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Does treatment with UV-C LED light reduce the number of *Salmonella* and *Campylobacter* on eggs?

Raw eggs can contain bacterial pathogens on their shell or on the inside. These include *Campylobacter* and *Salmonella*, which can lead to foodborne infections. They are usually accompanied by stomach cramps, diarrhoea and vomiting. Foodborne infections usually resolve on their own. However, in extreme cases they can be life-threatening for people whose body's immune systems are not yet fully developed or are impaired (small children, pregnant women and their unborn child, older people and people with pre-existing medical conditions).

Data from official food monitoring authorities show that the occurrence of *Campylobacter* and, less frequently, *Salmonella* on the shells of table eggs can be expected regularly in Germany. In addition to combating the occurrence of pathogens in laying hen farms, technical procedures can help to reduce the number of pathogens present on eggs. In the research project "UVegg", the German Federal Institute for Risk Assessment (BfR) has investigated whether the treatment of table eggs with UV-C-LED radiation is suitable as an additional measure to reduce the risk of foodborne infections caused by *Salmonella* and *Campylobacter*.

UV-C-LED treatment was generally able to reduce the number of artificially applied bacteria on egg surfaces in the project, but it was dependent on the amount of dirt on the egg surface and bacterial contamination level.

The project showed that UV-C-LED radiation reduces the number of bacteria on eggs with visually clean or slightly polluted surfaces. Larger amounts of pollution and higher numbers of bacteria sometimes significantly reduce the effect of UV-C-LED irradiation.

Consumers can reduce the risk of transmitting pathogens from chicken eggs by, among other things, storing and processing raw chicken eggs separately from other foods and thoroughly cleaning their hands and kitchen utensils after contact. Sensitive groups of people should only eat eggs and foods containing eggs when they have been fully heated (at least 2 minutes at 70 °C on all parts of the food).

1 Subject of the assessment

Successful control programmes for *Salmonella* in laying hen farms have significantly reduced the prevalence of *Salmonella* on table egg shells. Nevertheless, these pathogens are repeatedly detected on table eggs in official monitoring. *Campylobacter* is even more common on table egg shells in Germany.

Solutions are being sought to remove pathogens that may be present on the egg shells to minimise the risk of foodborne infection to consumers from contaminated table eggs. However, washing table eggs or treating them with chemical substances is not allowed. This is why some egg packing centres already use UV-C low-pressure mercury-vapour lamps to reduce bacterial burden on eggshells. The lamps emit UV light in the "C" wave range, which causes nucleic acid and protein damage in bacteria. However, the lamps contain mercury. The toxic properties of this heavy metal and its necessary disposal as hazardous waste threaten to contaminate humans and the environment, which is why the use of this technology is being phased out in the European Union (EU) (Regulation (EU) 2017/852). UV-C LED panels are a possible alternative to conventional UV-C lamps.

Experimental data on the impact of UV radiation on bacteria is available for various surfaces and matrices in the food sector. Among other things, conveyor belts and various contact surfaces are treated with UV light on which meat and meat products are subsequently transported (Morey, McKee, Dickson, & Singh, 2010).

Together with several project partners, the BfR carried out the research project UVegg ("Use of UV-C/UV-C-LED radiation to reduce microorganisms on eggs") from 2018 to 2021 to test whether the UV-C-LED panels have a comparable effectiveness compared to the UV-C low-pressure mercury-vapour lamps.

Based on the project results, the BfR prepared this opinion on the suitability of treating table eggs using UV-C LED radiation as an additional measure to prevent foodborne infections caused by *Salmonella* and *Campylobacter*.

2 Results

Salmonella or *Campylobacter* on table eggs pose a risk to consumers by contracting foodborne infections. Therefore, in addition to combating zoonotic pathogens in laying hen farms, suitable technical procedures are being sought to remove or kill pathogens present on table eggs before they are sold to consumers.

After evaluating the available publications and the research results of the UVegg project, the BfR concludes that UV-C light generated using LED panels (UV-C-LED) with a wavelength of 280 nanometres (nm), an intensity of ~ 2.4 milliwatts/square centimetre (mW/cm²) and an exposure time of 5 seconds is suitable for reducing *Salmonella* or *Campylobacter* present on visually clean eggshells by around one log level/cm^2. This means that the reduction in germs achieved by the UV-C-LED is lower than that of the conventional UV-C lamps currently used in packing centres. Visible dirt on table eggs further reduces the effect of UV-C-LED treatment.

Due to missing or insufficient data on the quantitative occurrence of *Salmonella* and *Campylobacter* on shells of naturally contaminated table eggs, the effect of UV-C-LED

treatment on the safety of table eggs can only be estimated with great uncertainty. Assuming that visually clean or slightly polluted chicken eggs have a little, if any, *Salmonella* or *Campylobacter* on the egg shells (less than 10 colony forming units (CFU)/cm^2 egg surface), the risk of foodborne infections could be reduced with the UV-C-LED treatment tested in the UVegg project, provided that recontamination of the table eggs is prevented.

It can be assumed that increasing the intensity of UV-C-LEDs increases the germ-reducing effect of the treatment since the conventional UV-C lamps currently used in packing centres achieve a higher energy density. Extending the exposure time to up to 50 seconds did not lead to any qualitative change in the eggs during the investigations in the UVegg project. This might boost the germ-reducing effect of the UV-C-LED treatment.

There is currently no evidence that the treatment of table eggs with UV-C-LED poses any health risks to consumers.

As a condition for the use of UV-C-LED processes for the treatment of table eggs, the food company should, from the BfR's point of view, meet the following conditions, among others:

- verify the efficacy of the treatment used,
- document the decontamination procedure,
- implement measures to prevent adverse effects on eggs in the event of technical malfunctions during UV-C treatment.

Suitable placement of the UV-C-LED panels in the egg packing centre is required (preferably at the end of the egg sorting process) to avoid recontamination of table eggs after UV-C-LED treatment has taken place.

3 Rationale

3.1 Risk assessment

3.1.1 Hazard identification

3.1.1.1 Salmonella

Salmonella spp. are gram-negative, predominantly motile, non-spore-forming rod-shaped bacteria. They belong to the bacterial family of Enterobacteriaceae and are one of the most important bacterial zoonotic pathogens. Biochemical and serological investigation has differentiated two species, namely *Salmonella* (*S*.) *enterica* and *S. bongori*. *S. enterica* is further divided into six subspecies. *Salmonella* isolates can be categorised using the White-Kauffmann-Le Minor scheme on the basis of their somatic (O) and flagellar (H) antigens, and assigned by means of their seroformula to one of over 2,600 serovars (strains with identical antigen combinations).

Bacteria from the *Salmonella genus* are widespread in nature. They are found in many cold- and warm-blooded animals and can be transmitted to humans, e.g. via food. Most of the 2,600 serovars can occur in humans and most animal species. Some serovars, such as *S.* Typhi*, S.* Paratyphi*, S.* Gallinarum and *S.* Dublin, are host-specific and typically found only in humans or chickens or cattle. Salmonella can survive for several months in the environment and in—or on—various kinds of foods. They are tenacious and can survive under extreme environmental conditions.

Compared with other bacteria, *Salmonella*'s requirements for growth are undemanding. *Salmonella* generally grow at temperatures ranging from 10 to 47 °C, and at a pH between about 4 and 9 (optimum pH is a value between 6.5 and 7.5).

While some *Salmonella* exhibit growth at higher temperatures (up to 54 °C), others exhibit an increased tolerance to cold (psychotropic characteristics) and also grow in food stored at between 2 and 4 °C. The water activity value at which growth takes place (minimal a_w value) lies between 0.92 and 0.95, depending on the substrate and temperature. In dry food, *Salmonella* can stay viable for a prolonged period of time even at low a_w values.

3.1.1.2 Campylobacter

Campylobacter are gram-negative, microaerobic, non-spore-forming, spiral-shaped bacteria. They grow under microaerobic conditions (increased CO_2 requirement and higher O_2 sensitivity). They are widespread in the intestines of warm-blooded animals, especially in poultry. While livestock are usually colonised without clinical symptoms, humans can contract campylobacteriosis or *Campylobacter* enteritis. The main causes of human campylobacteriosis are thermotolerant. This means that they cannot multiply below temperatures of 30 °C and have an optimum growth temperature of 37 - 42 °C (Doyle & Roman, 1982). These physiological requirements result in *Campylobacter* generally not being able to multiply in or on food. The most important species that can cause human illness are *C. jejuni* and *C. coli*. The optimum pH for *Campylobacter* is between 6.5 and 7.5; pH values below 4.9 or above 9 result in halted growth or even a decrease in bacterial counts (Doyle & Roman, 1981). When *Campylobacter* occur in an environment that is unfavourable for them, e.g. outside the intestinal tract of the host animals, they are exposed to oxidative stress as well as cold and desiccation stress. *Campylobacter* were culturally detectable in chicken faeces for 5-6 days with decreasing numbers (Ahmed, Schulz, & Hartung, 2013; Bui, Wolff, Madsen, & Bang, 2012). Under these conditions, the bacteria lose some of their vitality, but can also reach a state in which they are no longer culturable using classical methods, but may still exhibit infectivity (viable but nonculturable – VBNC) (Baffone et al., 2006; Bovill & Mackey, 1997; Cappelier, Minet, Magras, Colwell, & Federighi, 1999; Krüger et al., 2014).

3.1.2 Hazard characterisation

3.1.2.1 Salmonellosis

Salmonellosis is an infection caused by bacterial species in the *Salmonella* genus. The typhoidal form (typhoid fever and similar diseases) is primarily caused by the serovars *S.* Typhi*, S.* Paratyphi A, B and C.) Person-to-person transmission is possible. The pathogens are ingested orally and spread through the blood in the body. After an incubation period of a few days to three weeks, a severe, cyclical general infection with diarrhoea and high fever can occur. Organ damage can also occur to the gut, heart, liver, kidneys and gallbladder. In patients with gallstones, the pathogens can be excreted over long periods of time.

In humans, most other *Salmonella* serovars cause the 'enteritic' form of the infection (enteritis = inflammation of the intestines). The infectious dose for adult humans is 10,000 (10^4) to 1,000,000 (10^6) *Salmonella* cells. If *Salmonella* is present in very fatty foods such as cheese, hamburger, chocolate or salami, however, infections have been observed for an infectious dose as low as 100 (10^2) cells of *Salmonella*; the same is true for especially sensitive patients.

The incubation period for infections with enteritic *Salmonella* is 5 to 72 hours (a maximum of seven days) and depends on the infectious dose. In humans, salmonellosis typically starts suddenly with severe watery diarrhoea (which may become bloody in the course of the infection), often accompanied by fever, nausea, vomiting and stomach aches or headaches. Symptoms typically last a few hours or days. In severe clinical cases, chills, high fever, fainting and other systemic clinical symptoms will appear with a typhoidal progression. A mild or symptomatic progression is common, which may also be dependent on the quantity of pathogen ingested.

Patients excrete enteritic *Salmonella* for an average of 3 to 6 weeks, and several months in the case of infants. Long-term excretion exceeding six months is relatively rare.

Cases of severe clinical progression are rare, as are extra-intestinal infections, which may include pericarditis, neurological disorders, reactive arthritis, spondylitis or osteomyelitis. Salmonellosis is rarely fatal. High-risk groups include persons whose immune system is not yet fully developed (children under five years old) and people whose immune system is weakened as a result of old age or pre-existing conditions, for example.

Detection of *Salmonella* must be reported in accordance with the German Infection Protection Act (IfSG) (IfSG, Section 7 Detection of Pathogens Subject to Notification). A decreasing trend of salmonellosis cases was observed between 2001 to 2015 (from 76,990 to 13,876 cases per year). Between 2015 to 2019, the numbers were relatively constant in the range of about 13,000 to 14,300 illnesses per year. With 8,743 cases in 2020, the number of transmitted salmonellosis cases dropped dramatically compared to 2019 (13,696) (RKI, 2021b). However, these observations are associated with the COVID-19 pandemic, which has affected the incidence and recording infectious diseases subject to notification (Ullrich et al., 2021). As in previous years, the highest age-specific incidence was in children aged under five years, with maximum incidence in infants. Both sexes were affected nearly equally (RKI, 2021b).

In 2020, 37 % of cases reported with details of a specific serovar were caused by *S.* Enteritidis, and *S.* Typhimurium, also with 37 %. *S.* Infantis (3.8 %), *S.* Muenchen (2.21 %), *S.* Derby (1.71 %) and *S.* Brandenburg (1.3 %) followed far behind. In 2020, a total of 13 confirmed deaths in connection with salmonellosis were reported to the Robert Koch Institute (RKI) (18 cases in 2019). These included seven males and six females between 43 and 89 years old (overall median: 74 years). Four deaths could be linked to the serovars *S.* Enteritidis and *S.* Typhimurium respectively and one death to *S.* Infantis. Four deaths were reported without specific details of serovars (RKI, 2021b).

In accordance with Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003, a total of 37 salmonellosis outbreaks were reported to the EU by Member States for 2020. High-quality evidence indicates that these outbreaks were caused by the consumption of eggs and egg products. High-quality evidence is said to be available if the results of microbiological and/or epidemiological investigations have been able to determine a link between the identified food and the illness diagnosed that has a high degree of probability.

According to the report from the European Food Safety Authority (EFSA), most outbreaks of salmonellosis in humans are associated with the consumption of eggs and egg products, bakery goods, and meat and meat products (EFSA/ECDC, 2021).

The role of poultry products has been confirmed as a recurring risk for salmonellosis associated with a transnational outbreak of *S.* Enteritidis ST11 infections. In this outbreak, a total of 193 people were ill in eight EU countries and the United Kingdom between 2018 and 2020 (EFSA/ECDC, 2021).

Also in a study on the systematic review of risk factors for human salmonellosis (Guillier et al., 2021), eggs and egg products, mixed foods and meat (pork, red meat other than beef, and poultry), were identified as the foods most frequently associated with salmonellosis.

3.1.2.2 Campylobacteriosis

Human campylobacteriosis is an intestinal infection with abdominal pain and watery, occasionally bloody diarrhoea and fever (Dasti, Tareen, Lugert, Zautner, & Gross, 2010). Various authors showed in a self-experiment and in another study with test subjects that the infectious dose of *C. jejuni* is very low, at 500 - 800 CFU (Black, Levine, Clements, Hughes, & Blaser, 1988; Robinson, 1981). Detection of *Campylobacter* must be reported (IfSG, Section 7 Detection of Pathogens Subject to Notification). In Germany, about 60,000 - 70,000 cases (80 - 90 illnesses per 100,000 inhabitants) are reported annually. Therefore, *Campylobacter* is currently the most common bacterial cause of intestinal infections and human campylobacteriosis is the most frequently reported bacterial foodborne illness in Germany and the EU (EFSA/ECDC, 2021; RKI, 2018).

However, human campylobacteriosis can also lead to autoimmune diseases that occur several weeks after the acute symptoms have subsided. Late effects, such as irritable bowel syndrome (in approx. 4 % of cases), reactive arthritis (acute inflammation of joints, in approx. 2.9 % of cases), and also Guillain-Barré syndrome (in approx. 0.07 % of cases) can occur (Keithlin, Sargeant, Thomas, & Fazil, 2014). Guillain-Barré syndrome causes signs of paralysis in the peripheral nerves. Most of these autoimmune diseases are reversible, but irreversible damage and even death can occur in rare cases. In the case of human campylobacteriosis, all consumer groups are affected. Campylobacteriosis is particularly common in children under four years of age and in young adults aged 20 to 29 (Schielke, Rosner, & Stark, 2014). In 2020, five cases of *Campylobacter* enteritis were reported to the RKI in which the patients died from the illness. These were three men and two women aged between 78 and 87 (RKI, 2021b). In 2020, 46,519 cases of *Campylobacter* enteritis were reported to the RKI (RKI, 2021b).

EFSA data shows that a total of 317 campylobacteriosis outbreaks were reported in 2020, with a total of 1,319 cases, 112 hospitalisations and no deaths. Eleven outbreaks were reported with strong evidence and 306 with weak evidence. The most common foods blamed for foodborne campylobacteriosis outbreaks with strong evidence were chicken meat and raw milk (EFSA/ECDC, 2021). According to estimates, 50 to 80 % of cases with a partly unknown mode of transmission can be ascribed to chicken as the disease reservoir of *Campylobacter* (BfR, 2018b).

Other causes of *Campylobacter* infection can be contaminated surface water, pork or beef. Contact with domestic animals can also cause an infection. Chicken eggs can also transmit *Campylobacter* to humans, particularly if the eggs are visibly contaminated with chicken faeces (BfR, 2018b).

3.1.3 Exposure assessment

3.1.3.1 Prevalence of *Salmonella* in samples of table eggs

Salmonella can enter the egg via two different routes of contamination (Khan, McWhorter, Moyle, & Chousalkar, 2021). On the one hand, the inside of the egg can already be contaminated before the egg is laid, during egg formation in the oviduct of an infected chicken (primary or vertical contamination) (Bygrave & Gallagher, 1989; Shivaprasad, Timoney, Morales, Lucio, & Baker, 1990). Literature states that a number of 100 *Salmonella* cells per egg is seldom exceeded inside the egg after primary contamination (Gast & Beard, 1992; Gast & Holt, 2000; Humphrey, Whitehead, Gawler, Henley, & Rowe, 1991). It is more common that the egg is externally contaminated with *Salmonella* (secondary or horizontal contamination) during or after it is laid, e.g. with the faeces of infected animals (De Reu, Grijspeerdt, Herman, et al., 2006; W. Messens, Grijspeerdt, & Herman, 2005).

Quantitative data on the number of *Salmonella* on the egg surface in the case of natural contamination is not available in the literature for the European market. Contamination of table egg shells with *Salmonella* is sporadic (Ebel & Schlosser, 2000). A low level of bacterial contamination can be expected due to the successful control programmes for *Salmonella* in laying hen farms in the EU. The number of *Salmonella* on the egg surface in the case of natural contamination is therefore estimated by the BfR to be below 10 germs/cm^2 on average (based on the data collected for *Campylobacter*). *Salmonella* are able to survive on the egg shell. Viability on the shell extends at colder ambient temperatures, while unrefrigerated storage results in faster drying of the shell surface and, therefore, a faster death for *Salmonella* present there (W. Messens, Grijspeerdt, & Herman, 2006). If the egg is exposed to temperature fluctuations during storage (e.g. due to disruption of the cold chain), condensation will form on the shell surface. This damp environment, which is maintained for longer periods during cold storage, also increases the penetration of *Salmonella* through the eggshell (Khan et al., 2021). Like other bacteria, *Salmonella* are able to penetrate through the pores of the eggshell and shell membranes into the inside of the egg (Baker, 1990; Board, 1966; De Reu, Grijspeerdt, Messens, et al., 2006). Humid environmental conditions favour penetration into the egg interior (Chen, H., Anantheswaran, R., Knabel, S., 2002). The egg white is armed with substances that inhibit the growth of bacteria. Studies have shown (W. Messens, Duboccage, Grijspeerdt, Heyndrickx, & Herman, 2004) that *Salmonella* are nevertheless able to multiply in fresh egg whites at a temperature of 20 °C. Furthermore, *Salmonella* can survive in the egg white for a longer period of time, pass the yolk membrane and migrate into the nutrient-rich yolk (Baker, 1990; Braun & Fehlhaber, 1995). Inhibitory substances activity in the egg white decreases as a result of the egg's natural ageing process. Furthermore, the permeability of the yolk membrane increases, making it easier for bacteria to migrate into the yolk and also for nutrients to diffuse from the yolk into the egg white. In addition, the nutrients from the yolk facilitate the bacteria's ability to multiply. The time required to reduce the permeability of the yolk membrane is called "yolk membrane breakdown time (YMT)" and is temperature-dependent. When stored at 20 °C, the YMT is reached after about 18 days and the barrier function of the yolk membrane gradually decreases. In the event of cold storage, YMT is reached at a later time (J. Chen, S. Thesmar, & Kerr, 2005). The egg is exposed to natural cooling immediately after it has been laid (chicken's body temperature: 40 - 42 °C; cooler ambient temperature). This temperature difference causes negative pressure in the egg, possibly facilitating the penetration of bacteria through the shell (Board, 1966; Fromm,

1959). It has been determined (Miyamoto et al., 1998) that the penetration rate is highest up to three hours after laying (storage at ambient temperature) due to this thermo-osmotic effect. It has also been established that the penetration capacity decreases when the egg is actively cooled immediately after laying (Miyamoto et al., 1998). Furthermore, cooling the eggs as soon as possible after laying inhibits the ability of already existing or penetrated *Salmonella* in the egg to multiply.

The prevalence of *Salmonella* in samples of table eggs in Germany is recorded as part of systematic official monitoring. The prevalence calculated by the BfR on the basis of the notifications from the German federal states on monitoring carried out on routine samples was between 0.02 and 0.30 % (table eggs chicken, total) and 0.00 and 0.54 % (eggshells) between 2012 to 2017, whereby sampling was not exclusively carried out in the retail sector but also at the producer (Table 1). In the case of induced samples, the prevalence in the eggshell samples was between 0.00 and 1.89 % (Table 1). The serovar most frequently detected in table egg samples during food monitoring is *S.* Enteritidis.

Table 1: Data on the prevalence of *Salmonella* in samples of table eggs and eggshells in Germany from 2012 to 2020

Year	Reason for sampling	Matrix	Sample count	Number positive	Positive (in %)	Serovar (number)	Reference
2012	Monitoring	Table eggs chicken, total	8,370	5	0.06 %	*S.* Enteritidis (5)	(BfR, 2014a)
		Eggshell	831	0	0.00 %	-	
	Monitoring occasion-spesific samples	Eggshell	197	3	1.52 %	*S.* Enteritidis (3)	
2013	Monitoring	Table eggs chicken, total	5,915	1	0.02 %	*S.* Enteritidis (1)	(BfR, 2015)
		Eggshell	1,744	1	0.06%	*S.* Enteritidis (1)	
	Monitoring occasion-spesific samples	Eggshell	144	0	0.00 %	-	
2014	Monitoring	Table eggs chicken, total	4,737	8	0.17 %	*S.* Enteritidis (3) *S.* Kiambu (4) *S.* Indiana (1)	(BfR, 2016)
		Eggshell	1,589	6	0.38 %	*S.* Enteritidis (1) *S.* Kiambu (4) *S.* Indiana (1)	
	Monitoring occasion-spesific samples	Eggshell	794	15	1.89 %	*S.* Enteritidis (11) *S.* Group B (4)	
2015	Monitoring	Table eggs chicken, total	4,098	9	0.22 %	*S.* Enteritidis (6) *S.* Indiana (1) n.d. (2)	(BfR, 2018a)

		Eggshell	1,300	7	0.54 %	*S.* Enteritidis (6) *S.* Indiana (1)	
	Monitoring occasion-spesific samples	Eggshell	772	1	0.13 %	*S.* Typhimurium (1)	
2016	Monitoring	Table eggs chicken, total	3,728	3	0.08 %	*S.* Typhimurium (1) n.d. (2)	(BfR, 2019)
		Eggshell	1,680	0	0.00 %	-	
	Monitoring occasion-spesific samples	Eggshell	2,034	3	0.15 %	*S.* Indiana (2) *S.* sp (1)	
2017	Monitoring	Table eggs chicken, total	4,600	14	0.30 %	*S.* Enteritidis (5) n.d. (9)	(BfR, 2020)
		Eggshell	1,430	0	0.00 %	-	
	Monitoring occasion-spesific samples	Eggshell	1,859	2	0.11 %	*S.* Enteritidis (1) *S.* Ordonez (1)	
2020	Zoonoses monitoring	Pool sample from table egg shells	367	0	0	-	(BVL, 2021)
		Table egg shells at the entrance to the packing station	317	0	0	-	
		Table egg shells at the exit of the packing station	325	0	0	-	

n.d. = no data

Table eggs were examined nationwide in Germany in 2010 as part of zoonoses monitoring. The insides of the eggs were free of *Salmonella* in all pool samples of table eggs examined. Contamination only affected the egg shells. In pool samples of table eggs from the retail sector, an average of 0.7 % of the eggshells tested positive for *Salmonella*. Pool samples from table egg shells from the retail sector from caged laying hens showed 0.9 % contamination with *Salmonella* spp. Egg shells from table egg pool samples from cage-free and free-range hens were contaminated with *Salmonella* at 0.7 % and 0.8 %, respectively. *Salmonella* was detectable in 0.4 % of the pool samples from eggshells of table eggs from organic production. Pool samples of table eggs from the retail sector originating in Germany had a shell contamination rate of 0.8 %, while 0.5 % of pool samples of table eggs of non-German origin tested positive for *Salmonella* (BVL, 2012).

In 2020, no *Salmonella* was detected in samples of (table) eggshells from egg packing centres or from the retail sector as part of zoonoses monitoring (Table 1) (BVL, 2021).

3.1.3.2 Prevalence of *Campylobacter* in samples of table eggs

Contamination of eggshells with *Campylobacter* occurs via faecal excretions from laying hens. In Germany, laying hens are frequently colonised with *Campylobacter*. In 2009, *Campylobacter* were detected in 41.8 % of faecal samples from laying hen farms (BVL, 2010). Literature states that colonisation with *Campylobacter* in naturally infected laying hens generally lasts longer and is more severe than colonisation with *Salmonella* (Jones, Anderson, & Guard, 2012; Jones et al., 2016; Rukambile, Sintchenko, Muscatello, Kock, & Alders, 2019).

There is little experimental data on the viability of *Campylobacter* on eggshells. It confirms the relatively low stress tolerance of these bacteria to desiccation (Allen & Griffiths, 2001; Clark & Bueschkens, 1985). Nevertheless, the infection strain was detected in the intestinal tract of about 10 % of hatched, healthy chickens after hatching eggs infected with *C. jejuni* were culturally "*Campylobacter*-free" (Clark & Bueschkens, 1985). Vertical contamination has not been documented with *Campylobacter* (Sahin, Kobalka, & Zhang, 2003). In the rare cases where transmission from laying hen to hatching chick has occurred, faecal contamination of the shell, shell membrane and albumin of freshly laid fertilised eggs and subsequent oral ingestion by the hatching chick is assumed (Cox et al., 2012). There is hardly any quantitative data in the literature on the number of *Campylobacter* on eggshells in the case of natural contamination. A study by the BfR dealt with the quantification of *Campylobacter* on eggshells. *Campylobacter* was detected by real-time PCR on 80 % of the eggshells. *Campylobacter* could be detected with a mean concentration of 3.31 and 3.61 $\log_{10}$ CFU per 10 eggs respectively on visually clean or slightly polluted eggshells, (Stingl, 2021). A subset of the eggshells was also examined after being washed in peptone water for 30 minutes using a special real-time PCR that can distinguish living from dead bacteria. In this case, living bacteria were detected in 12 % of the eggshell samples. The mean value was 3.51 $\log_{10}$ CFU per 10 eggs, with a maximum of 5.17 $\log_{10}$ CFU per 10 eggs quantifiable as alive in one batch. Based on an average egg surface of 70 cm^2, this results in an average *Campylobacter* contamination of ~ 5 germs/cm^2 egg surface, with a maximum of 211 germs/cm^2. Data on germ transmission through short or longer contact times is still lacking.

In contrast to *Salmonella*, *C. jejuni* are not able to survive or multiply inside the egg for long periods (Fonseca et al., 2014; Paula, Fonseca, Silva, & Rossi, 2009). Penetration of *Campylobacter* through the eggshell has been observed occasionally in studies (Allen & Griffiths, 2001; Fonseca et al., 2014). For example, after inoculation for 24 hours in nutrient broth contaminated with *Campylobacter, C. jejuni* was able to penetrate the shell and detected on the shell membrane in 4.2 % of 48 fresh eggs (Allen & Griffiths, 2001). In another study, where eggs were placed for inoculation in wood chips contaminated with *C. jejuni*, *C. jejuni* was able to penetrate the shell of 20 % of fertilised eggs but not of table eggs (Fonseca et al., 2014). Although *Campylobacter* was detected at a frequency of 0.28 - 4 % on chicken egg shells in several studies, the pathogen was not detected inside the egg (Aziz, Bahobail, Hassan, & El-deeb, 2012; Doyle, 1984; Ge et al., 2016; Messelhäusser et al., 2011; Sulonen, Kärenlampi, Holma, & Hänninen, 2007). Only one Iranian study reported the detection of *Campylobacter* in 2 % in egg whites, 4 % in egg yolks and 7 % on shells out of a total of 100 chicken eggs examined (Jonaidi-Jafari, Khamesipour, Ranjbar, & Kheiri, 2016). A study from Japan, in which *Campylobacter* was detected in 27.9 % of samples of unpasteurised liquid whole egg and 36 % of unpasteurised liquid egg yolk, showed that

Campylobacter can often pass from the shell into the liquid egg contents during industrial egg-breaking (Sato & Sashihara, 2010).

There is little data on the prevalence of *Campylobacter* in samples of table eggs in Germany. In one study, 271 pool samples of 10 chicken eggs each were examined for the presence of *Campylobacter*. *Campylobacter* was detected in 4.1 % of the eggshell samples (Messelhäusser et al., 2011). Despite low total sample numbers, *C. jejuni* and *C. coli* were regularly detected on eggshells or whole table eggs between 2012 and 2017 (Table 2). In 2014, chicken egg shells were examined for the presence of *Campylobacter* in Germany as part of the zoonoses monitoring and a prevalence of 0.4 % was determined (BfR, 2016). The prevalence calculated by the BfR on the basis of the notifications from the German federal states on monitoring carried out on routine samples was between 0.99 and 10.55 % (Table 2), whereby sampling was not exclusively carried out in the retail sector but also at the producer.

Table 2: Data on the prevalence of *Campylobacter* in samples of table eggs and eggshells in Germany (2012-2020)

Year	Reason for sampling	Matrix	Sample count	Number positive	Positive (in %)	Strain	Reference
2012	Monitoring	Table eggs chicken, total	101	1	0.99 %	*C. jejuni* (1)	(BfR, 2014a)
2013	Monitoring	Table eggs chicken, total	39	2	5.13 %	*C. jejuni* (1)	(BfR , 2015)
2014	Monitoring	Table eggs chicken, total	265	6	2.26 %	*C. coli* (1) *C. jejuni* (2)	(BfR , 2016)
	Zoonoses monitoring	Pool samples of table egg shells	471	2	0.40 %	n.d.	
2015	Monitoring	Table eggs chicken, total	148	12	8.11 %	*C. coli* (2) *C. jejuni* (3)	(BfR, 2018a)
2016	Monitoring	Table eggs chicken, total	218	23	10.55 %	*C. coli* (9) *C. jejuni* (4)	(BfR , 2019)
2017	Monitoring	Table eggs chicken, total	329	27	8.21 %	*C. coli* (9) *C. jejuni* (5)	(BfR , 2020)
2020	Zoonoses monitoring	Pool samples of table egg shells	364	2	0.5 %	*C. jejuni* (1)	(BVL, 2021)
		Table egg shells at the entrance to the packing station	313	10	3.2 %	*C. coli* (4) *C. jejuni* (5)	
		Table egg shells at the exit of the packing station	320	4	1.3 %	*C. coli* (1) *C. jejuni* (1)	

n.d. = no data

3.1.3.3 Decontamination of table eggs using UV-C treatment

UV-C rays are electromagnetic waves in the wavelength range from 100 to 280 nanometres (nm). Inactivation of bacterial cells by UV rays is based on damage to DNA and RNA. Bacterial DNA transcription and replication is prevented due to DNA lesions. There is an increase in the formation of pyrimidine dimers and other photoproducts due to UV treatment (Rastogi, Richa, Kumar, Tyagi, & Sinha, 2010).

However, there are major differences between bacterial genera in terms of their resistance to UV-C treatment. Furthermore, several factors, such as the physiological status of bacterial cells and strain differences, contribute to differences in resistance to UV light (Hijnen, Beerendonk, & Medema, 2006). Limiting factors are also contamination (clouding phenomena) and the low penetration depth of the UV treatment. This is in the micrometer range, whereby it is heavily dependent on the matrix that is being treated (Geveke, Boyd, & Zhang, 2011).

Studies showed that UV-C treatment of eggs with no visible pollution can reduce the microbial load of the shell by at least one log level (Coufal, Chavez, Knape, & Carey, 2003; De Reu, Grijspeerdt, Herman, et al., 2006). Conversely, in eggs with visible faecal contamination, no significant reduction in the bacterial count was found (De Reu, Grijspeerdt, Herman, et al., 2006). There are several experimental studies available on the effect of UV-C treatment on eggs. Reduction rates of up to 3 $\log_{10}$ CFU/egg for the total microbial count, 4 $\log_{10}$ CFU/egg for *Salmonella* spp. and 4 - 5 $\log_{10}$ CFU/egg for *E. coli* have been achieved (Chavez, Knape, Coufal, & Carey, 2002; Coufal et al., 2003; De Reu, Grijspeerdt, Herman, et al., 2006; Wells, Coufal, Parker, & McDaniel, 2010). By rotating the eggs during UV-C treatment, the bactericidal effect could be increased by reducing this clouding effect (Kuo, Ricke, & Carey, 1997).

3.1.3.4 Results of the UVegg project on UV-C-LED treatment of table eggs

The aim of the BMEL-funded project “Use of UV-C/UV-C-LED radiation to reduce microorganisms on eggs” (UVegg) (2018 - 2021) was to demonstrate the efficiency of the reduction of (zoonotic) microorganisms and the harmlessness of treating table eggs using UV-C-LED radiation.

In different approaches, artificially contaminated table eggs were treated with UV-C-LED radiation (intensity: ~ 2.4 mW/cm², wavelength range: 280 nm). The rolling speed of the table eggs was 1.6 rotations per second, the UV-C exposure was 5 seconds and the distance to the UV-C LED was 5 cm. Visually clean, size M table eggs were used up to a maximum of 10 days before the best-before date.

The experimental contamination of the egg surface was carried out with defined bacterial suspensions (*S.* Enteritidis*, S.* Typhimurium) in three different recovery levels (100, 10,000 and 1,000,000 CFU/cm^2), each of which additionally had no (only phosphate-buffered saline – PBS), a low (3 grams (g) bovine serum albumin (BSA)/litre) or a high organic load (10 g BSA/L and 10 g yeast extract/L). The experiment with *C. jejuni* was only possible with a bacterial contamination of 100 CFU/cm^2 due to high methodological losses.

UV-C-LED treatment was generally able to reduce the number of artificially applied bacteria on egg surfaces in the project, but it was dependent on the level of pollution and bacterial contamination level.

Using UV-C-LED, *S.* Enteritidis (19-SA00302) and *S.* Typhimurium (18-SA01629) could be reduced by up to 1.32 $\log_{10}$ CFU/cm^2 and 1.42 $\log_{10}$ CFU/cm^2 respectively, with medium bacterial contamination (10,000 CFU/cm^2) and low organic load on the egg surface. With high bacterial contamination (1,000,000 CFU/cm^2) and a high organic load, lower reduction rates were achieved (0.57 $\log_{10}$ CFU/cm2 for *S.* Enteritidis and 0.34 $\log_{10}$ CFU/cm^2 for *S.* Typhimurium). In the case of low bacterial contamination (100 CFU/cm^2) without additional organic load, *Salmonella* could no longer be detected in half of the chicken eggs examined after treatment with UV-C-LED (methodological limit of detection: 5 CFU/cm^2).

The influence of UV-C-LED treatment of table eggs on the bacterial reduction of *C. jejuni* (DSM4688) could only be tested at a low bacterial contamination (100 CFU/cm^2) because of high methodological losses. The highest reductions of up to 1.69 $\log_{10}$ CFU/cm^2 could be achieved at a low organic load using UV-C-LED. The use of a high organic load generally contributed to significantly reduced reduction rates of 0.61 $\log_{10}$ CFU/cm^2. In the absence of an additional organic load, *C. jejuni* could no longer be detected in half of the chicken eggs examined after treatment with UV-C-LED (methodological limit of detection: 5 CFU/cm^2).

The use of the UV-C-LED panels was also investigated in practice in the UVegg project. To determine the reduction rates of relevant microorganisms, 35 table eggs were each taken from the conveyor belt directly before and directly after treatment with the UV-C-LED system on a total of five test days. However, the intensity of the UV-C-LED was 4.5 mW/cm^2 and the samples were taken before and after UV-C treatment at two different times. In the laboratory, the surfaces of the table eggs were examined for their natural contamination with suspected enterococci, suspected *Staphylococcus aureus* and suspected *Escherichia coli*. The eggshells' total aerobic mesophilic bacterial counts were also determined. The treatments using UV-C-LED panels resulted in reductions of about one log level for the table eggs' natural bacterial contamination. In contrast to the laboratory tests with *Salmonella* and *Campylobacter*, lower reductions were achieved in practice. The table eggs' natural bacterial and organic load could lead to individual pathogen groups being better protected from UV-C exposure. Shadowing/absorption effects due to load substances as well as increased bacterial contamination also led to significantly lower reduction rates of a maximum of one log level/cm^2 in the laboratory experiments. In addition, the natural bacterial load of table eggs, which is at least partially recorded in the aerobic mesophilic total bacterial count, consists of yeasts and spore-forming bacteria of the genus *Bacillaceae* and *Clostridiaceae* (Olsen et al., 2017), which can be more resistant to UV-C treatment than the bacteria tested in the laboratory experiments. Furthermore, a rapid recontamination of the table eggs after UV-C treatment in the production plant could be determined. This is due to a high particle load in the air as well as contact with conveyor belts.

3.1.3.5 Chicken egg consumption in Germany

In Germany, around 20 billion eggs were consumed in 2020, of which around 70 % were produced in Germany itself (BMEL, 2021).

According to a national consumption survey published in 2008 by the Max Rubner Institute (MRI), men in Germany consumed an average of 97 and women 73 eggs per year (excluding

egg-based dishes). In addition, 5 g of egg per day are consumed in dishes with eggs as the main ingredient, such as egg salads or pancakes. The amount consumed could possibly be lower than in other consumption surveys since the dishes in which eggs are not the main ingredient were not included (MRI, 2008).

Raw eggs or foods containing raw eggs are among the foods rarely consumed and are consumed across all age groups 1 - 2 times per quarter (children/adolescents/adults) or less frequently or less than once a month (infants/toddlers) (Golsong, Nowak, Schweter, & Lindtner, 2017; Mensink et al., 2007).

3.1.4 Risk characterisation

Data from food monitoring authorities on the detection of *Salmonella* and *Campylobacter* in chicken eggs samples show that in Germany the presence of *Campylobacter* and very rarely also *Salmonella* can be expected on table egg shells. When the eggs are broken, these pathogens can get into the food. Due to the very low infection dose in humans, this can cause campylobacteriosis if the food is not heated sufficiently before consumption (at least 2 minutes at 70 °C on all parts of the food). For an infection with *Salmonella*, it is usually first necessary for the pathogens to multiply in the food due to insufficient cooling. However, *Salmonella* can also enter the inside of the chicken eggs from the shell and potentially multiply in the yolk, especially if the table eggs are stored unrefrigerated for a longer period of time.

Both pathogens can also enter other ready-to-eat food via cross contamination. Another conceivable route of transmission is blowing contaminated eggs with the mouth.

Salmonella and *Campylobacter* infections can cause moderately severe gastrointestinal diseases. Children under the age of five and elderly people or those with pre-existing medical conditions are particularly at risk of contracting salmonellosis. Campylobacteriosis mainly affects children under five and young adults, and in individual cases can lead to permanent health impairments. Both diseases are very rarely fatal.

To minimise the risk of foodborne infection, class A chicken eggs must have a clean, undamaged shell and cuticle according to Regulation (EC) No 589/2008 (Article 2, Paragraph 1a) on marketing standards for eggs. In the case of visible contamination, the eggs are classified as class B and undergo processing operations with a heating stage. However, despite these regulations, table eggs with low to moderate faecal contamination, sometimes also with sticky feather residues, are also occasionally found in retail.

One risk mitigation measure already in practice is the treatment of table eggs with UV-C; therefore, the influence of a UV-C-LED treatment on the bacterial reduction of chicken eggs contaminated with *Salmonella* or *Campylobacter* is assessed on the basis of two scenarios.

Scenario 1: Table eggs are contaminated with *Campylobacter* and treated for 5 seconds using UV-C-LED.

Campylobacter are regularly detected on table eggs in Germany. If visually clean table eggs are treated with UV-C-LED, a reduction in the number of *Campylobacter* present on the eggshell by about 1.5 log levels/cm^2 can be expected. According to the results of internal laboratory tests, pathogen quantities of less than 10 CFU/cm^2 can be assumed for visually clean or slightly polluted eggshells contaminated with *Campylobacter*. This means that treatment using UV-C-LED is suitable for reducing the probability of pathogen transmission

and thus also of health impairment for humans. The condition, however, is that no recontamination of the treated eggs occurs. In the case of higher contamination of the eggshells with *Campylobacter* or other organic material, treatment with UV-C-LED would have a significantly lower or no effect at all on the probability of pathogen transmission and health impairments for humans.

Scenario 2: Table eggs are contaminated with *Salmonella* and treated for 5 seconds using UV-C-LED.

Thanks to the successful control of *Salmonella* on laying hen farms, *Salmonella* is rarely found on table eggs in Germany. If visually clean table eggs are treated with UV-C-LED, a reduction in the number of *Salmonella* present on the eggshell by about one log level/cm^2 can be expected. Assuming that only small amounts of pathogens (less than 10 CFU/cm^2) are present on visually clean or slightly polluted eggshells contaminated with *Salmonella*, treatment with UV-C-LED is suitable for reducing the probability of *Salmonella* transmission and thus also health impairments for humans. The condition, however, is that no recontamination of the treated eggs occurs. If the egg shells are more heavily contaminated with *Salmonella* or organic material, treatment with UV-C-LED would probably have little effect because *Salmonella* could continue to penetrate the egg yolk or enter the food when the table eggs are broken and multiply there.

3.1.4.1 Assessment of the data quality, need for further research

The quality of existing data and information related to the characteristics of *Salmonella* and *Campylobacter*, its transmission to humans and the diseases triggered by these pathogens, can be assessed as satisfactory. The data situation regarding the prevalence of *Salmonella* on chicken eggs is also satisfactory. The data on the number of *Salmonellae* on eggshells in the case of natural contamination is not satisfactory. There is a need for further research here to be able to better estimate the probability of pathogen transmission and thus also health impairments for humans.

There is only limited data on the prevalence of live *Campylobacter* on chicken eggs in Germany. *Campylobacter*, especially after exposure to stress, such as desiccation, oxygen stress and suboptimal temperatures, require complex laboratory conditions to be reliably quantitatively detected. Alternatively, they can be quantified via live/dead differentiating real-time PCR. Further research on improved sensitivity of cultivation-independent detection of live *Campylobacter* in the context of dead cells and an application of these methods in combination with classical official monitoring of chicken eggs for the presence of *Campylobacter* (qualitative and quantitative analyses) are necessary to improve the data situation.

The data quality for substantiating the efficiency of the reduction of zoonotic microorganisms using UV-C-LED is judged to be satisfactory. However, the data generated from the “UVegg” project mainly referred to the treatment of table eggs using UV-C-LED with a wavelength of 280 nm, an intensity of ~ 2.4 mW/cm^2, a distance from the eggs to the UV-C-LED of 5 cm and an exposure time of 5 seconds. Further research is needed to clarify the effect of exposure time, distance to the UV-C source and the different intensities and wavelengths of the UV-C-LED panels on the reduction of zoonotic microorganisms on table eggs.

3.2 Risk management options, recommended measures

As a condition for the use of UV-C-LED processes for the treatment of table eggs, food companies should, from the BfR's point of view, meet the following conditions, among others:

- verify the efficacy of the radiation used,
- document the decontamination procedure,
- implement measures to prevent adverse effects on eggs in the event of technical malfunctions during UV-C treatment.

The UV-C light generated by LED panels (UV-C-LED) is suitable for a minor reduction of bacterial contamination of chicken eggs at an exposure time of 5 seconds. Complete elimination of the pathogens was only possible under experimental conditions with low bacterial counts and without organic loads. It is therefore to be expected that with higher bacterial counts or visibly polluted chicken eggs, the pathogens cannot be satisfactorily reduced by the UV-C-LED treatment under the conditions given in the project. Testing an LED application with higher energy density and/or longer exposure should therefore be considered.

Suitable placement of the UV-C-LED panels in the egg packing centre is required (preferably at the end of the sorting process) to avoid recontamination of table eggs after UV-C-LED treatment.

The risk of transmission of pathogens from chicken eggs to humans can also be reduced using the following measures, which address the producer, but also consumers:

- It is imperative that faecal contamination on the eggshells is avoided during the production and packaging of chicken eggs.
- Raw chicken eggs should always be stored and processed separately from other foods.
- Kitchen utensils should always be cleaned thoroughly with hot water and washing-up liquid after contact with raw chicken eggs.
- Hands should be washed thoroughly after touching chicken eggs.
- Only clean chicken eggs should be used to make raw egg dishes and they should be cracked particularly carefully so that the egg contents have as little contact as possible with the egg shell.
- Commercially available egg yolk separators should be used to separate the egg whites from the yolks.
- Heating egg-containing food can kill pathogens. Therefore, especially people with impaired immune systems or those that are not yet fully developed (especially small children, sick and very old people) should only consume chicken eggs after they have been heated through completely (when the egg white and yolk are solid) to protect them from foodborne infections.
- If you want to minimise the risk of contracting a foodborne infection, you should refrain from blowing raw chicken eggs using your mouth or use an egg blower.
- Children in particular should not eat raw dough or whipped egg whites when baking or lick their fingers or any utensils used.

3.3 Other aspects

The results obtained in the UVegg project show that the treatment of table eggs with UV-C-LED leads to a slight reduction of bacteria present on the egg shells. This effect was not only demonstrated for *Salmonella* and *Campylobacter*, but also for the other bacteria used in the research project (*Enterococcus faecium*, methicillin-resistant *Staphylococcus aureus* and ESBL-producing *Escherichia coli*).

Enterococci, especially *Enterococcus (E.) faecium* and *E. faecalis*, are natural colonisers of the human and animal gut (Fisher & Phillips, 2009). They possess a variety of natural and acquired resistance determinants and are, therefore, used as indicator germs for the occurrence of antibiotic resistance in certain populations (BfR, 2003). Enterococci are found almost ubiquitously in chicken coops. In one study, *Enterococcus* spp. was detected in more than 96 % of the cloacae of German laying hens and on the shells of 53 % of the eggs examined. Of the isolated *E. faecalis*, 36 % of the isolates showed resistance to more than one antibiotic (Schwaiger et al., 2010).

The two species *E. faecium* and *E. faecalis* are also most frequently responsible for human infections with enterococci. Vancomycin-resistant enterococci (VRE), which account for a steadily increasing number of nosocomial infections (RKI, 2021a) are particularly significant.

Methicillin-resistant *Staphylococcus aureus* (MRSA) are resistant to certain antibiotics, such as penicillin and cephalosporins (BfR, 2014b). They appear as colonisers of the skin and mucous membranes of humans and animals, but can also cause serious infections and septicaemia in immunocompromised people (BfR, 2009).

The abbreviation ESBL stands for extended spectrum beta-lactamase, meaning these enzymes can destroy not only penicillin but also 3rd and 4th generation cephalosporins (BfR, 2011). This means that the bacteria that produce these enzymes are resistant to these antibiotics. In most cases, human colonisation with ESBL-producing *E. coli* is asymptomatic, as most of these bacteria are harmless intestinal flora. However, there are also ESBL-producing *E. coli* that can cause illness in humans, e.g. enterohaemorrhagic *E. coli* (EHEC). Furthermore, the genes that transmit the ESBL property are often located on mobile genetic elements so that they can easily be transferred between different bacterial species, therefore spreading resistance.

Further information on foodborne infections available on the BfR website

Consumer Tips for the Protection against foodborne Infections in private households (in German only)
https://www.bfr.bund.de/cm/364/protection-against-foodborne-infections.pdf

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