Position statement of the ZKBS on genetic engineering operations with enterohaemorrhagic *Escherichia coli* strains (EHEC)

1. Introduction

*Escherichia coli* are gram-negative bacteria that physiologically occur in the intestines of humans and many animal species. As facultative pathogens they can cause various diseases when they overcome the barrier function of the intestine, the skin, the respiratory tract or the urinary tract. A prerequisite is an anatomical or functional pre-damage of the respective barrier (e.g. by trauma, mechanical ventilation, antibiotic therapy). This is often the case with hospitalised patients. Accordingly, *E. coli* often cause wound healing disorders, peritonitis, cholecystitis, urinary tract infections, pneumonia and - with systemic spreading - sepsis or meningitis. They are feared as they cause nosocomial infections with increasing resistance to common antibiotics.

Depending on their genetic make-up of cell-bound or secreted virulence factors whose genes are located chromosomally or on transposons, plasmids, phages or pathogenicity islands, *E. coli* strains lead to a broad spectrum of intestinal and extraintestinal diseases.

Depending on phenotypic, genotypic and clinical features, a distinction is made between:

- Extraintestinal pathogenic *E. coli* (ExPEC): cause urinary tract infections, septicemia, peritonitis, neonatal meningitis, or infections of birds ¹
- Enteropathogenic *E. coli* (EPEC): cause diarrhea, especially in infants, due to damage to the epithelium of the small intestine (adhesins, secretory proteins)
- Enteroinvasive *E. coli* (EIEC): cause diarrhea due to damage to the epithelium of the colon (cell invasion, intracellular multiplication)
- Enterotoxic *E. coli* (ETEC): cause diarrhea by secretion of various toxins (heat-labile toxins LT I, LT II, heat-stable enterotoxin ST)
- Enteroaggregative *E. coli* (EAEC): cause often chronic diarrhea caused by auto-aggregative adherence fimbriae, biofilm formation, and secretion of serine proteases and enterotoxins.
- Enterohemorrhagic *E. coli* (EHEC): cause watery or bloody diarrhea (colitis) and hemolytic-uremic syndrome (HUS) by secretion of various toxins, especially shigatoxins 1 and 2 and hemolysin.

*E. coli* are transmitted by contact infection or via water or contaminated food between humans and animals.

¹ According to these clinical pictures, ExPEC have in the past been assigned to their own groups (eg UPEC, MNEC, APEC). However, these have since been united because they are indistinguishable phylogenetically, epidemiologically and by their repertoire of virulence factors [1].
Also worth mentioning are the *E. coli* laboratory strains K12 and B, which are considered apathogenic and widely used as model organisms in research into bacterial genetics, physiology and molecular biology.

One or more virulence factors or toxins are detected for diagnosis. The classification according to serological surface characteristics (O-antigens, H-antigens), which used to be common practice, is now used at best as a guide.

In order to respond to various requests, the ZKBS comments on the safety assessment of genetic engineering operations with EHEC strains and on the safety measures required according to GenTSV.

2. **EHEC infections**

EHEC cause watery or bloody diarrhea. As a complication, the hemolytic uremic syndrome characterised by hemolytic anemia, thrombocytopenia, renal failure, and central nervous system dysfunction can occur. The infectious dose for humans is very low with 100 germs. Transmission takes place through the consumption of contaminated food (meat, raw milk, water, fruit juices, plants fertilised with manure). During an outbreak in Germany and some European countries in 2011 caused by an *E. coli* strain of the serotype O104:H4, 3842 mostly adult people fell ill. 855 patients (22%) developed a HUS, of which 53 of these patients (4.1%) died [2]. The trigger of the epidemic is a hybrid strain of EHEC and EAEC, which is therefore also known as entero-aggregative-hemorrhagic *E. coli* (EAEHEC) [3; 4].

EHEC are highly persistent at low pH, high salt concentration and low temperatures. These properties, but especially the high acid resistance in combination with the low infectious dose, explain that EHEC are belong to the dreaded pathogens of foodborne infections today.

In acute EHEC infections, antibacterial chemotherapy is generally contraindicated. It can prolong bacterial excretion and contribute to increased toxin release. In asymptomatic chronic excretion, short-term therapy with azithromycin may be considered. The treatment of the symptoms of HUS can only be symptomatic (usually by forced diuresis, plasmapheresis and in the case of global renal insufficiency by hemo- or peritoneal dialysis).

There is no vaccine available for the prevention of EHEC infections in animals or humans.

Most EHEC bacteria have the following virulence factors:

- the phage-encoded ability to produce Shiga toxins (also known as verocytotoxins) Stx1 and/or Stx2 (rarely variant Stx2v),
- a virulence plasmid with the genes for EHEC-hemolysin and catalase / peroxidase,
- the adhesion protein intimin, whose gene eaeA together with genes for the type III secretion apparatus are on the so-called pathogenicity island LEE (*locus of enterocyte effacement*); these genes localized in LEE are necessary together for the effective attachment of EHEC to the intestinal epithelial cells.

Shiga / verotoxin-producing *E. coli* are generally referred to as STEC / VTEC. The EHEC represent a subgroup of the STEC, whereby in Germany all STEC from human isolates are called EHEC [5]. Virulent EHEC strains with the three virulence factors toxin production, EHEC-hemolysin and intimin are more commonly associated with EHEC enteritis or HUS than strains lacking these virulence factors.

STEC / VTEC without virulence plasmids and/or eaeA gene (intimin), which are often assigned to serovars other than *E. coli* O157, O26 and O111 and circulate in farm animals, especially cattle, usually have a significantly lower virulence for humans. However, in the past,
strains whose genome did not contain the eaeA gene typical for EHEC were also involved in HUS outbreaks [3; 6]. The best-known example is the above-mentioned EHEC outbreak in 2011. This outbreak was caused by an *E. coli* strain whose genome lacks an eaeA gene. However, it contains both a phage genome with the stx2a gene as well as genes for aggregative adherence fimbriae (AAF/1) that are not typical of EHEC but of EAEC.

3. Safety assessment

*E. coli* strains are assigned to **risk group 1** in the list of risk-assessed donor and recipient organisms for genetic engineering operations according to Article 5 para. 6 GenTSV, if it is proven that they are apathogenic. This applies, for example, to the well characterised laboratory strains *E. coli* K12 and B, but also to some commensal *E. coli* strains such as Nissle 1917. Commensal *E. coli* in general and pathogenic *E. coli* are assigned to **risk group 2**, as they can cause diseases that are usually well treatable. These include, for example, EPEC, EIEC, EAEC or ExPEC. Hybrid strains of these pathovars are assigned to **risk group 2** if no Shiga toxin genes are present in their genome.

In contrast, enterohemorrhagic *E. coli* strains, STECs or hybrids from EHEC and other *E. coli* pathogenic strains are assigned to **risk group 3** because they have a low infectious dose and can cause life-threatening HUS, but are not transmitted over aerosols.

The decisive factor for the classification is whether the relevant *E. coli* strains carry Shiga toxin genes and are capable of colonising host organisms. It cannot be ruled out that STEC or hybrids from EHEC and other pathogenic *E. coli* strains can trigger hemorrhagic diseases in humans even without expressing virulence factors typical for EHEC, such as intimin. For this reason, all STECs and hybrids of EHEC and other pathovars bearing Shiga toxin genes are considered potential EHECs. The following assessment refers to both EHEC in the strict sense and STEC and the above-mentioned hybrid strains from EHEC and other pathovars.

A. Genetic engineering with EHEC and EHEC hybrid strains as donor organisms:

a) If genes for virulence factors of EHEC or EHEC hybrid strains, such as Stx1, Stx2, Stx2v or other toxins such as hemolysin, are introduced into *E. coli* K12 and derivatives (risk group 1) as recipient organisms with the aid of vectors which fulfil the requirements for biological safety measures in accordance with Article 6 (5) GenTSV, the resulting genetically modified organisms (GMOs) are classified into **risk group 2**.

The genetic engineering operation is assigned to class 2.

Reasoning:

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2 The classification in risk group 3** was originally made by Directive 97/59/EEC of 1997, which adapted Annex III (list of organisms) to Council Directive 90/679/EEC on the protection of workers from risks related to exposure to biological agents at work to technical progress and assigned EHEC strains to risk group 3 and marked with (**): “For certain biological agents classified in Group 3 and marked with two asterisks (**) in the list, the risk of infection for workers is limited as airborne infection is not normally possible. Member States shall assess the safety measures applied to biological agents in order to determine whether, in the specific circumstances, specific measures can be waived, considering the nature of the activities involved and the quantity of biological agent concerned.”
Single or multiple genes coding for virulence factors of EHEC are transferred to strains such as *E. coli* K12. The GMOs show no or, compared to EHEC, only a weaker virulence, since the other virulence factors responsible for the pathogenesis of EHEC are missing, such as adhesins.

b) If genes for other virulence factors of EHEC, such as intimin or other genes located on the LEE pathogenicity island, are individually introduced into *E. coli* K12 and derivatives as recipient organisms with the aid of vectors which fulfil the requirements for biological safety measures according to § 6 para. 5 GenTSV, the resulting GMOs are classified in **risk group 1**.

The genetic engineering operation is assigned to **class 1**.

**Reasoning:**

Individual genes located on a pathogenicity island are transferred from EHEC to strains such as *E. coli* K12. They encode secreted proteins belonging to the type III secretion system, or interact with bacterial effectors (e.g. intimin) to ensure colonisation and survival of EHEC in the host. Individually, these genes cannot be effective. For the development of virulence further genes of a pathogenicity island are needed. Since *E. coli* K12 laboratory strains lack pathogenicity islands, it is not to be expected that virulence proteins expressed can be effective.

**B. Genetic engineering with EHEC and EHEC hybrid strains as recipient organisms:**

If genetic modifications are introduced in EHEC strains (mutations, insertions) that do not alter the virulence or do not lead to the attenuation of virulence, the GMOs are assigned to **risk group 3**. This classification also applies to genetically modified EHEC strains which do not exceed the hazard potential of EHEC wild-type strains (e.g. O157: H7, O104: H4 or O103: H2) or patient isolates.

One mutation that certainly reduces pathogenicity is the deletion of the Stx genes.

Genetic engineering operations with genetically modified EHEC strains or STEC strains or hybrids from EHEC and other pathovars must always be carried out in compliance with **class 3 safety measures** according to Annex IIIA GenTSV (see TRBA 100, No. 5.4.2). If the restricted transmission possibilities of organisms of the risk group 3** are taken into account, the following measures are not necessary for genetic engineering work with EHEC:

- air lock
- Negative air pressure in the laboratory
- Sealability of the laboratory for the purpose of fumigation
- Filtration of exhaust air from the laboratory
- Autoclave in the laboratory (an autoclave must be present within the building and the transport of waste thereto must be in accordance with GenTSV Annex III Part A III, Level 3, sentence 15)
- higher fire resistance class of walls, windows and doors compared to genetic engineering facilities with safety measures of level 2
- external emergency plan.

However, the above classification of genetic engineering operations with EHEC strains as recipient organisms and the safety measures laid down here do not release the competent state
authorities from the obligation to obtain a statement from the ZKBS (see Article 10 para. 7 in conjunction with Articles 8 para. 1 and 9 para. 3 GenTG). Such work must also be submitted to the ZKBS for consultation/evaluation.

If a genetic modification has demonstrably led to a weakening of virulence (e.g. deletion of the Shiga toxin genes), or if a weakening is to be expected on the basis of an individual case assessment, the GMO is classified in risk group 2. The genetic engineering operation is assigned to class 2.

In the case of infection of animals with the above-mentioned genetically modified EHEC strains and the keeping of these animals, safety measures of class 3 are sufficient according to Annex V GenTSV, provided that the animals do not release other organisms of higher risk groups. Protective clothing must be worn that is adapted to the test animal.

However, the following measures are not required:

- air lock
- emergency power supply
- Negative pressure and high-efficiency particulate air filter for filtration of exhaust air
- An autoclave in the livestock room.
- Wearing a respirator
- Filter on insulators or insulated rooms.

**Reasoning:**

EHEC strains have a combination of different virulence factors. However, the risk of infection for persons involved in the work is limited as the infectious agents are normally transmitted only by oral or contact infection, but not by air. However, due to the low infectious dose and the risk of triggering life-threatening diseases, special care is required in dealing with EHEC strains. Endangerment of the persons involved in the work by the aerogenic or parenteral transmission of the GMOs can be ruled out without observing the level 3 safety measures mentioned above if a good microbiological practice is maintained.

**4. References**


