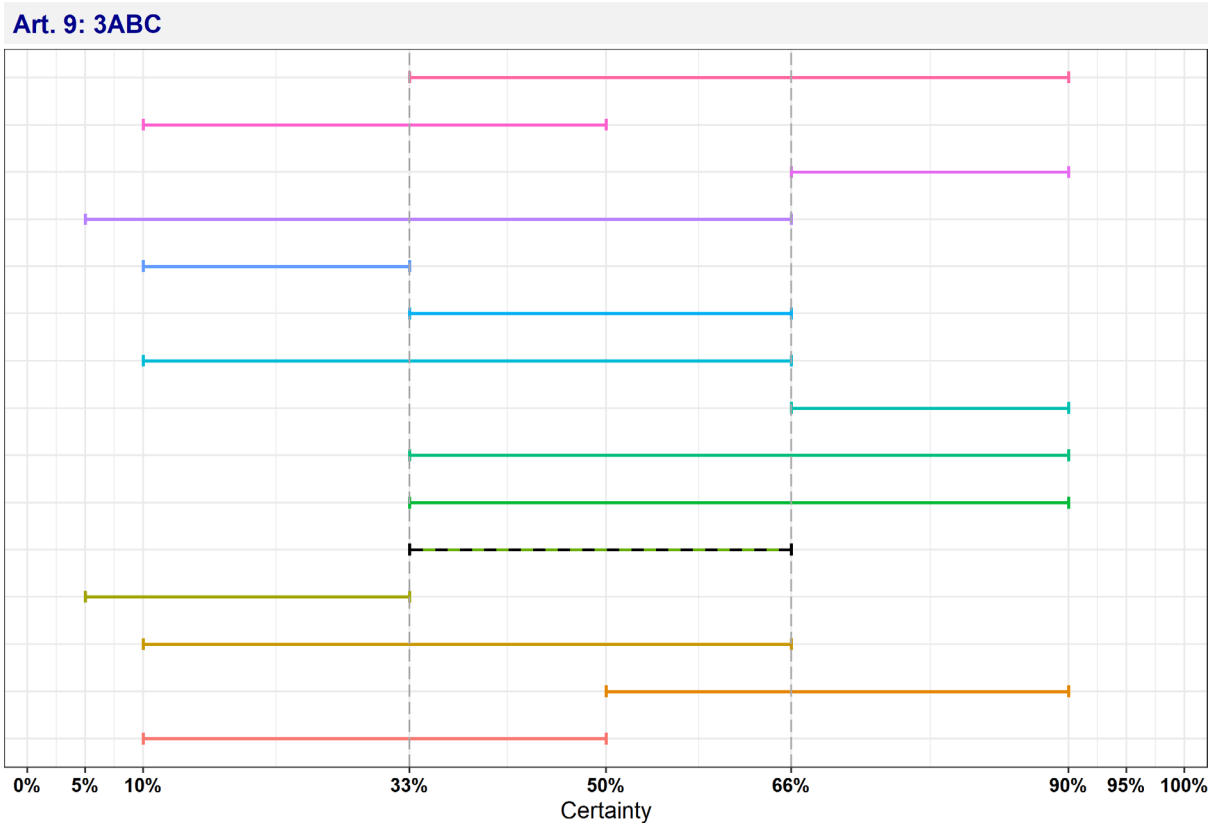
Collective Assessment



The median range is displayed as a dashed line.

Figure B.9: Individual probability ranges reflecting uncertain outcome on criterion 3AB (the disease has a zoonotic potential with significant consequences for public health, including epidemic potential or possible significant threats to food safety) after the collective judgement

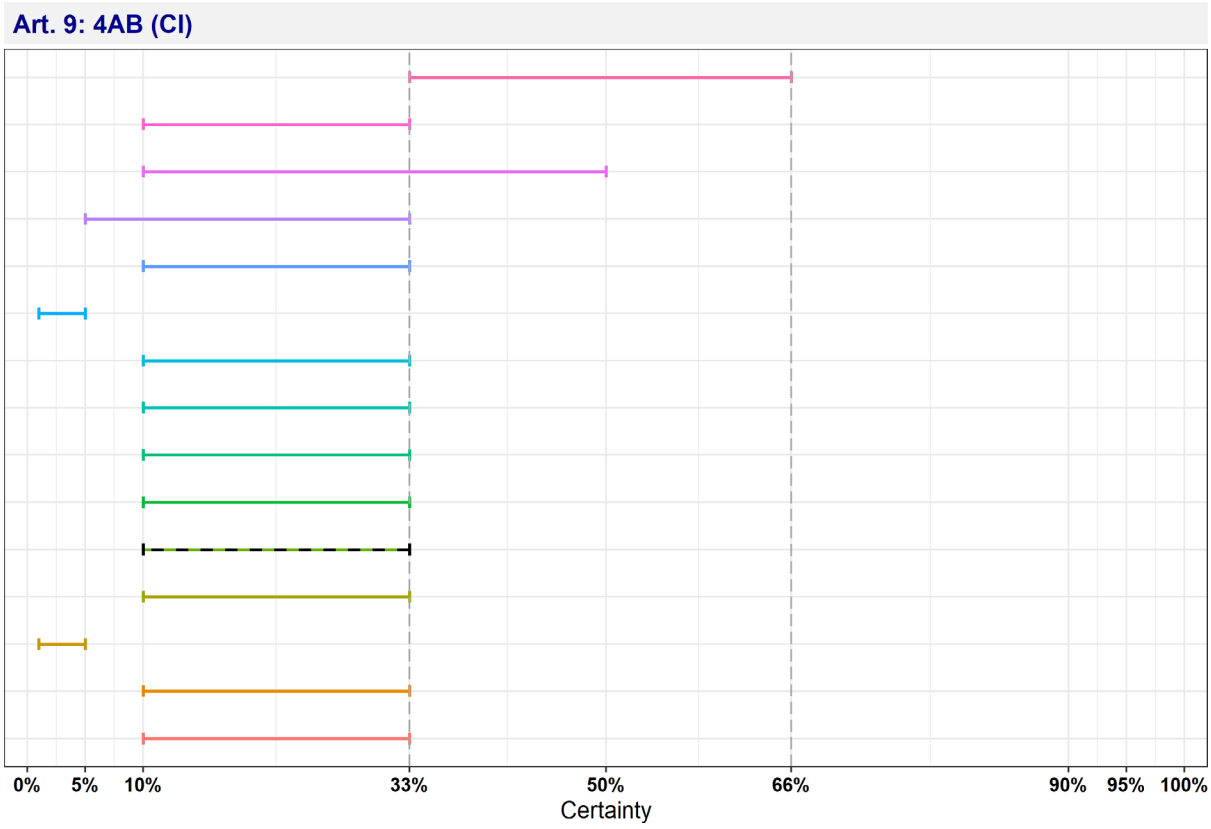
Collective Assessment



CI: current impact.
The median range is displayed as a dashed line.

Figure B.10: Individual probability ranges reflecting uncertain outcome on criterion 3ABC (the disease has a zoonotic potential with significant consequences for public health or possible significant threats to food safety) after the collective judgement

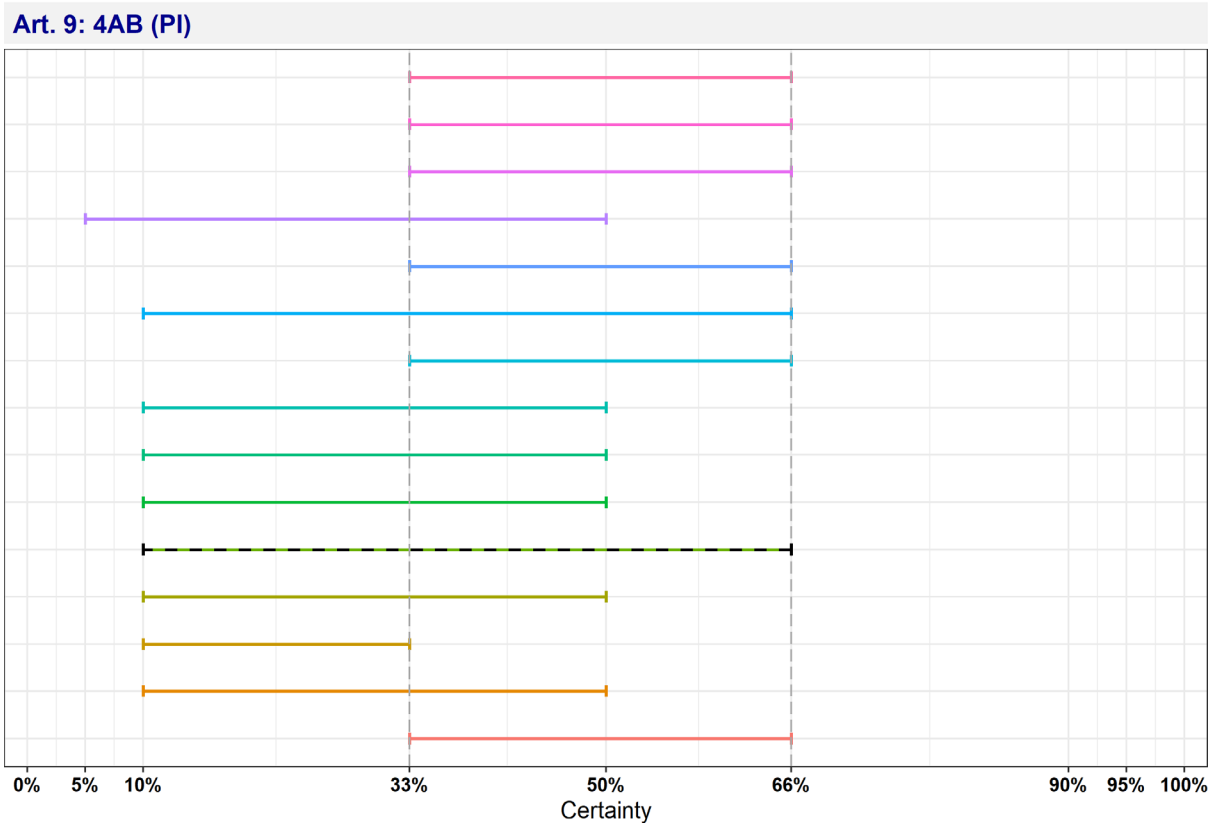
Collective Assessment



CI: current impact.
The median range is displayed as a dashed line.

Figure B.11: Individual probability ranges reflecting uncertain outcome on criterion 4AB (current impact) (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) after the collective judgement

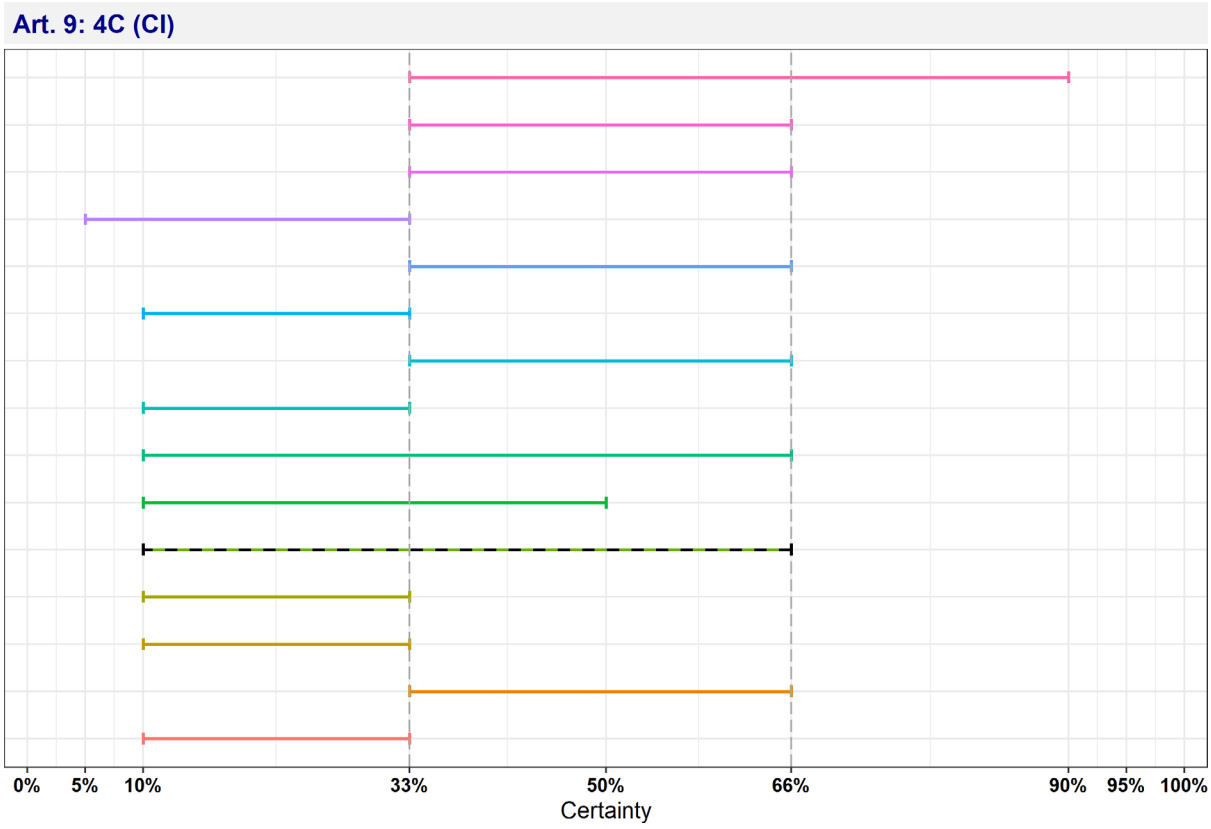
Collective Assessment



PI: potential impact.
The median range is displayed as a dashed line.

Figure B.12: Individual probability ranges reflecting uncertain outcome on criterion 4AB (potential impact) (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) after the collective judgement

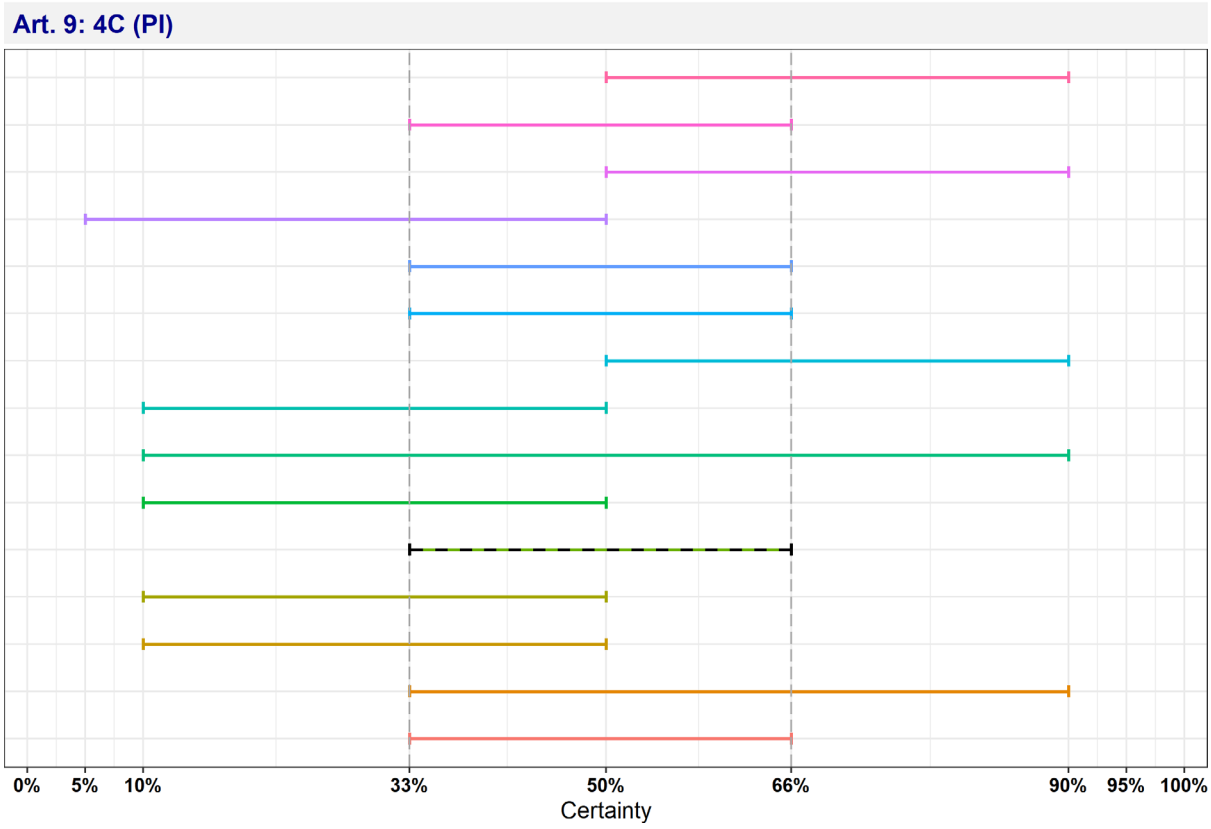
Collective Assessment



CI: current impact.
The median range is displayed as a dashed line.

Figure B.13: Individual probability ranges reflecting uncertain outcome on criterion 4C (current impact) (the disease has a significant impact on the economy of the Union, mainly related to its direct impact on certain types of animal production systems) after the collective judgement

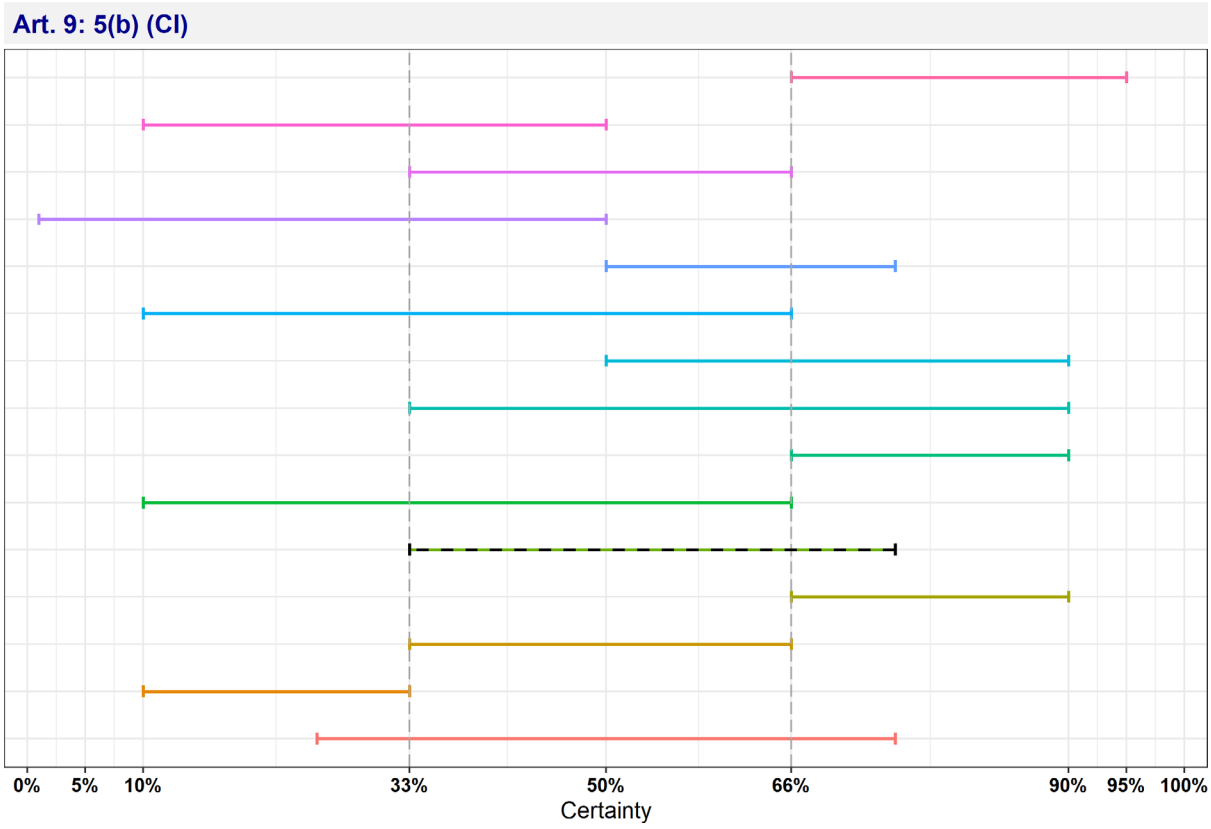
Collective Assessment



PI: potential impact.
The median range is displayed as a dashed line.

Figure B.14: Individual probability ranges reflecting uncertain outcome on criterion 4C (potential impact) (the disease has a significant impact on the economy of the Union, mainly related to its direct impact on certain types of animal production systems) after the collective judgement

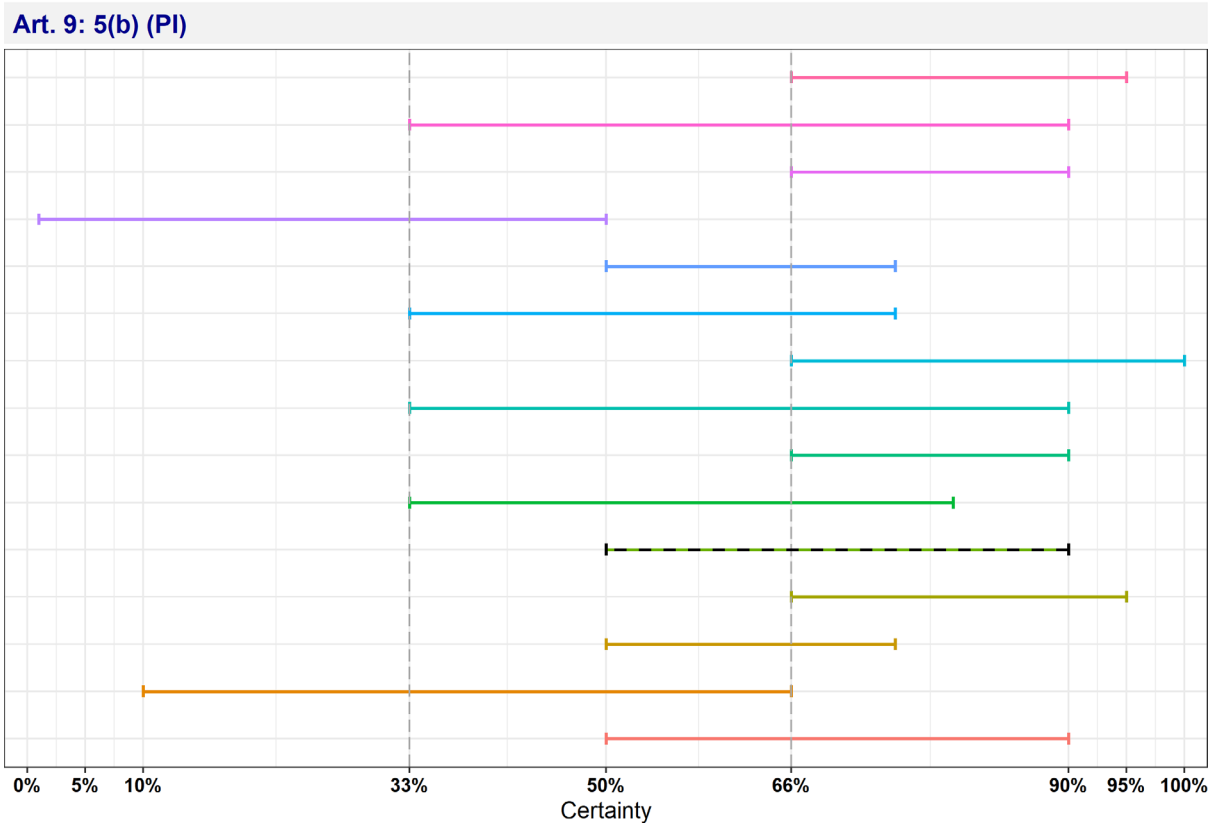
Collective Assessment



CI: current impact.
The median range is displayed as a dashed line.

Figure B.15: Individual probability ranges reflecting uncertain outcome on criterion 5(b) (current impact) (the disease has a significant impact on animal welfare, by causing suffering of large numbers of animals) after the collective judgement

Collective Assessment



PI: potential impact.
The median range is displayed as a dashed line.

Figure B.16: Individual probability ranges reflecting uncertain outcome on criterion 5(b) (potential impact) (the disease has a significant impact on animal welfare, by causing suffering of large numbers of animals) after the collective judgement