

Characterisation of illegally imported meat and meat products and identification of *E. coli* in the samples confiscated at the Croatian borders

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ABSTRACT

Prevention and control of foodborne diseases is an international public health goal. Legal imports of food of animal origin are monitored for contamination, and alerts are reported regularly, but food brought into the country illegally could represent a potential threat to consumers' health. In order to assess the level of the risk for public health from uncontrolled imported food of animal origin entering the Republic of Croatia, 83 meat samples were collected at 6 border points between Croatia and third countries. The origins of all confiscated samples were third countries, and the final destinations were EU countries and Switzerland. The weight of the confiscated samples ranged from 500 g to 108 kg. Special attention was focused on the presence of *E. coli* and its antimicrobial susceptibility. From altogether 83 confiscated meat samples, 8 were positive for *E. coli*. Although STEC and/or O157, as well as multidrug resistant *E. coli* were not found in the examined samples, illegally imported meat should be monitored continuously to prevent possible foodborne bacterial infections.

Key words: meat; confiscation; borders; *Escherichia coli*

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Introduction

Microbial food safety is still a major concern in many European Union (EU) member states due to repeated outbreak episodes and contamination problems. The global influence and growth of the European market affect the food supply chain that is becoming increasingly complex and requires professional risk management systems to ensure consumer protection. Legal imports are well monitored for contamination, and alerts are registered through the RASFF notification systems (ANONYM., 2008). On the other hand, the amount of meat and dairy products confiscated from passengers in the EU in the 2009-2011 period was between 273 and 280 tons, respectively (ANONYM., 2012a; as cited by JANSEN et al., 2016). Also, between 67,003 and 81,397 of illegal consignments of meat and dairy products were noticed during the 2009-2011 period (ANONYM., 2012a; as cited by JANSEN et al., 2016). The reported weight of the illegally imported food per passenger at German airports was on average between 3.22 kg and 3.44 kg (JANSEN et al., 2016) and at Paris airport, the largest confiscated meat package weighed 51 kg (CHABER et al., 2010).

Uncontrolled food imports present the risk of transferring new strains of different pathogens from third countries to the EU (BEUTLICH et al., 2015; NAGY et al., 2015; RODRÍGUEZ-LÁZARO et al., 2015; CHABER and CUNNIGHAM, 2016). Many particular species of bacterial pathogens, such as *Salmonella*, *Campylobacter* or *Listeria*, have been isolated from transcontinental or local outbreaks (NEWELL et al., 2010; CIOLACU et al., 2015). According to the EFSA report for 2016, zoonotic food-borne diseases are still of major concern for public health (ANONYM., 2017). One of the bacteria that also raises an alert is *Escherichia coli*. In particular Verocytotoxigenic *E. coli* (VTEC) O157:H7 and Shiga toxin-producing *E. coli* (STEC) may colonize the gastrointestinal tract of different animals, and potentially contaminate the meat during processing (TAMMINEN et al., 2018; WILSON et al., 2018). The bacteria VTEC was isolated from food samples confiscated at German airports, as well as other pathogenic bacteria, such as *Salmonella*, *Listeria* and *Yersinia* (BEUTLICH et al., 2015). *E. coli*, isolated from animals and food products, resistant to numerous antimicrobial agents could represent a reservoir of resistant genes that could be transferred to opportunistic pathogenic bacteria (SÁENZ et al., 2004; SUNDE, 2005; BEN SLAMA et al., 2010). Major concerns are raised by the antimicrobial resistance of commensal *E. coli* in poultry production, especially due to the fact that broilers have become one of the most important meat sources worldwide (ROTH et al., 2018). According to the ANONYM. (2018), *E. coli* isolated from broilers, fattening turkeys and their meat, was frequently highly resistant to ampicillin, (fluoro)quinolones, tetracyclines and sulfonamides. The level of multi-drug resistant (MDR) *E. coli* was also overall very high (50.2%) (ANONYM., 2018). MDR *E. coli* was also observed in food samples in the EU (JAKOBSEN et al., 2010; NAGY et al., 2015; JANS et al., 2018). The geographical position of the Republic of Croatia is such that it is located on a corridor where many travellers transit through on their way

to the EU. Also, the borderline that the Republic of Croatia has with third countries is longer than 1300 kilometres, of which 1052 kilometres is with Bosnia and Herzegovina alone. Illegal import of food of animal origin entering Croatia from third countries may represent a risk to public and animal health.

In order to assess the level of risk and to detect the hazard, movement of illegally imported meat and meat products was tracked at 6 land border points with third countries (Bosnia and Herzegovina, Serbia, and Montenegro). The total weight and the country of origin and final destination were recorded. Testing of the microbiological quality of confiscated meat samples was carried out with specific attention paid to the presence and determination of *E. coli* and its antimicrobial susceptibility.

Since public awareness of the global dimension of food safety is increasing, the main aim of this research was to investigate if the food of animal origin, mainly different meat products, found illegally imported into Croatian territory, may represent a potential risk for public and animal health.

Material and methods

Collection of samples. A total of 83 samples of different meat and meat products were confiscated by border veterinary inspectors in the period from November 2013 to May 2014 (Table 1.). The samples were collected at four road border points between Croatia and Bosnia and Herzegovina, one road border point between Croatia and Serbia, and one road border point between Croatia and Montenegro. For all confiscated samples, data were recorded about the total quantity of the product, the origin and final destination, as well as information about whether the product was intended for personal or commercial use. All confiscated samples were sent immediately, or after storage at +4 °C for a maximum of 24 hours, to the laboratories at the Faculty of Veterinary Medicine of the University of Zagreb.

Microbiological examination. *E. coli* bacteria were detected by standard bacterial examination and the appropriate ISO methods. The taxonomic classification of the isolated bacteria was based on colony morphology, and microscopic and biochemical characteristics. Gram staining, catalase and oxidase reactions were conducted as described by BROWN (2005).

The isolated *E. coli* were sent to the Veterinary Medical Research Institute of the Hungarian Academy of Sciences, where they were further tested according to ISO guidelines 16654:2001 (ANONYM., 2001) for O157 VTEC or ISO/ TS13136:2012 for non-O157 VTEC (ANONYM, 2012b).

Antimicrobial sensitivity testing using the disc diffusion method was conducted according to the instructions given by Clinical and Laboratory Standards Institute (CLSI). The antimicrobial agents used were as follows: gentamicin, amikacin, streptomycin, tobramycin, kanamycin, ampicillin, ceftazidime, aztreonam, amoxicillin, imipenem, cefotaxime, chloramphenicol, nalidixic acid, ciprofloxacin, sulphamethoxazole,

sulphonamides, tetracycline and trimethoprim. *E. coli* ATCC 25922 was used as the referent strain.

Results and discussion

In total, 83 samples of different meat and meat products were confiscated at the Croatian border points (Table 1). All confiscated samples arrived from non-European countries. The final destination for all the confiscated products were EU countries and Switzerland. The majority of samples were processed (dry) meat products (sausages, ham, ribs and bacon). The veterinary inspectors also confiscated fresh and frozen meat products (beef, poultry and pork). Although the confiscated meat products were designated for personal use and were found in personal luggage, the weight of samples from 500 g to 108 kg raised the concern that some of those food items were not intended for personal use only, but could also be placed on Croatian local markets, or consumed by a larger group of people in other EU countries (*i.e.* 20 kg of dry cured pork greaves, or 108 kg of dry cured pork bacon).

Table 1. Description of confiscated meat and meat products at Croatian border crossings

Sample	Number of confiscated samples	Total amount of the original sample
Processed (dry) meat products		
Dry cured pork ham	17	5.5-33.4 kg
Dry cured pork sausage	12	4.0-20.0 kg
Dry cured pork bacon	9	8.8-108.0 kg
Dry cured pork meat	7	10.0-26.0 kg
Dry cured beef meat	4	7.0-33.0 kg
Dry cured pork rib	3	3.0-6.10 kg
Dry cured pork greaves	2	8.0-20.0 kg
Other pork products	2	6.15-10.0 kg
Dry cured sheep meat	1	2.20 kg
Fresh meat samples		
Beef	8	3.0-14.4 kg
Chicken	7	0.5-14.4 kg
Pork	2	10.0-25.0 kg
Veal	1	4.5 kg
Minced meat	1	6.1 kg
Frozen meat samples		
Chicken	5	4.0-10.0 kg
Beef	2	8.5-10.0 kg

Bacteria *E. coli* was isolated from altogether 8 meat samples: from four fresh beef samples, three samples of frozen chicken meat, and one sample of fresh chicken meat, respectively. Altogether 6 isolates of *E. coli* that were further tested by ISO methods were not identified as O157 or STEC. Similar results were also obtained by KAUSHIK et al. (2018), where no *E. coli* O157 was found in poultry meat samples examined. Although it was not the case in our study, the majority of STEC or O157 *E. coli* was previously isolated from beef (HUSSEIN, 2007; NOBILI et al., 2017; VARCASIA et al. 2018).

Table 2. Antimicrobial susceptibility of *E. coli* isolates

Antimicrobial agent	Origin					
	<i>E. coli</i> isolates (sample ID)					
	1	2	3	4	5	6
	Fresh beef meat	Fresh chicken meat	Fresh chicken meat	Frozen chicken meat	Fresh beef meat	Fresh beef meat
Gentamicin	S	S	S	S	S	S
Amikacin	S	S	S	S	S	S
Streptomycin	I	S	S	S	S	S
Tobramycin	S	S	S	S	S	S
Kanamycin	S	S	S	S	S	S
Ampicillin	S	S	S	S	S	S
Ceftazidime	S	S	S	S	S	S
Aztreonam	S	S	S	S	S	S
Amoxicillin	S	S	S	S	S	S
Imipenem	S	S	S	S	S	S
Cefotaxime	S	S	S	S	S	S
Chloramphenicol	S	S	S	S	S	S
Nalidixic acid	S	S	S	S	S	S
Ciprofloxacin	S	S	S	S	S	S
Sulphamethoxazole	S	S	S	S	S	S
Sulphonamides	R	S	S	S	S	S
Tetracycline	R	S	S	R	S	S
Trimetoprim	S	S	S	S	S	S

Regarding antimicrobial susceptibility, one of the *E. coli* isolates, found in fresh beef meat, was resistant to sulphonamides and tetracycline, and intermediately resistant to streptomycin. Another isolate from frozen chicken meat was only resistant to tetracycline (Table 2). KAUSHIK et al. (2018) reported similar findings regarding the antimicrobial sensitivity of *E. coli* isolated from poultry meat, with the highest degree of susceptibility observed against gentamicin, amikacin and ciprofloxacin. However in our case, it

was also susceptible to 11 other agents. It is known that in general organically raised chickens are less likely to be contaminated with antibiotic-resistant *E. coli* (DAVIS et al., 2018). Although we can only speculate about the origin of the meat (conventional or organic animal production), the lack of MDR *E. coli* in the examined samples could be related to the small scale family or organic production.

As previously described, the majority of illegal meat and meat products found at the border crossings of the Republic of Croatia were negative or contained only saprophytic or facultative pathogen bacteria (PRUKNER-RADOVČIĆ et al., 2014), so it may be concluded that the illegal import of food of animal origin represents only a minor risk for consumers. The highest number of the samples in this investigation were confiscated during the Christmas and Easter holidays and were mainly dry cured (processed) meat products. For such meat products it is known that, due to their low pH and water activity, they are only occasionally linked to food poisoning (CHACON et al., 2006). On the other hand, an outbreak of *E. coli* O157:H7 food poisoning occurred in the USA, when the consumers consumed dry-cured salami (ANONYM., 1995).

Conclusion

Although in the meat and meat samples confiscated at the Croatian borders no pathogenic or multidrug resistant *E. coli* was found, it could be reasonable to continue such monitoring for a longer period of time and on larger amount of samples, especially focusing on assessment of the persistence of pathogenic bacteria in dairy and fresh meat products. Also, there is a clear need for the use of modern molecular methods in such investigations, to be able to detect the subtypes, virulence and resistance of genes in the isolated bacteria.

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HORVATEK TOMIĆ, D., L. KOZAČINSKI, M. LUKAČ, I. TOTH, S. GUTIĆ, E. PRUKNER-RADOVČIĆ: Struktura ilegalno uvezenoga mesa i mesnih proizvoda i pojavnost bakterije *Escherichia coli* u uzorcima zaplijenjenima na graničnim prijelazima Republike Hrvatske. Vet. arhiv 89, 123-132, 2019.

SAŽETAK

Prevenција i kontrola bolesti uzrokovanih hranom među glavnim su ciljevima javnoga zdravstva diljem svijeta. Legalan uvoz hrane redovito se prati službenim kontrolama i o tome se redovito izvješćuje. S druge strane, hrana koja se nezakonito unese u zemlju mogla bi biti potencijalna prijetnja zdravlju potrošača. Kako bi se procijenila razina rizika za javno zdravstvo, ukupno 83 uzorka mesa i mesnih proizvoda različita podrijetla oduzeta su na šest graničnih prijelaza između Hrvatske i trećih zemalja. Posebna je pozornost bila usmjerena na prisutnost *E. coli* i njezinu antimikrobnu osjetljivost. Podrijetlo pošiljki bile su tzv. treće zemlje, a konačna destinacija zemlje EU-a i Švicarska. Težina zaplijenjenih pošiljki bila je od 500 g do 108 kg. *E. coli* pronađena je u ukupno 8 zaplijenjenih pošiljki. Iako u ispitivanim uzorcima nisu pronađeni STEC/O157 ni multirezistentna *E. coli*, nezakonito unesenu hranu svakako treba kontinuirano pratiti i pregledavati kako bi se spriječilo širenje bakterijske infekcije uzrokovane hranom.

Ključne riječi: meso; konfiscirani uzorci; granični prijelazi; *Escherichia coli*
