# Assessment of the occurrence and the molecular diversity of *Escherichia albertii* in Switzerland

**Background:**
*Escherichia albertii*, a close relative of *E. coli*, is an emerging zoonotic foodborne pathogen associated with watery diarrhea mainly in children and immunocompromised individuals. *E. albertii* was initially classified as *eae*-positive *Hafnia alvei*, however as more genetic and biochemical information became available it was reassigned to its current novel taxonomy. Shiga toxin-producing *E. albertii* are among the eight emerging risks according to a report of the European Safety Authority (EFSA) in 2023.

## WP1: Microbiological and epidemiological aspects of *Escherichia albertii* - an emerging foodborne pathogen – a review

**Muchaamba et al.** (2022): Microorganisms 10(5):875. https://www.mdpi.com/2076-2607/10/11/2265

**Barmetter and Stephan** (2023): Rundschau für Fleischhygiene und Lebensmittelüberwachung.

## WP2: Occurrence in livestock and pets in Switzerland

### 1.1 poultry

We assessed the occurrence of *E. albertii* in 150 pooled faecal samples collected at slaughterhouse level from poultry flocks (n=150) in Switzerland. Using an *E. albertii*-specific PCR targeting the *Eacdt*-gene, all samples tested negative.

**Barmettler et al.** (2022), Microorganisms, 10,2265. https://doi.org/10.3390/microorganisms10112265

### 1.2 pig, cattle, sheep

A total of 515 caecal samples from sheep, cattle, calves, and pigs were collected between May 2022 and August 2022 at abattoir level. Depending on the herd size, from each herd two to five animals were sampled. In total, 44 different farms were sampled. Using an *E. albertii*-specific PCR targeting the *Eacdt*-gene, 51 (23.7 %) out of 215 pigs, belonging to 24 different farms, were positive. One (1%) out of 100 calves showed a weak positive PCR result. All samples of sheep and cattle were PCR negative. Strain isolation was attempted on the PCR positive samples by subculturing the broth cultures onto Xylose-MacConkey-plates. Isolation was possible on 8 of the 51 *Eacdt*-PCR positive pig samples. All eight *E. albertii* whole-genome sequenced swine isolates belonged either to ST4619 (n = 3) or ST2087 (n = 5). The two swine-associated clusters were distantly related but characterized by the shared presence of a virulence plasmid harboring the *sitABCD* and *iuc* genes. Here, we demonstrate that swine constitute an important *E. albertii* reservoir in Switzerland. Moreover, our study suggests that swine are a reservoir for specific *E. albertii* lineages.

**Barmettler et al. (2023),** Schweizer Archiv für Tierheikunde 165, 299–306. https://doi.org/10.17236/sat00393.

### 1.3 pets

Fecal samples from dogs (n = 51) and cats (n = 49) were collected between October 2022 and November 2022 and incubated overnight at 42°C in *Enterobacteriaceae* Enrichment (EE) broth (Becton Dickinson). One cat sample (2 %) and three dog samples (6 %) were PCR positive for *Eacdt*. For two of those (one cat and one dog sample), *E. albertii* isolates could be recovered. The strains were sequenced.

**Biggel et al. (2023),** Microbiology Resource Announcements, 23:e0135622. https://pubmed.ncbi.nlm.nih.gov/36815777/

## WP3: Occurrence in the food/ feedchain

### 2.1 pork meat

We assessed the occurrence of *E. albertii* in 50 raw pork meat samples (meat origin Switzerland) collected between May and June 2023 at retail level in Switzerland. 10 g of each sample was enriched overnight in *Enterobacteriaceae* Enrichment (EE) broth. Using an *E. albertii*-specific PCR targeting the *Eacdt*-gene, all samples tested negative.

Tan, J. (2023). student project

### 2.2 raw hamburger patties and tartare

We assessed the occurrence of *E. albertii* in 112 samples collected at from a food producing company performing self control tests for Shigatoxin-produing *E. coli* in these products. Using an *E. albertii*-specific PCR targeting the *Eacdt*-gene, all samples tested negative after an enrichment step in *Enterobacteriaceae* Enrichment (EE) broth.

### 2.3 game meat

We assessed the occurrence of *E. albertii* in 93 wild game meat samples collected during November 2021. The collection comprised meat samples of red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and wild boar (*Sus scrofa*). The meat samples were either supplied by Swiss hunters from animals shot during the hunting season of 2021 or purchased at Swiss butcher shops and retail stores. In addition, meat samples were obtained from a large game meat processing establishment located in Slovenia. The establishment processes domestic and imported hunted game animals and produces game meat and meat products for the European market, including for Switzerland. Using an *E. albertii*-specific PCR targeting the *Eacdt*-gene, all samples tested negative after an enrichment step in *Enterobacteriaceae* Enrichment (EE) broth.

### 2.4 milk products

In progress

### 2.5 herbs and ready-to-eat salads

We assessed the occurrence of *E. albertii* in 100 herb samples and 100 ready-to-eat salads samples collected during 2023 at retail level in Switzerland. 10 g of each sample was enriched overnight in *Enterobacteriaceae* Enrichment (EE) broth (Becton Dickinson). *E. albertii* was detected using an *E. albertii*-specific PCR targeting the *Eacdt*-gene. One herb sample (Minze from Italy) and one ready-to-eat salad (Nüsslisalat from Switzerland) tested PCR positive. From the herb sample the strain could be isolated and was sequenced.

**ST58i (Minze from Italy): *E. albertii* ST5399, *eae* positive, *stx*, *iuc* and *sit* negative**

### 2.6 seafood and oysters

We assessed the occurrence of *E. albertii* in 50 seafood and oyster samples collected at retail level in Switzerland. Using an *E. albertii*-specific PCR targeting the *Eacdt*-gene, all samples tested negative after an enrichment step in *Enterobacteriaceae* Enrichment (EE) broth..

### 2.6 BARF samples

We assessed the occurrence of *E. albertii* in 59 RMBD products which were purchased from ten different suppliers either on site in pet food stores or from certified Swiss RMBD producing enterprises, or in online stores of suppliers located in Switzerland and Germany. The products were purchased frozen or shipped frozen to the laboratory and stored until analysis according to the recommendations of the suppliers. The tested products contained either pure muscle or pure organ meat, mixed muscle and organ meat products, or meat supplemented with plant ingredients. Products were categorized into those originating from of beef cattle, poultry, horse, lamb, rabbit, venison, and fish. Types of meat within these categories included beef (including rumen and liver) (n=17), chicken (n=7), duck (n=1), quail (n=1), turkey (n=5), ostrich (n=1), horse (n=8), lamb (n=6), rabbit (n=4), reindeer (n=1), moose (n=1), unspecified venison (n=2), salmon (n=4),

and perch (n=1). Using an *E. albertii*-specific PCR targeting the *Eacdt*-gene, all samples tested negative after an enrichment step in *Enterobacteriaceae* Enrichment (EE) broth..

## WP4: Occurrence in the environment

### 3.1. wild birds

We assessed the occurrence of *E. albertii* in faecal samples from wild birds (n=130) in Switzerland. Using an *E. albertii*-specific PCR targeting the *Eacdt*-gene, 23.8% (31/130) of the samples tested positive for *Eacdt*. The positive samples originated from 11 bird species belonging to 8 families. Strain isolation was attempted on the PCR positive samples by subculturing the broth cultures onto Xylose-MacConkey-plates. Isolation was possible on 12 of the 31 *Eacdt*-PCR positive samples. Whole genome sequencing revealed that the stains belonged to 9 distinct sequence types, with ST13420 and ST5967 being represented by two and three isolates, respectively. All strains harboured the *eae* gene, while two strains **were also positive for *stx2f***. Our study thus shows that *E. albertii* is present in the Swiss wild bird population, which can potentially act as a source of this pathogen to humans, other animals and the environment.

**Barmettler et al. (2022),** Microorganisms, 10,2265. https://doi.org/10.3390/microorganisms10112265

### 3.2 wild boars

Fecal samples from 52 wild boars were collected between December 2022 and January 2023 and incubated overnight at 42°C in *Enterobacteriaceae* Enrichment (EE) broth (Becton Dickinson). Five samples (9.6%) were PCR positive for *Eacdt*. For four of those, *E. albertii* isolates could be recovered. The strains were sequenced. This is the first study showing that wild boars are a reservoir for *E. albertii*.

**Biggel et al. (2023),** Microbiology Resource Announcements 12(5):e0013523. doi: 10.1128/mra.00135-23

### 3.3 river water

We assessed the occurrence of *E. albertii* in 59 river samples collected during 2023. The samples were filtered and enriched overnight in *Enterobacteriaceae* Enrichment (EE) broth (Becton Dickinson). *E. albertii* was detected using an *E. albertii*-specific PCR targeting the *Eacdt*-gene. Five river samples (8.5%) tested positive. From two sample the strains could be isolated and were sequenced.

**KBF51: *E. albertii* ST5268, *eae* positive, *stx*, *iuc* and *sit* negative**
**KBF58: *E. albertii* ST5967, *eae* positive, *stx*, *iuc* and *sit* negative**

## WP5: Humans as asymptomatic carriers and clinical human samples

### 5.1 Healthy humans as asymptomatic carriers

For this study, we assessed the occurrence of *E. albertii* in 500 stool samples from healthy employees in the food industry received in September 2022. The samples were randomly selected. Using an *E. albertii*-specific PCR targeting the *Eacdt*-gene, all samples tested negative.

### 5.2 clinical human samples

We assessed the occurrence of *E. albertii* in 206 stool samples from patients with diarrheal disease, which were sent to the National Reference Laboratory for Enterpathogens (NENT) for STEC testing. 50% of the patients were hospitalized, 50% of the patients were not hospitalized. Using an *E. albertii*-specific PCR targeting the *Eacdt*-gene, all samples tested negative for *Eacdt*.