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SISTEMSKO VREDNOTENJE IN PREPREČEVANJE VIRUSNE OKUŽBE S HRANO V ŽIVILSKO PREDELOVALNI OSKRBOVALNO PREHRANSKI VERIGI

DOKTORSKA DISERTACIJA

SYSTEMIC EVALUATION AND PREVENTION OF VIRAL FOODBORNE INFECTION IN FOOD SUPPLY CHAIN

DOCTORAL DISSERTATION

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AI Oskrba z varno hrano, ki ne ogroža zdravja potrošnikov, je temelj zdrave prehrane in pomemben dejavnik varovanja zdravja kot javnega interesa. V preteklosti je bila mikrobiološka varnost živil osredotočena predvsem na nadzor bakterijskih okužb. Virusi, preneseni s hrano, so čedalje pogostejši povzročitelji bolezni in epidemij. Namen naloge je bil ugotoviti poznavanje virusov kot možnega dejavnika tveganja v živilsko predelovalni oskrbovalno prehranski verigi. Z rezultati raziskave želimo izboljšati razumevanje pojmovanja virusov vzdolž verige. Pridobljeni rezultati lahko pripomorejo k razvoju varnosti živil na področju obvladovanja virusnih okužb. Z namenom ugotavljanja dejanskega stanja obvladovanja virusnih okužb vzdolž živilsko predelovalne oskrbovalno prehranske verige so bila uporabljena kvantitativna in kvalitativna metodološka orodja (vsebinska analiza, SWOT analiza, anketne raziskave, polstrukturirani intervju). Na vseh analiziranih stopnjah verige so ugotovljene pomanjkljivosti pri razumevanju in obvladovanju virusnih okužb, prenesenih s hrano kot dejavnikov tveganja. Ugotovitve potrjujejo zastavljene hipoteze, da niti proizvajalci niti potrošniki ne prepoznajo virusov kot dejavnikov tveganja, in da zagotavljanje virološke varnosti živil v živilsko predelovalni oskrbovalno prehranski verigi ni doseženo z obstoječimi dobrimi praksami, saj le-te operativno temeljijo na mikrobioloških tveganjih. Poleg tega smo izpostavili dobro higiensko prakso, kot jo postavlja Codex Alimentarius kot primerno osnovo za razvoj sistema zagotavljanja virološke varnosti živil, saj zajema vse člene v verigi. Interaktivna komunikacija vseh deležnikov, nadziran sistemski pristop, dobre prakse in izvajanje izobraževanja so bistvenega pomena pri zagotavljanju virološko varnega živila.

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AB Supply of safe food is the foundation of public health. In the past, the microbiological food safety management systems focused primarily on the control of bacterial infections. In the last decades, several foodborne viruses have been identified as etiological agents of viral gastroenteritis. The aim of this study was to determine the actual state of the viral food safety management practices at all stages within food supply chain. The obtained results may contribute to the development of viral food safety management practices. Various quantitative and qualitative methodological tools were used, including content analysis, SWOT analysis, questionnaires and structured interviews. We came to conclusion that deficiencies in understanding and control of foodborne viruses were found at all analysed stages of the food supply chain. The findings of this study confirm that neither food handler neither consumer is aware of foodborne viruses as distinctive food safety hazards and objectives of existing food safety management practices within food supply chain are not focused on the control of viral infections. The results pointed out that Codex Alimentarius guidelines for the control of viruses in food serves as firm basis for ensuring viral food safety due to its role in national legislation and food safety management system development and implementation. The findings suggest the need to establish controlled system approach, where communication and education among stakeholders along food supply chain are essential to ensure viral food safety and thus contribute to the overall strategy of ensuring food safety.

KAZALO VSEBINE

KAZALO ZNANSTVENIH DEL

KAZALO SLIK

KAZALO PRILOG

OKRAJŠAVE IN SIMBOLI

CDC	Center za preprečevanje in obvladovanje bolezni (angl. Centers for Disease Control and Prevention)
DNA	Deoksiribonukleinska kislina
E. coli	*Escherichia coli*
EFSA	Evropska agencija za varnost hrane (angl. European Food Safety Authority)
ES	Evropska Skupnost (angl. European Community)
FAO	Svetovna organizacija za hrano in kmetijstvo (angl. Food and Agriculture Organization of the United Nations)
HACCP	analiza tveganja in ugotavljanja kritičnih kontrolnih točk (angl. Hazard Analysis and Critical Control Points System)
RNA	ribonukleinska kislina
RT-PCR	reverzna transkripcija s sledečo verižno reakcijo s polimerazo
SWOT analiza	Analiza prednosti, pomanjkljivosti, priložnosti, nevarnosti (angl. Strengths, Weaknesses, Opportunities, Threats)
UVHVVR	Uprava Republike Slovenije za varno hrano, veterinarstvo in varstvo rastlin
WHO	Svetovna zdravstvena organizacija (angl. World Health Organization)
ŽPO	živilska/prehranska/oskrbovalna veriga
ŽPOP	živilsko predelovalna oskrbovalno prehranska veriga

1 PREDSTAVITEV PROBLEMATIKE IN HIPOTEZE

1.1 PREGLED OBJAV

Visoka raven varovanja javnega zdravja je eden od temeljnih ciljev živilske zakonodaje (Regulation 178/2002 …, 2002). Varna hrana in varovanje interesov potrošnika je čedalje večja skrb javnosti, (ne)vladnih organizacij, strokovnih združenj in trgovinskih organizacij ob primarni skrbi državnih organov (Raspor in Ambrožič, 2012). Za varnost oz. zdravstveno ustreznost živil je bistven celosten pristop, ki upošteva dejstvo, da je oskrba z živili vezana v živilsko predelovalno oskrbovalno prehransko (ŽPOP) verigo, ki sega od »polja do vilic«. Raspor in Jevšnik (2008) navajata, da če želimo natančno pojasniti in razumeti slogan, ki se v angleškem jeziku glasi »From Farm to Fork«, v slovenskem pa »od polja do mize« ali »od vil do vilic«, je nujna sprememba oz. zamenjava obstoječega termina. Termin »živilska veriga« je potrebno preimenovati v »živilsko/prehransko/oskrbovalno (ŽPO) verigo«, ki izpostavi tri pomembna področja: živilstvo, prehrano in oskrbo, ki jih povežejo dobre prakse na poti od polja do mize v krogotok zagotavljanja varnih in zdravih živil za potrošnika. Prejšnja poimenovanja niso vključevala pomembne komponente, kot je zdravje ljudi (Raspor in Jevšnik, 2008).

Veriga preskrbe s hrano vključuje pridelavo oziroma prirejo, predelavo, distribucijo, veleprodajo oziroma maloprodajo do končnega potrošnika in je trajnostno naravnana, saj s svojim obstojem in delovanjem vpliva na gospodarstvo, zdravje in okolje (Zakon o kmetijstvu, 2008). Verige preskrbe s hrano so v praksi zelo raznolike in vključujejo deležnike od kmetov, zadrug, živilsko predelovalne industrije, trgovinskih verig in potrošnikov. Predelava živil je danes globalna dejavnost (Varuh odnosov v verigi preskrbe s hrano, 2016), zato so lahko posledice okužb zelo široke in povzročajo resno škodo zdravju ljudi po vsem svetu. Pri tem pa posledice okužb rušijo verodostojnost proizvajalcev in zakonodajalcev ter vplivajo na ugled samih procesov predelave živil in celotne verige preskrbe s hrano. V verigi je potrebno ozavestiti prav vse deležnike, da za našo prehransko varnost potrebujemo vitalno in razvijajočo se verigo, kjer je vsak člen nosi svoj del odgovornosti v procesu zagotavljanje varnosti živil. V ta namen smo termin »ŽPO veriga« razširili v ŽPOP verigo, ki poudarja pomembnost predelovalne dejavnosti v verigi od polja do mize. Varnost živil je širok pojem, ki se razteza od tehnologije do zakonodaje in od prehrambenika do potrošnika živil (Raspor, 2004). Zagotavljanje varnosti živil opredeljujejo različna sistemska orodja in pristopi ter številne dobre prakse, ki določajo smernice varnega rokovanja z živili. Iz teoretičnega zornega kota se zdi, da je varnost živil tako obvladovana in zagotovljena.

Praktične izkušnje pa odkrivajo nekatera odstopanja in nova tveganja, ki niso zadostno obvladovana. Danes zagotavljamo varnost živil z različnimi dobrimi praksami, ki so posledica kulture, zgodovine in načina življenja (Raspor in Jevšnik, 2008). Dobre prakse opredeljujejo pridelavo, predelavo, proizvodnjo, transport in skladiščenje posameznih živil ali skupin živil, ki so bistvenega pomena za zagotavljanje varnosti in ustreznosti živil za prehrano (Raspor, 2008). Pomembno pa je, da je vsak člen v ŽPOP verigi definiran, nadzorovan in obvladovan. Kljub vsemu pa se izkaže, da se veriga varnosti živil lahko hitro pretrga zaradi različnih ovir ali enostavno zaradi nesodelovanja in posledično nezaupanja med členi v ŽPOP verigi.

V obdobju globalizacije in razvoja potrošniške družbe postaja potreba po zdravem prehranjevanju vse pomembnejša, a je zaradi sodobnega načina življenja pogosto zapostavljena. Tradicionalno razumevanje ŽPOP verige se neprestano spreminja, kakor tudi incidenca bolezni, povezanih s hrano (Havelaar in sod., 2015; Raspor, 2004). Bolezni, povezane s hrano, predstavljajo veliko gospodarsko breme (Havelaar in sod., 2015; Hoffmann in sod., 2015; Havelaar in sod., 2010; Kuchenmüller in sod., 2009), kakor tudi ovire za družbeno-gospodarski razvoj po vsem svetu. Zaradi tega države poskušajo s pravnimi predpisi in drugimi ukrepi, ki opredeljujejo pogoje in način notranjega in uradnega nadzora živil, izboljšati varnost živil in tako obvladovati bolezni, ki so povzročene oziroma se prenašajo s hrano in pitno vodo. Cilj živilske zakonodaje je varovati interese potrošnikov in zagotoviti potrošnikom podlago za obveščeno izbiro v zvezi z živili, ki jih uživajo (Regulation 178/2002 …, 2002). Medtem ko imajo nacionalne »živilske« zakonodaje razmeroma dolgo tradicijo, je trenutna evropska živilska zakonodaja nastala kot posledica razvoja v zadnjih štiridesetih letih in odraža mešanico znanstvenih, družbenih, geografskih, političnih in gospodarskih vplivov (Raspor in sod., 2012). Pomembnost Uredbe Evropske Skupnosti 178/2002 (Regulation 178/2002 …, 2002) je predvsem v uniformiranosti zakonodaje na celotnem območju Evropske Skupnosti (ES), saj le na ta način lahko zagotavljamo prost pretok varnih in kakovostnih živil ter visoko raven varovanja življenja in zdravja ljudi.

Hrana ni bila nikoli varnejša, kot je danes, a hkrati tudi nikoli ni bila bolj izpostavljena dejavnikom tveganja. Zaradi kriz v zadnjih letih na področju živilstva postaja varnost in kakovost živil pomembna tema v vsakodnevnem življenju potrošnika (Banati, 2011). Nevarnosti izbruha bolezni zaradi onesnažene hrane so se zavedali že davno, kar je mogoče zaslediti že v bibličnih zapisih (Lasztity in sod., 2004). Človek je skozi stoletja uživanja hrane kot proizvoda narave spoznal tiste izdelke, ki škodujejo njegovemu zdravju. Posledično se je instinktivno skozi izkustva naučil, kateri proizvodi predstavljajo tveganje za njegovo zdravje. Dolžina poti, ki jo hrana prepotuje »od njive do mize«, je lahko zelo različna. Veriga je danes bistveno daljša, kot je bila nekdaj, ko si je človek še sam prideloval hrano, s tem pa so nastale tudi nove poti v ŽPOP verigi, ki lahko prinašajo nova tveganja ali obujajo stara (Wilhelm in sod., 2015; Oyarzabal, 2012; Tauxe, 2002). Z

globalizacijo trga se povečuje dostopnost izdelkov iz celega sveta. Tako so poti čedalje daljše in vse bolj zapletene ter odvisne od zunanjih partnerjev v verigi, kar še dodatno zapleta sledljivost ter preglednost. V ŽPOP verigi obstaja niz tveganj, ki vzpodbujajo proizvajalca, da si prizadeva ponuditi potrošniku varno in kakovostno živilo. Tveganje je vsako nesprejemljivo onesnaženje biološke, kemijske ali fizikalne narave, ki lahko ogroža zdravje potrošnika (Regulation 178/2002 …, 2002). Ta ista tveganja na drugi strani vzpodbujajo potrošnika k treznemu razmisleku, za katero živilo se bo odločil v svojem izboru in pri tem svoje zdravje izpostavil najmanjšemu tveganju. Varnost in kakovost živila pomeni za potrošnika tisto dobrino, ki mu lahko omogoči ohranitev zdravstvene in fizične kondicije za nadaljnje delo in življenje. Torej se mora tveganj, ki se jim izpostavlja, zavedati in jih obvladovati (Raspor in sod., 2012).

Mikrobiološka varnost živil je bila kot pomemben javnozdravstveni in ekonomski problem (Havelaar in sod., 2015; Havelaar in sod., 2010) v preteklosti osredotočena predvsem na nadzor bakterijskih okužb. Z vidika virusnih okužb je področje varnosti živil še razmeroma neraziskano. V zadnjih letih pa je tako v znanstveni kot strokovni literaturi možno zaslediti, da so virusi, vedno pomembnejši povzročitelj alimentarnih bolezni. Mikrobiološke analize kažejo porast virusnih infekcij v primerjavi s klasičnimi bakterijskimi infekcijami (CDC, 2015; EFSA, 2015; EFSA, 2014; Kirk in sod., 2014; Koopmans, 2012; Scallan in sod., 2011; EFSA, 2006, Mead in sod., 1999). Po ocenah Svetovne zdravstvene organizacije (WHO) je bilo v letu 2010 na svetu skupno 600 milijonov primerov alimentarnih bolezni, med katerimi so bili norovirusi odgovorni za 120 milijonov primerov, virusi hepatitisa pa za 14 milijonov (Havelaar in sod., 2015), kar kaže na resnost in razsežnost zdravstvenega problema v svetu s stališča javnega zdravja. Za države članice ES je poročanje o preiskavah izbruhov, prenesenih s hrano, vključno z virusi, obvezno od leta 2005. V članicah držav ES je bilo med letoma 2005 in 2014 opaziti porast prijavljenih, s hrano prenesenih izbruhov, povezanih z virusi, iz 6 % na 20,4 % (EFSA 2015; EFSA, 2006) vseh prijavljenih izbruhov. V ES so bili v letu 2014 virusi odgovorni za 20,4 % prijavljenih, s hrano povzročenih izbruhov, kar jih po številu prvič uvršča na prvo mesto. Na drugem mestu so bile salmonele, ki so bile odgovorne za 20,0 % izbruhov. Pri tem pa je treba poudariti, da veliko primerov sploh ni zabeleženih, saj so bolezenski znaki v mnogih primerih blagi in kratkotrajni ter jih večina ljudi ne prepozna kot okužbe s hrano. V virusne izbruhe so bili najpogosteje vpletene skupine živil, kot so »raki, školjke, mehkužci in proizvodi iz njih« (44,7 % izbruhov), »samopostrežni obroki« (15,8 %), »mešana hrana« (13,2 %) ter »sadje« in »jagodičevje in sokovi« (obe 5,3 %) (EFSA, 2015).

Zaradi vse bolj prepoznavnih in pogostejših izbruhov virusnih okužb, prenesenih z živili, se pojavljajo zahteve po vključitvi virusov v smernice dobre prakse (še posebej v dobro proizvodno in higiensko prakso) in ne nazadnje tudi v sisteme vodenja varnosti živil (Boxman, 2013; Koopmans and Duizer, 2004) kljub pomanjkanju znanstvenih podatkov za

pripravo ocen tveganj (Bouwknegt in sod., 2015; de Roda Husman in Bouwknegt, 2013; EFSA, 2011; FAO/WHO, 2008). Vse bolj se razvijajo metode za dokazovanje prisotnosti virusov v živilih kot tudi v vzorcih kužnin (la Rosa in Muscillo, 2013; Rodriguez-Lazaro in sod., 2013; Rzezutka in Cook, 2009; Goyal, 2006), kar pomembno prispeva k opredelitvi, preprečevanju, nadzoru in zmanjševanju tveganja za pojav virusnih okužb z živili. V zadnjih letih je možno opaziti porast raziskav, v katerih so bili uporabljeni različni postopki za inaktivacijo virusov iz hrane in vode (Ha in sod., 2016; Ryu in sod., 2015; Li in sod., 2013; Rodríguez-Lázaro in sod., 2012; Baert in sod., 2009).

Virusi so zelo majhni, preprosto grajeni mikroorganizmi, ki povzročajo epidemije vnetja želodčne in črevesne sluznice (gastroenteritis) ali pa celo vnetje jeter (hepatitis). Virusni genom (FAO/WHO, 2008; Koopmans in Duizer, 2004) je lahko sestavljen iz deoksiribonukleinske kisline (DNA) ali ribonukleinske kisline (RNA) v obliki enojne ali dvojne vijačnice z dodatkom beljakovin, pomembnih za razmnoževanje. V hrani in vodi se virusi za razliko od bakterij ne razmnožujejo, saj za razmnoževanje potrebujejo žive celice (Koopmans in sod., 2002). Na eni strani nizka infektivna doza (10 do 100 virusnih delcev), na drugi strani pa izločanje iz obolelega organizma z blatom in izbruhanino v velikem številu ter njihove visoke odpornosti na zunanje dejavnike olajšuje hiter prenos med ljudmi. Zaradi tega so zato lahko izvor okužbe poleg obolele osebe tudi onesnaženi prostori in predmeti, prek katerih se okužba lahko širi (Greening, 2006).

Virusi, ki povzročajo bolezni prenesene s živili, večinoma spadajo v skupino enteričnih virusov, med katerimi so najpogostejši povzročitelji okužb norovirusi, virusi hepatitisa A, virusi hepatitisa E, rotavirusi, astrovirusi in enterični adenovirusi (EFSA, 2011, Koopmans in sod., 2004).

Norovirusi iz družine Caliciviridae so genetsko zelo heterogena skupina sorodnih, enovijačnih RNA virusov brez ovojnice in povzročajo najpogosteje nalezljivo črevesno bolezen (Todd in Greig, 2015; Patel in sod., 2009). Norovirusi se prenašajo s stikom med ljudmi, preko onesnaženih živil in vode ali z dotikanjem onesnaženih površin. Virus povzroča vnetje želodca in/ali črevesja (akutni gastroenteritis) pri osebah vseh starosti. Na splošno je bolezen blaga, vendar lahko povzroči zaplete in celo smrt pri rizičnih skupinah, kot so starejši in otroci. Norovirusi se pogosto prenašajo v dnevnih centrih, šolah, vrtcih, zdravstvenih ustanovah, domovih starejših občanov ter na območjih, kjer je povečana koncentracija ljudi. Čas od okužbe do pojava bolezni je običajno od 24 do 48 ur. Začetek bolezni je lahko nenaden ali pa se razvije postopoma. Pojav simptomov po okužbi z norovirusi je pogosto označen z nenadnim začetkom bruhanja in/ali z enim do več dni trajajočo drisko ter slabostjo in želodčnimi bolečinami. Okužimo se lahko z zaužitjem onesnaženih živil in pijače, z dotikanjem onesnaženih površin ter s telesnim stikom z okuženimi. Norovirus je lahko prisoten v blatu, še preden se pojavijo prvi znaki obolenja. Prav tako virus ostane prisoten v blatu še 14 dni ali dlje po prenehanju driske. Največja

možnost za prenos virusa obstaja ob začetku bolezni in prvih nekaj dni po ozdravitvi. Okužbe se preprečuje (NIJZ, 2016):

- s pravilno in zadostno higieno rok,
- s pranjem sadja in zelenjave oziroma zadostno toplotno obdelavo živil,
- s čiščenjem in dezinficiranjem onesnaženih površin in
- tako, da otroci, ki imajo drisko ali bruhajo, ne obiskujejo vrtca ali šole.

Virusi hepatitisa A povzročajo pri človeku črevesno nalezljivo bolezen, imenovano hepatitis A. Virus hepatitisa A je poleg norovirusov najpogostejši virusni povzročitelj okužb z živili v svetu. Incidenca okužb z virusi hepatitisa A med državami in znotraj njih se močno razlikuje (Todd in Greig, 2015; Rodriguez-Lazaro in sod., 2012). Tveganje za okužbo je obratno sorazmerno s stopnjo urejenosti splošnih higienskih razmer in nivojem osebne higiene. V večini držav v razvoju, v katerih prevladuje nizek higienski standard, je hepatitis A endemičen. V razvitih državah z visokim življenjskim standardom so okužbe z virusom hepatitisa A in izbruhi bolezni redki. Večina bolnikov ozdravi po nekaj tednih brez zapletov. Zelo redko hepatitis A poteka s težko klinično sliko s smrtnim izidom. Prebolela bolezen zapušča trajno imunost. Z virusom hepatitisa A se zaradi neurejenih higienskih razmer in nehigienskega rokovanja lahko onesnažijo vsa živila na poti od njive do mize. Glavni vir okužbe z virusom hepatitisa A je blato obolelih ljudi, ki je zelo kužno. Z nehigienskim ravnanjem in razmerami se lahko virusi hepatitisa A prenesejo na živila, pitno vodo, površinske vodotoke in morje. Prenos okužbe je lahko tudi z neposrednim stikom z izločki obolelega. Surove ali nezadostno toplotno obdelane školjke so pomemben vir okužbe z virusi hepatitisa A (Bellou in sod., 2013).

Virus hepatitisa E povzroča podobno klinično sliko kot hepatitis A. Tvegana živila so onesnažena pitna voda, nezadostno oprana zelenjava, surovo neolupljeno sadje, nezadostno toplotno obdelane školjke ter nezadostno toplotno obdelano meso. Znano je, da je zelo velik delež komercialnih in domačih čred prašičev okuženih z virusom hepatitisa E, ki lahko povzroči hepatitis pri ljudeh (Todd in Greig, 2015; Meng, 2011). Okuženi prašiči so običajno videti zdravi in celo veterinarji ne prepoznajo simptomov bolezni. Zaradi tega je mogoče okužene prašiče poslati v zakol in okuženo meso bo vstopilo v ŽPOP verigo. Virusi hepatitisa E so lahko prisotni v zelo velikem številu v blatu, urinu, jetrih, žolčniku in žolču okuženih prašičev. Širijo se lahko po klavnici in predelovalnem obratu, kar lahko privede do navzkrižnega onesnaženja mesa okuženih in neokuženih prašičev. Nekateri primeri hepatitisa E pri ljudeh so povezani z uživanjem svinjine, jeter ter izdelkov iz svinjine, kot so jetrne klobase, ki se uživajo surove (Berto in sod, 2013). Svinjino in prašičja jetra se sicer ponavadi pred zaužitjem toplotno obdela, vendar neprimerna toplotna obdelava prinaša tveganje za okužbo potrošnika.

Rotavirusi so razširjeni po vsem svetu. So pomembni povzročitelji epidemij drisk, ki se najpogosteje pojavljajo na oddelkih bolnišnic, v vrtcih, domovih za ostarele … Največ

obolenj je pri otrocih. Zelo pogosto se z rotavirusi okužijo starši, ki negujejo bolnega otroka – okuži se kar polovica staršev, tretjina pa ima znake okužbe (NIJZ, 2016). Najpogostejši način širjenja bolezni je prek onesnaženih rok obolelih oseb (Todd in Greig, 2015; FAO/WHO, 2008). Bolezen se širi tudi prek okuženih predmetov in hrane, glede na odpornost virusa pa je možno širjenje tudi z onesnaženo vodo. Glede na način širjenja so tvegana predvsem tista živila, ki niso toplotno obdelana pred uživanjem, kot so sadje, zelenjava in gotove jedi (Todd in Greig, 2015; Rodriguez-Lazaro in sod., 2012).

Adenovirusne driske (NIJZ, 2016; Rodriguez-Lazaro in sod., 2012) se prenašajo fekalno-oralno. Glavni vir okužbe je blato obolelih ljudi. Z adenovirusnimi driskami obolevajo predvsem otroci do dveh let starosti, lahko pa zbolijo tudi starejši otroci in odrasli. Z nehigienskim ravnanjem in razmerami (zlasti pomanjkljivo umivanje rok) se virus zanese v živila in pitno vodo. Glede na način širjenja virusa so tvegana živila predvsem tista, ki pred zaužitjem niso toplotno obdelana. Največ okužb je z uživanjem gotovih živil ter školjk. Adenovirusne driske preprečujemo z izvajanjem osebne higiene, higienskim rokovanjem z živili ter vzdrževanjem čistoče v sanitarnih in drugih prostorih (NIJZ, 2016).

Astrovirusne driske (NIJZ, 2016; Todd in Greig, 2015) se pojavljajo predvsem pri otrocih, lahko pa zbolijo tudi odrasli. Veliko okužb poteka brez bolezenskih znakov (asimptomatsko). Do prenosa okužbe lažje prihaja v zaprtih kolektivih. Človek se lahko okuži z astrovirusi z onesnaženo vodo in hrano. Astrovirusi se v velikem številu izločajo z blatom okuženih oseb. Od tod se lahko z nehigienskim ravnanjem ali neurejenimi higienskimi razmerami virusi zanesejo v pitno vodo in onesnažijo hrano, površinske vode in predmete (npr. kljuke, mize, telefone, igrače ...). Pogost vzrok okužb z astrovirusi je pomanjkljiva osebna higiena pri rokovanju z živili.

Enterični virusi se razmnožujejo v celicah črevesnega epitelija in se tako prenašajo po fekalno oralni poti kot tudi aerogeno z aerosoli bruhanja. Od tod lahko z nehigienskim ravnanjem ali neurejenimi higienskimi razmerami viruse zanesemo v živila, pitno vodo ter prostore in predmete (npr. kljuke, gospodinjski pripomočki ...), preko katerih se lahko bolezen širi. Glavni vir onesnaženja živil, pitne vode in ostalega okolja so odplake ter iztrebki, okuženi delavci in okužene živali (Todd in Greig, 2015; Boxman, 2013; Verhoef in sod., 2013; Koopmans, 2012; Rodriguez-Lazaro in sod., 2012; Patel in sod., 2009; FAO/WHO, 2008; Koopmans in Duizer, 2004).

Živila se z virusi lahko okužijo že v fazi pridelave, lahko pa do okužbe pride naknadno pri obdelavi, predelavi ali pri distribuciji hrane. Glavni dejavniki, ki vplivajo na tveganje okužb pridelkov, so kakovost uporabljene vode ter stopnja osebne higiene delavcev, ki rokujejo z živili. Živila, za katera obstaja največja verjetnost, da pride do okužbe, so školjke, sveže sadje in zelenjava ter že pripravljena jedila, katera pred zaužitjem ni

potrebno dodatno obdelati (Todd in Greig, 2015; Koopmans, 2012; Rodriguez-Lazaro in sod., 2012; Patel in sod., 2009; FAO/WHO, 2008; Goyal, 2006; Koopmans in sod., 2002).

Enterični virusi so lahko odporni na različne okoljske dejavnike, kot so visoke ali nizke temperature, ekstremne pH vrednosti ter postopki dezinfekcij. Zaradi teh lastnosti jih običajni tehnološki postopki in postopki konzerviranja ne uničijo (Papafragkou in sod., 2006). Odpornost na okoljske dejavnike jim omogoča tudi prehod skozi prebavno pot. Te lastnosti jim prav tako omogočajo preživetje v kisli, slani in začinjeni hrani, zamrznjeni hrani ali ne dovolj toplotno obdelani hrani (Greening, 2006; Rzezutka in Cook, 2004).

Zaradi naraščajoče porabe surovega in/ali minimalno predelanega sadja in zelenjave, školjk ter že pripravljenih jedil, katerih pred zaužitjem ni potrebno dodatno obdelati, so virusi čedalje bolj prepoznaven vzrok alimentarnih obolenj. Razloge lahko vidimo v tem, da so ti proizvodi pogosto uvoženi z območij, ki nimajo strogih higienskih ukrepov in pogosto pridejo v stik s potencialno onesnaženo vodo, ledom ter neprimernim rokovanjem (Verhoef in sod., 2013; EFSA, 2011; FAO/WHO, 2008; Goyal, 2006).

Školjke so živilo, ki je vedno pogosteje tudi na krožniku slovenskega potrošnika (Poklar Vatovec in sod., 2014). Globalizacija poti v ŽPOP verigi tudi pri školjkah povečuje zapletenost verig v primerjavi z lokalnim trgom, kjer so poti pregledne, kratke in enostavne. Obolenja, povezana z zaužitjem školjk, so poznan pojav glede toksinov, manj pa je znanega o virusih. Školjke se prehranjujejo s filtracijo vode in posledično koncentrirajo virusne delce v fekalno onesnaženi vodi ter toksine mikroalg. Poleg tega so školjke tudi na udaru zaradi onesnaženja z virusnimi delci (Henigman, 2012) s strani zaposlenih pri pripravi živil. V zadnjih nekaj letih je bil dosežen velik napredek na tem področju, vendar kljub temu področje še ni regulirano z evropsko zakonodajo. Mikrobiološko vzorčenje školjk temelji na rednem vzorčenju školjk preko celega leta (Regulation 853/2004 …, 2004; Regulation 854/2004 …, 2004). Vzorči se jih na gojitvenih površinah na školjčiščih. Ustrezni ukrepi, ki naj bi zmanjšali možnost onesnaženja školjk, so povezani zlasti s mikrobiološko kontrolo zdravstvene ustreznosti vode in odplak, za kar pa je potrebno tudi ustrezno oblikovanje in izvajanje predpisov. Pri pripravi hrane mora biti zagotovljena ustrezna higiena, zlasti rok, uporaba rokavic, izvajanje sanitarnih ukrepov, vakcinacija osebja in izločanje okuženih oseb iz procesa priprave oz. proizvodnje hrane. Pomembna je tudi zadostna toplotna obdelava školjk pred zaužitjem (FAO/ WHO, 2008).

V slovenskem prostoru ni pristopa, ki bi določal ali predlagal, kako potrošniku zagotavljati virološko varno živilo. Slednje dejstvo odpira raziskovalno vprašanje o celoviti analizi vzrokov neobvladovanja virusnih okužb vzdolž ŽPOP verige.

1.2 NAMEN RAZISKAV IN HIPOTEZE

1.2.1 Namen raziskav

Z načrtovano raziskavo želimo pojasniti vzroke neobvladovanja virusnih okužb vzdolž ŽPOP verige ter s tem prispevati k razvoju varnosti živil ter izboljšati razumevanje pojmovanja virusnih okužb. Ugotovitev stanja in zavedanja o pomembnosti zagotavljanja varnosti živil tudi na področju obvladovanja virusnih okužb vzdolž ŽPOP verige je osnova za izdelavo nadaljnjih strategij za učinkovito zagotavljanje varnosti živil. Namen raziskave je bil:

- preveriti, ali je obvladovanje virusnih okužb v ŽPOP verigi doseženo z obstoječimi dobrimi praksami na področju živilstva;
- oceniti, ali je dobra higienska praksa za nadzor virusnih okužb po Codex Alimentarius, primerna osnova za razvoj sistema zagotavljanja varnosti živil na področju preprečevanja in obvladovanja virusnih okužb;
- preveriti, ali se tako potrošniki kot zaposleni, ki rokujejo z živili, zavedajo nevarnosti virusnih okužb, ki se prenašajo s živili;
- z ovrednotenjem celovitega sistema zagotavljanja varnosti živil pokazati kritične kontrolne točke, ki so značilne za virusne dejavnike tveganj v ŽPOP verigi.

1.2.2 Raziskovalne hipoteze

Postavili smo naslednje hipoteze:

- Dobre prakse obvladovanja virusov v ŽPO verigi niso dosežene z obstoječimi dobrimi praksami v ŽPO verigi, ki temeljijo na mikrobioloških tveganjih.
- Dobra praksa, kot jo postavlja Codex Alimentarius, je primerna osnova za razvoj sistema zagotavljanja varnosti živil, ki temelji na upoštevanju virusov kot specifičnih agensov kontaminacije, ki niso vključeni v sedanja mikrobiološka tveganja z relevantnimi metodami dela.
- Niti proizvajalci niti potrošniki se ne zavedajo celovito potencialne nevarnosti virusov na področju varnosti živil.
- Celoviti sistem zagotavljanja varnosti živil bo popolnejši s ponovnim vrednotenjem tradicionalnega HACCP sistema, saj bo pokazal kritične kontrolne točke, ki so specifične za virusne kontaminante in niso enake kot pri mikrobioloških tveganjih.

2 ZNANSTVENA DELA

2.1 OBJAVLJENA ZNANSTVENA DELA

2.1.1 Nedosledna uporaba izrazoslovja na področju varnosti živil: stalni dejavnik tveganja?

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Koncept razumevanja pojma varnosti živil zajema vse stopnje od polja do mize, tako na globalni kot tudi na lokalni ravni. Pomembno je, da se je vsak člen v verigi »od vil do vilic« sposoben sporazumevati z drugim. Cilj raziskave je bil analizirati pojavljajoče se ključne besede, povezane z varnostjo živil v obdobju 1969–2008 v najpomembnejših (spletnih) podatkovnih bazah na področju varnosti živil, in sicer: “PubMed” in “Food Science and Technology Abstracts” (FSTA) z uporabo kvalitativne vsebinske analize. Rezultati so pokazali nedosledno uporabo izrazoslovja na področju varnosti živil. Pokazalo se je, da strokovnjaki na tem področju ne komunicirajo z enakim pojmom, ki odraža stanje, vzrok ali posledico. Ta članek izpostavlja potrebo po nujni uporabi enotnega in enoličnega izrazoslovja, saj je uporaba neskladnega izrazoslovja zavajajoča in predstavlja tveganje tako na strokovnem kakor tudi znanstvenem področju. Z namenom poenotenja izrazoslovja na področju varnosti živil smo na eni strani izpostavili možne dejavnike tveganj, na drugi pa ukrepe za obvladovanje teh tveganj, ki predstavljajo temelj dobrih praks in opredeljujejo živilsko predelovalno oskrbovalno prehransko verigo.

Dovoljenje za objavo članka Ambrožič in sod. (2010) v tiskani in elektronski obliki je v Prilogi A.

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Inconsistent terminology in food safety field: a permanent risk factor?

MATEJA AMBROŽIČ – MOJCA JEVŠNIK – PETER RASPOR

Summary
Food safety understanding is a concept, which begins with technologies and goes all the way to the legislation, from producer to consumer and it is very important that each single link in the food chain is able to communicate with each other. The aim of this study was to analyse terms connected to "food safety" in the period from 1969 to 2008 in major on-line databases for the field, namely, "PubMed" and "Food Science and Technology Abstracts" (FSTA), using qualitative content-analysis as a methodological tool. Main results revealed inconsistent use of "food safety" terminology in the food safety field. It has been shown that professionals do not speak the same language. With regard to global food safety in the food supply chain, this paper points to the need for uniformed terminology, because inconsistent terminology is deceptive and can be one of the risk factors at professional and scientific levels. With an intention to unify the terminology, we expose two influencing categories with seven subcategories, which create the basis of Code of Good Practices and defined food supply chain terminology.

Keywords
food safety; good practice; terminology; qualitative content analysis

Food is essential to life, but if contaminated it can cause illness and even death. Due to recent food crisis, consumer concern about treats associated with food is growing worldwide. With important changes in lifestyles and demography and with globalization in the food trade, we see the food supply growing ever rapidly in size and diversity [1]. Food safety understanding is a concept, which begins with technologies and goes all the way to the legislation, from producer to consumer [2]. Today we ensure "farm to fork" food safety through Hazard Analysis Critical Control Point (HACCP) system and its supporting programmes (prerequisite programmes or good practices) from producer to consumer through entire food supply chain [3]. Food safety is one of the highest priorities of public health at national, community and international level. In order to achieve food safety for consumers, industry and government regulatory sectors have to follow recommendations of the United Nations Codex Alimentarius Commission (CAC) by implementing appropriate quality assurance systems, HACCP system, risk management programmes and ensuring that the world's food supply is sound, wholesome, free from adulteration and correctly labelled [4–6].

Good practices are described in several different Codes of Good Practice designed by producers' organizations, importers and retailers' consortia, as well as government bodies representing consumers [7]. They define the production, processing, manufacturing, transport and storage practices for individual foods or groups of foods that are considered essential to ensure the safety and suitability of food for consumption [8]. Codex Alimentarius codes of practice is a collection of internationally adopted food standards presented in a uniform manner. The first Codex code of practice was adopted in 1969 and was representing a firm foundation for ensuring food hygiene.

The majority of microbial food-borne illnesses are thought to be preventable if food safety principles are understood and practiced throughout the entire food chain from production to consumption [9]. Despite the efforts of food safety information campaigns and educational efforts, food remains a prevalent vehicle of disease [10]. BAŞ and co-workers [11] found out that complicated

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terminology is one of the most common barriers to ensure food safety in food businesses.

Despite the agreed terminology by Food and Agriculture Organization (FAO), World Health Organization (WTO), EU regulation and Codex Alimetarius, a lack of clarity in terminology connected with food safety can be noticed in various scientific and professional articles, which is very confusing for understanding and application in further use. JEVŠNIK and co-workers [12] reported that authors of scientific and technical papers use many different terms for similar barriers to successful HACCP and its supporting programmes implementation, which complicates their ranking and further systematic handling. We were motivated by doubts about the inconsistent use of food safety terminology in the food safety field and consequently we conducted a qualitative content analysis of selected documents covering this particular topic from various aspects.

Time scarcity, the feeling of not having enough time, has been implicated in changes in food consumption patterns such as decrease in food preparation at home, an increase in the consumption of fast food, a decrease in family meals, increase in the consumption of convenience or ready-prepared foods on one side, and both trends in the global food production and concern for health on the other side, such as preference for fresh and minimally-processed foods and the increasingly longer period between processing and consumption of foods [13, 14]. Institutionalizing children in schools and childcare facilities, a growing number of elderly persons in hospitals and nursing homes, and eating outside one's home means that food for many is prepared by a few and can therefore be a source of major food-borne disease outbreaks [15, 16].

In spite of all preventive programmes against food-borne diseases in the food supply chain and in spite of all invested efforts, the number of food-borne diseases is raising and killing approximately 2.2 million people annually [17]. Food-borne diseases not only significantly affect people's health and well-being, but they also have economic consequences for individuals, families, communities, business and countries. Not only that bacterial infections are a problem [3, 18], but lately enteric viruses have been increasingly recognized as important cause of food-borne disease, because of the increasing consumption of shellfish, ready-to-eat foods, and raw and/or minimally processed fruits and vegetables. FAO/WHO reported [19] that food-borne viral infections are very common in many parts of the world despite the measures already in place to reduce bacterial contamination through the use of tools such as HACCP. In Europe, viral agents were responsible for 13.1% of the food-borne outbreaks in 2008 and were identified as the second most common causative agent, after *Salmonella* [20]. The reason is that products are often imported from areas lacking strict hygienic measures, they are often eaten uncooked, and they often come to contact with potentially contaminated water, ice, human hands and surfaces from "farm-to-table" continuum [19].

The main purpose of all good practices in the food safety circle is to provide consumers with safe, healthy and quality food. RASPOR and JEVŠNIK [3] described basic good practices in food supply chain and suggested that, for solving the existing barriers, it is necessary to link-up all relevant good practices "from farm to the table" to one practice named Good Nutrition Practice, which also involves consumer, because the studies in the last years highlighted gaps in food safety knowledge and some critical safety violations regarding food handling at home [21–24].

Safe food handling is important anywhere along the food chain. Therefore the consistent terminology is essential for conveying accurate information. Codex Alimentarius and other international groups have tried to codify the terminology, yet observations indicate inconsistencies that impair accurate communication. Thus this study investigated how prevalent the inconsistencies are.

The aim of this study is to analyse the food safety terminology in the last decades, and to detect possible interactions among the identified and analysed terms and their application in both scientific and professional literature. In the field, there is neither clearly defined food safety terminology concept, nor its use in the professional and scientific work. The latter evolution of the language and different kind of terminology concepts is arising, which are not unambiguous anymore, and this leads to misinterpretation and consequently to incorrect activities in the food safety arena. In this study, we want to show the potential conflict points in the food safety assurance, which are not caused by incorrect activities, but rather by misunderstanding of the food safety terms in the scientific and technical literature.

METHODS

The research method of qualitative content analysis was used to collect and analyse the selected studies dealing with food safety. Content analysis is a research method that has come into wide use in health studies in recent years, and is

a set of qualitative and quantitative methods for collecting and analysing data from verbal, print or electronic communications with numerous applications [25]. Bernard BERELSON [26] defined content analysis as "a researcher technique for the objective, systematic and quantitative description of manifest content of communication". Content analysis has much strength, including the abilities to use retrospective data and to track changes over time, and lower costs compared to other methods of research. As with any research method, it has got limitations, too. These include limits to the inferences drawn, the inability to access causality and the labour-intensive nature of the research [25].

Food Science and Technology Abstracts (FSTA; IFIS, Reading, United Kingdom) thesaurus is a search aid for users of FSTA in all its forms (electronic and printed version) and in this study, it was used as searching code for both used online databases for the field. Studies were selected by defined descriptor and keywords searching. FSTA Thesaurus 2004 [27] defines descriptor as a term currently used in indexing key points of the documents in an information retrieval system. Keyword is a main or often repeated word (usually a noun) that is closely linked to, or describes/defines, a particular subject [28]. Documents were selected in two separated parts. First descriptor "food safety" was used to get familiar with studies dealing with food safety. Studies were selected in a specialized data collection for the field, named "PubMed" (U. S. National Centre for Biotechnology Information; U.S. National Library of Medicine; National Institutes of Health, Bethesda, USA) and FSTA from the period from 1969 to 2008. We chose these two databases due to their high respect and tradition in food, nutrition and health area. During last decades, their reliability has been proven as leading databases in the field. Using this approach, more than 56000 studies were obtained and further selection was needed. In the second part, the search pool was narrowed by using keywords "food safety and good practice*", which allocated 2294 appropriate studies. Keyword searching had to be chosen because there is no existing descriptor for "good practice*" in FSTA Thesaurus 2004. Because of the comprehension of the obtained data, content of selected studies was further evaluated on the basic of total subject in the abstracts.

Criteria for exclusion were defined to eliminate unsuitable documentation sources from further analysis. The chosen sources were selected on the basis of prerequisites that the abstracts were published in English, published between years 1969, when the first Codex codes were published, and 2008. Criteria for selection of abstracts according to their content included citation of synonyms for terms connected to "food safety" and "good practice*". Because of inconsistent use of the terms "good practice" or "good practices", we used unlimited truncation, which retrieves all possible suffix variations of the root word indicated. There are different truncation characters in different databases. In database FSTA, the truncation character is $ and in database PubMed it is *. In order to have better and easier overview, we use truncation character * in this study. All abstracts obtained in this way were analysed comprehensively and their keywords were determined. The determination of each keyword was performed after multiple examinations of selected abstracts in consensus among all co-authors. Authors received their trainings through their practice in the food safety field as well as from additional trainings in social sciences. Besides that, they also track novelties in the food safety field, which more and more consider qualitative methods. At first step, clustering of identified keywords into groups was performed. Determined keywords were sorted into thirty groups, named elements. In the second step, classification of elements to make subcategories and categories was conducted. Elements were further sorted into seven subcategories named "hazards", "food hygiene", "epidemiology", "consumers", "regulations", "food law" and "education and training". Subcategories were once more sorted into two main categories named "causative agents" and "tools". With this classification of determined keywords and its interlinking, we tried to create the basis of Code of Good Practice, which is a fundament of any quality assurance programme.

RESULTS AND DISCUSSION

The history of food safety is probably nearly as old as human history itself. Synonymously with food hygiene, it embraces anything in the processing, preparation or handling of food to ensure it is safe to eat. It may have started with the recognition and subsequent avoidance of foods that were naturally toxic [29]. Nowadays, we master food safety at different levels of food production, distribution and consumption with different good practices which are the consequence of human culture, history and lifestyle [7].

Based on results of the analysed 2294 abstracts, we were able to clarify some observations. Analysis of the term "food safety" over the last century can be illustrated in three evolution periods. At the beginning of last century, food

safety meant nutritional insurance and prevention of food adulteration [30]. In the industrial age, food safety regarded problems arisen in large part of the technological progress in food production with new equipment such as cleaners, sanitizers and lubricants; processing with a long and lengthening series of chemical adjuvants; and distribution with improved packaging materials [31]. In the consumers age, food safety refers to ensuring that food, at all points along the food supply chain, is kept safe for consumption in order to reduce food-borne disease regarding microbiological, chemical and physical contaminant hazards [32]. This illustrates the inconsistency in the food safety definition. It is even more complicated when we go for its understanding and connection to other terms and activities, which are related to this term. Good practices are the term closely connected in scientific and in practical terms. In general, good practice means activity of the quality assurance, which ensures that food products and food related processes are consistent and controlled to assure quality procedures in food systems [3].

Different types of organizations mentioned in the introduction unified the terminology concept in food supply chains that could be followed. In this study, we tried to analyse the existing disharmony, which appears in abstracts of journal articles in the food safety field. Regarding the roles in food safety chain, elements were sorted in two main categories named "causative agents" which present the barriers that affect food supply chain and "tools", which should prevent these barriers. Interlinking two main categories "causative agent" and "tools" represents very important codes of good practices (Tab. 1), which are fundamental for ensuring food quality. Keywords related to the meaning of "farm to the table" food supply chain were sorted into thirty groups, named "elements" (Tab. 2; Tab. 3).

In "causative agents", we have linked together barriers that affect food supply chain under the subcategories "hazards", "food hygiene", "epidemiology" and "consumers".

Subcategory "hazards" (hazard is defined as a biological, chemical or physical agent in, or condition of, food or feed with the potential to cause an adverse health effect [33]) means inclusion of elements "contamination", "risk analysis" and "enteric viruses". Regarding the increased incidence of food-borne diseases caused by viruses, we selected only enteric viruses as representatives of the hazards, although there are not many articles, which would discuss this problem in the food safety field, in comparison to other microbiological hazards. In this subcategory, we noticed the use of different keywords in the element "contamination", where derivative of the word contamination mostly occurred (Tab. 2).

Subcategory "food hygiene" called "hygiene", which is defined as measures and conditions necessary to control hazards and to ensure fitness for human consumption of a foodstuff taking into account its intended use [33], is a fusion of elements named "cleaning", "disinfection", "personnel", "food environment", "food handling" and "sanitation", where a frequent use of words cleaning and sanitation in different phrases can be noticed (Tab. 2).

Elements "outbreaks", "surveillance", "foodborne diseases", "foodborne poisoning", "food allergy" and "health" were linked into the subcategory "epidemiology", which is defined as the study of occurrence, distribution and control of disease in populations [34].

The last subcategory "consumers", who are defined as a final food stuff who will not use the food as part of any food business operation or activity [35], describe consumer comprehension under elements "awareness", "domestic preparation", "education and knowledge" and "population groups". However, elements "contamination" and "food environment" were found to have the biggest pool of keywords (Tab. 2).

In the second main category "tools", we linked together keywords under the subcategories "food law", "regulations", "education and training". Elements "institutions", "legislation" and "inspection" were linked into subcategory "food law", which is defined as laws, regulations both administrative provisions governing food in general, and food safety in particular, whether at Community or national level, and it covers any stage of production, processing and distribution of food, and also of feed produced for, or fed to food producing animals [35]. In the subgroup "food law", keywords FDA (U.S. Food and Drug Administration) and EFSA (European Food Safety Authority) pre-

Tab. 1. Determination of two influencing categories with seven subcategories which create the basis of Code of Good Practices and define food supply chain terminology.

Food supply chain terminology		
Causative agent	CODE OF GOOD PRACTICES	Tools
• Hazards • Hygiene • Epidemiology • Consumers		• Legislation • Regulations • Education and training

Tab. 2. Most frequently identified keywords in analysed abstracts, integrated into elements under the category "Causative agents".

Category	Subcategory	Elements	Identified keywords
Causative agents	Hazards	Contamination	cross-contamination errors; cross-contamination prevention; contaminants; contaminated feed; contamination pattern; contamination of water supplies; pre- and post-harvest contamination; post-process contamination; viral contamination; contamination control; bacterial contamination; fungal contamination; toxin contamination; insects; parasites; pesticides; antibiotics; cleaning chemicals; allergens; toxic metals; veterinary residues; chemical additives; glass; wood; metal; stones; plastics
		Risk analysis	risk assessment; risk management; risk communication; risk avoidance; risk factors; risk information; risk perception; food hygiene risk
		Enteric viruses	caliciviruses; noroviruses; Norwalk like viruses; adenoviruses; enteroviruses
	Food hygiene	Cleaning	chlorine washing; cleaning frequency; cleaning procedures; cleaning methods; cleaning protocols; cleaning regimes; cleaning practices; cleaning cloths; cleaning records; cleaning schedules; cleaning standards; cleaning work surfaces;
		Disinfection	disinfection; disinfection of sponges; disinfection residue; disinfection procedures; disinfection protocol; disinfectant resistance
		Personnel	hands-, fingernails-, clothes-, toilet- employee hygiene; employee opinion; employee motivators; employee awareness; employee intention; employee perception; employee knowledge
		Food environment	cutting boards; cutting blocs; clothes; knives; thermometers; refrigerators; aprons; gloves; processing-, sink-, food-environment; abattoir; slaughterhouses; markets; stores; restaurants; canteens; hotels; hospitals; long term care facilities; domestic-, satellite-, central- kitchens
		Food handling	irrigation water; fertilization; sewage disposal; products-, ingredients-storage; products-, ingredients-distribution; transport; household hygiene
		Sanitation	sanitary characteristics; sanitary conditions; sanitary education; sanitary effectiveness; sanitizing equipment; sanitation programs; sanitation behaviours; sanitation practices; sanitation procedures; sanitization procedures
	Epidemiology	Outbreaks	/
		Surveillance	/
		Foodborne disease	foodborne illness; foodborne sickness; foodborne zoonoses; foodborne infections; vomiting; diarrhoeal diseases; intestinal diseases; animal diseases; gastroenteritis; viral food-borne illness; foodborne infection; bacterial foodborne illness
		Foodborne poisoning	algal toxins; natural toxins; emetic toxins; mycotoxins (ohratoxins, aflatoxins, patulin, fusarium toxins)
		Food allergy	allergen control plan; allergen cross- contact; allergenic ingredients; allergy information; allergen database; allergenic foods; allergenic potential; allergenicity
		Health	health hazards; health problems; health standards
	Consumers	Awareness	instructions; labels; shell-life; quality; safety; eating habits
		Domestic preparation	cooking; reheating; thawing; cooling; chilling; defrosting; home kitchen; domestic kitchen; domestic gardens
		Education/knowledge	training; TV shows; web; schools; magazines, newspapers; cooking courses
		Population groups	elderly; children; school children; young adults; adolescents; mothers; men; women; pregnant women; immunocompromised people

Tab. 3. Most frequently identified keywords in analyzed abstracts, integrated into elements under the category "Tools".

Category	Subcategory	Elements	Identified keywords
Tools	Regulations	Standards	ISO 9000; ISO 22000; EUREP; IFS; BRC; BRI; Codex standards; documentations, keeping records; SSOP; SOP; education/knowledge; trade requirements; food hygiene requirements; international requirements; instructions
		Good practices	prerequisite programs; good hygiene practice; good manufacture practice; good storage practice; good trade practice; food safety practice; handling practice; foot hygiene practice; hand hygiene practice; cleaning practice; dietary practice; cooling practice; cooking practice; domestic food handling practice; domestic refrigeration practice; good trade practice; farm-, manure handling practice; farming practices; feeding practice; field to fork codes of practice; formula preparation practice; general hygiene practice; good agricultural practice; good educational practice; farm management practice; good preventive practice; good slaughtering practice; good training practice; knife cleaning practice; labelling practice; meat handling practice; personnel hygiene practice; pasteurization practice; processing practice; egg handling practice; food handling behaviours; domestic hygiene practice; mothers practice; refrigeration storage practice; good distribution practice; good storage practice; good catering practice; lifestyle practices; good nutritional practice; guidelines; hands-, equipment-, washing practice; washing behaviours
		HACCP	critical control point; PR/HACCP; Codex HACCP; HACCP barriers; HACCP hygienic code; temperature-, time-monitoring; traceability
		Codex Alimentarius	/
	Food law	Institutions	EFSA; FDA
		Government agencies	/
		Legislation	Food law: general principles and requirements of food law; EC178/2002; hygiene of foodstuffs; EC 852/2004; EC 853/2004; EC 854/2004; EC 882/2004; EU hygiene package; Directives: residues of veterinary medicinal products; pesticide contamination directives, radioactive contamination directives; biological safety; packaging
		Inspection	inspection system; inspection service; food hygiene inspection; violations
	Education & Training	Management personnel	managers
		Food professionals	food worker; food handler; employee; care takers; food service personnel
		Inspectors, regulators	government employees

vail over accurately quotation of named existing food laws (Tab. 3). Elements "standards", "good practices", "HACCP" and "Codex Alimentarius" were linked together in a subcategory named "regulations", which should guarantee quality and safety standards and norms. We found the biggest disharmony and confusion at the element "good practices" (Tab. 3).

The last subcategory "education and knowledge", definition of which is described in ISO 22000:2005 [36] as the food safety team and the other personnel carrying out activities having an impact on food safety, who shall be competent and shall have appropriate education, training, skills and experience, contains elements "management personnel", "food professionals" and "inspectors and regulators". Keywords clustering into elements is presented in detail in Tab. 2 and the Tab. 3.

For the elements "outbreaks", "surveillance", "Codex Alimentarius" and "government agencies", the identified keywords were the same as the names of the elements. At first sight, it looks like that food safety is determined by different tools and standards. But unfortunately this is not true, because the importance of exact terminology in food safety field it is frequently forgotten. With regard to our results, not enough attention is paid to food safety terminology in the food safety field as one of potential risk factors.

We have faced with several problems when searching for appropriate articles. One of the problems was that different databases did not have the same system of indexing descriptors, which was unclear and was hard to follow through different databases. Index heading "food safety" has been used in FSTA Thesaurus not earlier than 1992 [27], before that it was included under the index heading "health". The biggest problem we have faced was that no descriptor exists in FSTA Thesaurus 2004, which would define good practices [27]. Due to this fact, searching was complicated and time consuming. If good practices were defined in FSTA Thesaurus 2004, the searching would be easier and simpler. The major problem with descriptors is that they are not created by authors and therefore do not allow a researcher to find the appropriate literature considering the existing knowledge, and that is why it is better to use keywords searching then descriptor searching. JACOB, MATHIASEN and POWELL [9] stated that food safety messages found to be effective are relevant to the target audience, contain reliable information and are easily received and understood. Scientific findings have to make impact on practical work, professional communication and education if we want to fill up major gaps in the approach "from stable to table".

JEVŠNIK, HLEBEC and RASPOR reported [12] that research results are strongly associated with the time of observation and old data often prove irrelevant in present situations, which were also noticed in our study. With regard to a food safety programme, which should be able to identify all hazards, analyse them, evaluate the likelihood of their occurrence and identify measures for their control [37], we tried to identify the potential risk factors, which contribute to the application of food safety programme along the food supply chain. There are two main factors in the food safety programme, which have significant influence on food safety along the food supply chain. On one side there are barriers, which negatively affect entire food supply chain. In our study barriers are represented in four subcategories. On the other side there are tools, which should prevent or reduce occurrence of all hazards and in this study are divided then in three subcategories. Interlinking all seven categories forms good practices, which have a very important intention to provide consumers with safe and quality products.

Common language among food safety professionals should be worldwide equally understood and should enable their consistent application. However, BAŞ, YÜKSEL, and CAVUŞOĞLU [11] already reported that complicated terminology is one of the main barriers for food safety in the food business. We find the biggest disharmony and confusion at subgroup "regulations", where element "good practices" is highlighted. Identified keywords include both general phrases such as prerequisite programmes, good hygiene practice, good manufacture practice, good storage practice, good trade practice, food safety practice, good agricultural practice etc. (Tab. 3), and unusual phrases such as foot hygiene practice, hand hygiene practice, domestic refrigeration practice, field to fork codes of practice, knife cleaning practice, refrigeration storage practice, food handling behaviours etc. (Tab. 3), which are all included in the element "good practices". This confusion within the element "good practices" can be a consequence of the absence of communication with neighbouring or related good practices among food supply chain [7]. In our study we noticed numerous different interpretations and conceptualization of elements such as "contamination", "food environment" and "good practices" without regard to main categories. This phenomenon can be ascribed to researchers, who set up their own explanations and definitions.

Besides all findings, we should not ignore the chaos in the food safety field regarding interpretation, understanding and use of terms "food safety" [37], "food security" [38] and "food defence" [39], the last also called "food-borne bioterrorism" [40]. In 1996, countries at the World Food Summit in Rome agreed that "food security" exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life [38]. Outcome of the events of September 11th heightened a perception that food is vulnerable and it can be used as a vehicle for the dissemination of harmful agents that could threaten public health. That is why experts introduced the definition of the term "food defence", which can be defined as protecting the nation's food supply from deliberate or intentional acts of contamination or tampering [39]. This is distinguished from "food safety", which means protection of food products from unintentional contamination. Fortunately, the actual likelihood of intentional jeopardizing our food currently seems to be low compared to that of naturally emerging agents [41]. Nevertheless, we have to be aware of the existence of all three terms, the difference between them and use them correctly.

Inconsistent application of food safety terminology is observed at international level and consecutively also in national languages. This contributes to general confusion, which was already

identified by JEVŠNIK, HLEBEC and RASPOR [12]. We show that authors of scientific articles are using inconsistent food safety terminology concept and vocabulary, although a common language unified among food safety professionals is available and could be followed.

CONCLUSIONS

Food safety is a result of several factors: legislation should lay down minimum hygiene requirements, official controls should be in place to check food business operator's compliance, and food business operators should establish and operate food safety programmes and procedures based on HACCP principles. Each factor brings unique challenges to the development and transmission of food safety terminology. In order that all links in food supply chain are functioning altogether, each link should speak the same language. But unfortunately, we showed in this study that uniformed terminology is not in use in the food safety field, which means that food professionals do not speak the same language. Problem arises with development of new terms, which are normally from scientific level translated to professional level. With translation into practical level, it normally gathers new or at least additional meaning. This problem can be solved with unequivocal demand from science to define the term. Terms should also be consistently applied and that is only possible if consensus is reached. Consensus to the new terms could be entrusted to international organizations, which cover the scientific field. And where the food is in question, then we have two main organizations, which should bring about consensus. One of them is International Union of Food Science and Technology (IUFoST), and another is International Union of Microbiological Societies (IUMS). When application is in question, the translation can be assigned to agencies like EFSA or FDA, which are sound foundations for policies, legislation and to support the government in taking effective and timely risk management decisions. Further, agencies should also make the matter clear to all, who resume the terminology concept. Terminology concept should not be shattered in various documents, but should be gathered together and clearly defined in one place. In this way, the lexicon of food safety field can be established. Additionally, organizations like FSTA and PubMed should harmonize the terminology and their definitions in a consultation process with the previously mentioned associations. With regard to global food safety, all links in the food supply chain have to use just one food safety terminology concept, which would represent a firm foundation for further use in practice. Our findings contribute to systematic planning of further research, directed towards appropriate strategies for elimination of inappropriate terminology and to development of common language among food safety professionals.

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2.1.2 Skladnost predlaganih smernic Codex Alimentarius za obvladovanje virusnih okužb z načeli dobrih praks

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Zaskrbljenost javnosti zaradi tveganj, ki jih prinašajo živila, narašča. Večina pozornosti je namenjena sami proizvodnji živil in gostinski dejavnosti. Virusno onesnaževanje živil se lahko pojavi kjerkoli v živilsko predelovalni osrbovalno prehranski verigi. Večina virusnih okužb, prenesenih z živili, se izsledi do okuženih oseb, ki so rokovale z živili, ki niso bila toplotno ali kako drugače obdelana. Glede na vse večjo pojavnost virusnih okužb, ki se prenašajo z živili, je Codex Alimentarius izdal osnutek smernic dobre higienske prakse za nadzor virusnih okužb. Izsledki SWOT analize so pokazali omejitve na področju izrazoslovja, detekcije in preglednosti samega dokumenta.

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COMPLIANCE OF PROPOSED CODEX ALIMENTARIUS GUIDELINES FOR VIRUS MANAGEMENT WITH PRINCIPLES OF GOOD PRACTICE

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Public concerns relating to food safety remain high, with most attention focused on manufactured foods and those served in catering operations. The viral contamination of food can occur anywhere in the food supply chain from farm to fork, but most food-borne viral infections can be traced back to infected persons who handle food that is neither heated nor otherwise treated. Regard to the increasing incidence of food-borne viral infections, the Codex Alimentarius Committee on Food Hygiene issued an international draft on a Code of Hygienic Practice for the control of viruses in foods. Using SWOT analysis as a methodological tool, the main results of the analysis revealed limitations of the document regarding language terminology, detection methodology and transparency.

Keywords: food safety, food-borne viruses, Codex Alimentarius, SWOT analysis

Food-borne diseases encompass a wide spectrum of illnesses which are a growing public health problem worldwide. In recent decades, changes in food production and consumption have affected the safety of food. Trends in global food production, processing, distribution, and preparation present new challenges for food safety. These new challenges in reducing the incidence of food safety hazards due to typical changes in food supply chains (e.g. longer chains, booming food service establishments), health and demographic situations (e.g. more vulnerable groups, rapid urbanization), social situations (e.g. increased consumption of ready-to-eat foods, increased travel and exposure to unsafe foods) and environmental conditions (e.g. increased pollution) underpin the need for an effective food safety management system (Motarjemi & Käferstein, 1999; Crerar, 2000; Van Der Spiegel et al., 2003; Woteki & Kineman, 2003).

To achieve food safety for consumers, industry and government regulatory sectors have to follow the recommendations of the Codex Alimentarius Commission, which is the global reference point for consumers, food producers and processors, national food control agencies and the international food trade (CAC, 2006). The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have strong interests in promoting food control systems that are based upon scientific principles and guidelines as reflected in the priorities of the Codex Alimentarius Commission, because this is the only way that participating states and other subjects in international food law can develop a common language. These Codex recommendations do not represent legally binding norms

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but models for legislation used by governments to determine and refine the policies and programmes under their national food control systems.

In addition to the formally accepted food standards, the Codex Alimentarius is a collection of standards, codes of good practice, guidelines and other recommendations. Good practices are described in several different Codes of good practice designed by producers' organizations, importers' and retailers' consortia and government bodies representing consumer safety (Raspor, 2008). They define the production, processing, manufacturing, transport and storage practices for individual foods or groups of foods that are considered essential to ensure the safety and suitability of foods for consumption in a uniform manner (CAC, 2006).

The broad spectrum of food-borne infections has changed dramatically over time, as well-established pathogens have been controlled or eliminated, and new ones have emerged (Tauxe, 2002). Microbial food safety is considered a significant public health issue but historically has focused mostly on control of bacterial contamination. Lately enteric viruses have been increasingly recognized as an important cause of food-borne disease (Cook & Rzezutka, 2004; FAO/WHO, 2008), because of the increasing consumption of shellfish, ready-to-eat foods and raw and/or minimally processed fruits and vegetables. The reason is that such products are often imported from areas lacking strict hygienic measures, they are often eaten uncooked, and they often come into contact with potentially contaminated water, ice, human hands, and surfaces in the farm to table chain (Goyal, 2006; FAO/WHO, 2008). Consequently it is necessary to emphasise the significance of food-borne viruses as important microbiological hazards in food safety programmes. Food safety management systems, safety guidelines and good practice documents also need to be revised to include viral food safety. For this purpose the 40th Session of the Codex Committee on Food Hygiene in December 2008 set in motion the first international work on a Code of Hygienic Practice for the control of viruses in food (CAC, 2009a). This document was later entitled "Proposed Draft Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food" (CAC, 2010b).

With regard to the necessity to set up viral food safety control, the aim of this study was to analyse the forthcoming Codex Alimentarius proposed draft Guidelines on the application of general principles of food hygiene to the control of viruses in food at step 3 (CAC, 2010b) and its comparison to other globally important codes of good practice. In this study the findings were aimed at suggested augmentation and amendments to the draft of the forthcoming new code of good practice for control of viruses in foods.

1. Materials and methods

The research method of SWOT analysis (Andrews, 1971) was used to analyse the forthcoming Codex Alimentarius Guidelines to control viruses in foods. SWOT (Strengths, Weaknesses, Opportunities and Threats) analysis is a strategic planning tool that can be used to identify and assess strengths and weaknesses, as well as the opportunities and threats likely to be encountered.

SWOT analysis is a tool consisting of two parts (Hill & Westbrook, 1997; FAO, 2006):

1. An analysis of the internal strengths and weaknesses: any internal asset or a deficit (coordination, equipment, finance, motivation, procedures, staff skills ...), which enables or prevents the code of good practice from carrying out its mission effectively.

2. An analysis of the external environment: the external environment is defined as any external circumstance or trend (international cooperation, increased consumer awareness of food safety, global trade, transboundary food-borne disease…), which enables or prevents the role and operation of the code of good practice.

The analysis was performed in two parts. First, a screening and review of the FDA's Food Code 2009 (FDA, 2010), the Codex Alimentarius Recommended Codes of Good Practices (CAC, 2010a) and Food Code of Australia and New Zealand (FSANZ, 2000) was conducted. The review was focused only on viruses as currently the most important human food-borne pathogens in each of the above-quoted codes. In the second part a SWOT analysis of the Codex Alimentarius Proposed draft guidelines on the application of general principles of food hygiene to the control of viruses in food at step 3 was prepared. All current documents accessible to the public from FDA, the Codex Alimentarius and the Food Standards of Australia and New Zealand web pages were obtained.

Through SWOT analysis we determined five conceptual elements, which highlight the intention of Codex Alimentarius Guidelines for control of viruses in foods. A qualitative approach enabled an in depth analysis of perceptions in an attempt to grasp the complexity of the problems, especially in the phase of its creation. The results presented in this study suggested augmentation and amendments to the draft of the forthcoming new document for control of viruses in foods.

2. Results and discussion

Codex guidelines fall into principles that set out policy in certain key areas and guidelines for the interpretation of these principles, or for the interpretation of the provisions of the Codex general standards. In the case of food additives, contaminants, food hygiene and meat hygiene, the basic principles governing the regulation of these matters are built into the relevant standards and codes of practice (CAC, 2006). The document analysed was developed and transformed from the "Code of hygienic practice for the control of viruses in food" (CAC, 2009a) to "Guidelines on the application of general principles of food hygiene to the control of viruses in food" (CAC, 2010b). The evolution of the text reflects its alteration to a more general document, which is concerned with general principles and not specific for practical application within the food supply chain.

The SWOT analysis related to the Codex Proposed draft is developed in Table 1 and presented below regarding important elements like transparency, food safety terminology, control measures, environmental and personal hygiene, and consumer food safety. The analysis includes only those parts of the document which are written specifically for viruses. The requirements in the Proposed draft are listed in sections 1 to 10 (Table 1), which follow the same format as the Recommended international code of practice-general principles of food hygiene (CAC, 2010a).

2.1. Transparency

The primary purpose of these Guidelines is to minimize the risk of illness arising from the presence of human enteric viruses, with emphasis on Hepatitis A virus (HAV) and Noroviruses (NoV) as being the greatest concern from a food safety perspective with a focus on ready-to-eat food, which is one of the weaknesses identified (Table 1). The restricted focus on only the two viruses most frequently found in food-borne infections could be misleading, because

Table 1. SWOT analysis with its conclusions divided into conceptual elements related to the Codex Alimentarius Proposed draft guidelines on the application of general principles of food hygiene to the control of viruses in food at step 3

	Strengths	Weaknesses	Opportunities	Threats	SWOT analysis conclusions
Section 1: Objectives	Advice on the framework for the control of enteric viruses.	To minimize the risk of illnesses arising especially from the presence of NoV and HAV in foods with a focus on ready-to-eat food.	Document only for the control of human enteric viruses.	Not designed for consumers.	– Transparency – Consumer's affairs
Section 2: Scope, use and definition	For all kinds of foods from farm to table.	Inconsistent terminology concept.	To form a uniform common language in the field.	Conflict with other essential documents of older dates	– Transparency – Terminology process
Section 3: Primary production	To describe setting and identifying aspects of production processes.	No foreseen strict control procedures.	Development and/or implementation of effective measures.	Inefficient and limited routine detection methods.	– Control measures – Environmental and personal hygiene – Terminology process
Section 4: Establishment: Design and facilities	Assurance that surfaces can be cleaned and disinfected.	No inclusion infrastructure of irrigation water.	Selection of appropriate location, setting up appropriate building with appropriate equipment setting.	Inappropriate location with relevant infrastructure.	– Control measures – Environmental and personal hygiene
Section 5: Control of operation	To control processing operations.	Gaps in monitoring and review control procedures.	Development of efficient procedures.	Inefficient and limited routine detection methods.	– Control measures – Environmental and personal hygiene
Section 6: Establishment: Maintenance and Sanitation	Preventive maintenance and sanitation procedures after an event.	Precaution procedures before an event occur.	Development of effective cleaning and disinfection procedures.	Inefficient cleaning and disinfection procedures.	– Control measures – Environmental and personal hygiene
Section 7: Establishment: Personal hygiene	To prevent food handlers contaminating food due to poor hygienic practice.	Control of personal hygiene practice.	Awareness of the importance of healthy and clean food handlers.	Inappropriate personal hygiene behaviour.	– Environmental and personal hygiene – Consumer's affairs – Terminology process
Section 8: Transportation	/	/	/	/	
Section 9: Product information and consumer awareness	Assurance of sufficient product information.	Limited knowledge about the risk of infection when consuming raw or lightly cooked foods.	Importance of appropriate labels.	Low confidence of consumers in the safety and quality of food.	– Consumer's affairs
Section 10: Training	Those engaged in food operation should be trained and instructed	More emphasis on importance of periodically renewed refresher training.	Emphasis on acquisition of specific knowledge and skills.	No official education training for consumers.	– Consumer's affairs

other viruses could also be present in food (Vasickova et al., 2005; FAO/WHO, 2008). A significant rise in the consumption of fresh produce for their health benefits and major shifts in consumption trends have been reflected in a sharp increase in the popularity of minimally processed convenience foods such as fresh produce and shellfish. These two virus-commodity combinations are also captured in two supplemental annexes.

The document analysed lays a foundation for ensuring food hygiene and provides advice to governments on a framework to control human enteric viruses and also provides information that will be of interest to the food industry, consumers and other interested parties, which is one of the strengths (Table 1) of these guidelines. These Guidelines are intended to control viruses from farm to fork in all kinds of foods, which represents its next strength (Table 1), but only in conjunction with Good Hygiene Practices (GHPs) as specified in other essential recommended Codex Alimentarius codes of good practice (CAC, 2010b) of older dates (Table 1). These are necessary for a full understanding and interpretation, but they are not optimised for the prevention viral infections. However, they should not compromise controls already in place for any other pathogens. Beside that it is necessary firstly to be familiar with all relevant documents for using these guidelines, which can otherwise lead to a loss of transparency and to the misinterpretation of the document.

The document analysed is the first general document dealing only with food-borne viruses through the entire food supply chain, which represents one of the most important opportunities of the document (Table 1). The document also highlights the key hygiene safeguards at each stage in the chain for control of viruses. In other relevant codes, such as the FDA's Food Code 2009 (FDA, 2010) and FSANZ Food Code, food-borne viruses are listed as one of the food-borne pathogens which have high infectivity via contamination of food and should be prevented.

With regard to transparency, we also observed frequent repetition of requirements and general facts in different sections, which is unnecessary and confusing. This observation can be demonstrated by the requirement "exclusion of persons with illness symptoms", which is mentioned in both sections "Primary production/harvesting area" and "Establishment: personal hygiene".

2.2. Food safety terminology

For understanding and interpretation of the relevant documents, a consistent terminology at national and international level should be used (Jevšnik et al., 2006) and therefore the terminology should be uniformly developed and consistently applied for all documents in this field (Ambrožič et al., 2010). The use of inconsistent terminology represents one the weaknesses observed (Table 1) in these Guidelines. These Guidelines are supplemental to the Recommended International Code of Practice-General Principles of Food Hygiene, designed only for control of viruses in the food supply chain and as such should reflect the unified terminology (Table 1) used in other Codex Alimentarius documents. The terminology in all Codex Alimentarius documents (CAC, 2010b) should be carefully defined and its use at professional and scientific level should also be uniform. Our analysis showed that several expressions, such as "agricultural worker (CAC/RCP 53-2003)", "grower (CAC/RCP 53-2003)", "harvester (CAC/RCP 53-2003)", "food handler (CAC/RCP 1-1969; CAC/RCP 39-1993; FSANZ Food Code)" and "food employee (Food Code 2009)" (FDA, 2010) are used in different documents for defining people who handle food. The next issue the study revealed is also the application of different definitions for identical terms. This is illustrated by the expression "food handler". "Food handler" in the Recommended International Code of

Practice-General Principles of Food Hygiene (CAC, 2010a: CAC/RCP 1–1969) is defined as any person who directly handles packaged or unpackaged food, food equipment and utensils, or food contact surfaces, and therefore is expected to comply with food hygiene requirements. In the Recommended International Code of Practice-Code of Hygienic Practice for Precooked and Cooked Foods in Mass Catering (CAC, 2010a: CAC/RCP 39–1993) a "food handler" is defined as every person handling or coming into contact with food, or with any equipment or utensil used in food handling. The FSANZ Food Code (FSANZ, 2000) defines a "food handler" as a person who directly engages in the handling of food, or who handles surfaces likely to come into contact with food, for a food business. In these Guidelines also inconsistent use of the expressions "food handler" and "food worker" was observed. The expression "food worker" from the previous version of the document from July 2009 (CAC, 2009a) was replaced with the expression "food handler" (CAC, 2010b) and as such should be used throughout. With regard to the understanding and application of this document, these Guidelines should also contain differentiation between and explanation of the expressions "food-borne virus", "human enteric virus" and "zoonotic virus", as was already mentioned in the 41st Session of the Codex Committee on Food Hygiene (CAC, 2009b; 2009c). The document defines "human enteric virus" as viruses that replicate in the gastrointestinal tract or in the liver, are excreted in faeces from humans and are transmitted mainly by the faecal-oral route and are infectious of humans and as such should be used throughout and not just as "enteric virus", as can be observed in the document. The food supply chain has become longer and longer and there are more and more actors in the food supply chain, who see themselves just as resellers and are not aware of also being consumers. It would be reasonable to stress the importance of these two expressions in the relevant documents.

2.3. Control measures

Foods, traditionally eaten raw or receiving minimal processing, provide an ideal route for the transmission of viruses. Viral contamination of food can occur pre- or post-harvest at any stage in the food harvesting, processing and distribution chains. The major routs of viral contamination of foods are contacts with faeces or faecally contaminated water, faecally soiled materials, vomit or water contaminated with vomit, environments in which infected people were present, aerosols generated by infected people and animals containing zoonotic viruses (Koopmans & Duizer, 2004; FAO/WHO, 2008).

The document on one hand identifies production processes to control viral contamination, but on the other does not foresee strict control procedures for the processes identified, which is a weakness (Table 1) of the analysed document. Attention to good hygienic design and construction, appropriate location and the provision of adequate facilities is necessary to enable hazards to be effectively controlled. An important way of controlling viral contamination is the ability to select an appropriate location and setting up an appropriate premises with appropriate infrastructure and equipment with regard to the branch of food industry concerned. The successful introduction of quality assurance systems into the production of food and the food trade can be ensured by adequate technical and hygienic solutions for the external environment of the building, as well as for the building itself. In addition, the technical processing and characteristics of the materials used, the sequence of premises and areas, the microclimatic requirements, technical solutions for the supply of drinking water and waste management, requirements for cleansing, maintenance and for protection from insects and rodents have to be considered. Inappropriate locations for personal hygiene facilities and toilets with their relevant infrastructure may also result in viral contamination.

Hygienic design is critical in promoting the ability to clean the environment and to assure that high quality and safe products can be routinely produced. The equipment and facilities should be designed, constructed and laid out to ensure that surfaces can be easily cleaned, and if needed, disinfected (Table 1), which the document analysed also takes into consideration. Cleaning and disinfection of surfaces are of major importance in the prevention of enteric viral disease, and that is why activities should be oriented towards developing specific cleaning and sanitation procedures to prevent viral contamination. Many disinfection agents recommended for use in food establishments are not effective against enteric viruses (CAC, 2010b). Inefficient cleaning and disinfection procedures can lead to the development of resistance to disinfection and the growth of new pathogens, which is also one of the threats identified (Table 1).

Fruits and vegetables can potentially become contaminated before harvesting by agricultural practices (i.e. irrigation water, manure, biosolids and other natural fertilizers, soil, agricultural chemicals) and post-harvest by contaminated washing water. The irrigation or fertilization of food crops and washing with faeces or faecally contaminated water has been known to play a significant role in the transmission of enteric microorganisms (Papafragkou et al., 2006; FAO/WHO, 2008). That is why the method used for delivery of water (Table 1) should be defined in the Guidelines.

Food business operators are obliged to control processing operations to prevent contamination of food with different kinds of hazards. The control of food hazards in relation to viral contamination as one of the identified strengths (Table 1) implies comprehensive identification of steps critical to food safety, implementation of effective control procedures in these steps, monitoring control procedures to ensure their continuing effectiveness and reviewing control procedures periodically and whenever operations change. Control procedures at critical steps should be related to poor hygienic practice and the water resources used. The opportunities (Table 1) of this document are oriented towards promoting development and implementation of effective measures for regular viral control and developing instructions for protection against viruses. The document also includes general control strategies, such as recall procedures for contaminated products and training courses for personnel, because trained and competent personnel are one of the most important elements of food safety efficiency.

Since most food-borne viruses cannot be cultured in vitro, detection methods are based on molecular amplification techniques, even though they are unable to discriminate between infectious and non-infectious viruses (FAO/WHO, 2008). Molecular methods are less time- and labour-intensive and facilitate the analysis of large numbers of samples. Besides, the routine use of virus detection methods for foods is limited to a few laboratories in a few countries and there is a need to transfer this knowledge to other locations in an effort to promote its more routine use. Once validated, these methods will be useful in outbreak investigations as well as in auditing and monitoring procedures (FAO/WHO, 2008; CAC, 2010b). Today inefficient and limited detection methods of viral contamination can result in failure to comply with the required needs. The threats identified in the document (Table 1) are connected with the limited procedures for routine detection of viruses in/on foods and food contact materials.

2.4. Environmental and personal hygiene

Unlike bacteria, viruses cannot replicate in food or water, but only a few infectious viral particles are needed to infect and cause illness (Greening, 2006). When virus contamination

of foods occurs, the number of infectious virions will not increase during processing and storage. The ability of contaminated food to serve as a vehicle of infection therefore depends on virus stability, degree of initial contamination, the method of food processing and storage, the viral dose needed to produce infection, and the susceptibility of the host (KOOPMANS et al., 2002). High temperatures may be effective in some instances, but recommended time-temperature combinations are virus-specific and will vary with the food commodity (PAPAFRAGKOU et al., 2006). Low temperatures are not reliable means to reduce enteric viruses in contaminated foods, because cooling or freezing will not affect virus infectivity (PAPAFRAGKOU et al., 2006). Washing with water of drinking quality, both with or without the addition of chemical disinfectants, may also reduce the virus load in contaminated items, but decontamination again varies with both virus and produce type (KOOPMANS & DUIZER, 2004; PAPAFRAGKOU et al., 2006). The weaknesses (Table 1) are furthermore connected to the degree of personal hygiene and to the absence of applicable routine detection methods to control viral contamination.

The aim of the section "Establishment: Personal Hygiene" is to prevent food handlers from contaminating food due to poor hygienic practice, which is, at the same time, one of the strengths of this document. Good Hygiene Practice (GHP) consists of fundamental principles, procedures, personal sanitation and the means necessary to design and sustain an appropriate environment for producing food of an acceptable quality. Food handlers may transmit viruses to foods from a contaminated surface, from another food or from contaminated hands (KOOPMANS & DUIZER, 2004; PAPAFRAGKOU et al., 2006). Hands can become contaminated by direct contact with any virus-containing fluid from oneself or others; or they may also become individually contaminated by contacting virus-contaminated surfaces or objects (SATTAR et al., 2002). The ultimate source of viral contamination is usually human faecal matter, although vomit may also contain infectious viruses (PAPAFRAGKOU et al., 2006). For all food commodities preventing direct contact with human faecal material is obviously the first consideration in controlling virus contamination. Hands are believed to play an essential role in the spread of viruses. For hand decontamination to be successful in controlling viral food-borne disease outbreaks, three elements must be in place: an effective disinfecting agent, adequate use instructions and regular compliance (SATTAR et al., 2002). Compliance to hand hygiene is important at all times and that is why hand washing instructions and the wearing of gloves are fundamental in prevention of viral food contamination. But an identified weakness (Table 1) is the lack of control of personal hygiene practice.

In order to reduce the likelihood that certain viral and bacterial agents will be transmitted from infected food handlers to food, the section "Employee health" in Food Code 2009 (FDA, 2010) describes the responsibilities of persons in charge and employees, restrictions and exclusions in detail. One of the most important components is an exact reporting system, which enables identification of employees who present a risk of transmitting food-borne pathogens to food or to other employees. Food Code 2009 (FDA, 2010) introduces a list of the five most important pathogens which have high infectivity via contamination of food by infected food handlers, including NoV and HAV. This principle was also adopted by the FSANZ Code (ANZFA, 2001).

At the moment the only practical solution for the control of viruses in foods is stringent personal hygiene, which should be based on strict control by supervisors. This approach is used in the FSANZ Food Code and FDA's Food Code 2009 (FDA, 2010), where the structure of the responsibilities, restrictions and exclusions are designed to reduce the likelihood that certain bacterial and viral agents will be transmitted from an infected food handler into

food. This approach offers the maximum protection of public health, where the reporting requirement is the most important component.

2.5. Consumer food safety

In the last decade consumers have become very critical about food quality and food safety due to several incidents with contaminated food and a generally increased awareness about food and health matters. Microbiological hazards and the food-borne diseases they cause are an increasingly important public health problem. In the European Union in 2008 a total of 5 332 outbreaks were reported. The largest number of reported food-borne outbreaks were caused by Salmonella (35.4% of all outbreaks), followed by viruses (13.1%), bacterial toxins (9.8%) and Campylobacter (9.2%) (EFSA, 2010). Consumers and other parties are increasingly concerned about the continuing sequence of food scandals and incidents. The focus on various national and international food safety issues over the last few decades has arguably resulted in a decline in public trust in food safety regulation and management inside and outside Europe (Houghton et al., 2008). These Guidelines are not purposely designed for informing consumers, although studies in the last few years have highlighted gaps in food safety knowledge and some critical safety violations regarding food handling at home (Unusuan, 2007; Jevšnik et al., 2008a; 2008b; Redmond & Griffith, 2009; Sanlier, 2009; Byrd-Bredbenner et al., 2010). Private households are responsible for 38% of all verified outbreaks in the EU and represent the main setting of verified outbreak occurrence (EFSA, 2010). However, outbreaks of food-borne illness occurring in private homes are less likely to be reported than those in commercial and public premises and it is believed that infections attributed to the home are three times more frequent than those attributed to canteens and public eating places (Borneff, 1998; Scott, 2003).

An increasingly important role for food control systems is the delivery of information, education or advice to participants in the farm to table continuum. Insufficient product information can lead to their mishandling at later stages in the food chain, which can result in food-borne illness, and that is why assurance of sufficient product information is one of the strengths (Table 1) needed. Today we are faced on one hand with insufficient knowledge and awareness of food safety issues among food handlers, and on the other we have consumers insufficiently informed about food safety principles in the home (Sanlier, 2009). Limited knowledge among consumers about the risk of viral infection when consuming raw or lightly cooked foods represents the next observed weakness (Table 1). This weakness can be eliminated by product information via labelling.

Activities in the framework of this document are also aimed at improving the awareness of the importance of education, training and good hygiene practices, because as mentioned above, there are no routine methods for virus detection and all precautions are based only on maintenance of good personal and environmental hygiene. Farm workers often come from diverse cultural backgrounds, not all of which stress proper personal hygiene as an important behavioural value. To prevent food related diseases and assure safe food efficiency, suitable working environments from the hygienic-technical point of view and motivated, satisfied and qualified personnel must be assured (Jevšnik et al., 2008b).

Food hygiene training is fundamentally important (Jevšnik et al., 2006). All personnel involved in the process have to be aware of their role and responsibility in protecting food from contamination. Those engaged in food operation who come directly or indirectly into contact with foods should be trained and/or instructed in the control of enteric viruses to a level appropriate to the operations they perform (Table 1). An important element in the

effectiveness of food hygiene training is the support given by managers, both pre- and post-training, to motivate food handlers to enact the safe food handling practices learnt during training (Seaman & Eves, 2010). But Seaman and Eves (2010) in their study suggest that many managers do not have sufficient awareness or the correct attitude towards food hygiene training to promote an effective learning environment within the workplace. The analysed Guidelines exactly describe the content of training programmes for viral control. Periodic assessment of the effectiveness of training and instruction programmes should be made, as well as routine supervision and checks to ensure that procedures are being carried out effectively. That is why the managers and supervisors of food processes also need to have necessary knowledge of food hygiene principles and practices. Training and instruction has to be periodically renewed by all food operators and in accordance with the observed weaknesses (Table 1) more emphasis on refresher training should be assigned. For these types of activities it is desirable to outsource relevant experts. Public training courses can be organised for consumers too, because consumers have inadequate knowledge about the measures needed to prevent food-borne illness in the home and from shop to home (Jevšnik et al., 2008a). For this reason food safety public health campaigns to enhance household food safety awareness would be a suitable solution. The US national public campaign Fight BAC® (FIGHT BAC®, 2009) and the New Zealand national public Food Safe Partnership campaign (NZFSA, 2006), were established for this purpose, with the aim of promoting food safety in consumers and educating them on how to handle and prepare food safely.

3. Conclusion

There are still too many cases, outbreaks and epidemics of food-borne illness or just malpractices leading to food contamination. The food chain is only as strong as its weakest link and the responsibility for food safety lies not only with producers and processors of food, but also on governments and the consumers themselves. The role of consumers in the occurrence of food-borne illness is substantial, because consumers are the final line of defence and hence play an important role in the prevention of food-borne illness. The reasons for the increase in the incidence of food-borne diseases are most likely the improved diagnostic assays that have enhanced detection of some viral groups and changes in food processing and consumption patterns that have lead to the worldwide availability of high risk food. These conditions are creating an environment in which both known and new food-borne diseases can become prevalent. For this reason food regulation in the main is aimed at protecting the consumer's health, increasing economic viability and engendering fair trade in foods within and between nations.

The result of the five conceptual elements that highlight the efficiency of the Guidelines analysed is reflected through the strengths, weaknesses, opportunities and threats identified. The main advantage of this document for the control of viruses in foods is that it is one of the first documents dealing with the current situation in viral area. But the document has some crucial limitations regarding terminology, detection methodology and transparency. The main weakness is connected with the fact that some important information is integrated in the document in different sections, which detracts from the clarity of the communication. Terminology has already been shown to be one of the main barriers to food safety in the food business. The document is not consistent in offering solutions on exactly how relevant activities should be applied. It is not based consistently on the same principle because, the

document specifies sometimes what and sometimes why. This can be explained by the lack of routine detection methods for majority of viruses. Since the code was transformed to guidelines in the title, one would expect that the annexes could be easily included in the general text under the relevant sections. This would bring better transparency to the document which would be then more easily understood by relevant professionals with different level of knowledge and skills in food supply chain.

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2.1.3 Molekularna karakterizacija norovirusov, ugotovljenih v mediteranskih klapavicah (*Mytilus galloprovincialis*) iz slovenskih školjčišč

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Norovirusi so eden izmed glavnih povzročiteljev virusnega gastroenteritisa pri ljudeh. Med živili, ki so pogosto povezana z izbruhi svetovnih razsežnosti, so tudi školjke. V raziskavi, ki je bila opravljena v letih 2006–2008 ter 2010–2012, smo ugotavljali prisotnost norovirusov v dvesto osemtridesetih (238) vzorcih mediteranskih klapavic (*Mytilus galloprovincialis*) iz vseh treh slovenskih školjčišč: Seče, Strunjana in Debelega rtiča. Z metodo RT-PCR v realnem času smo ugotavljali prisotnost norovirusov genske skupine GI in GII. Med letoma 2006–2008 je bila ugotovljena prisotnost norovirusne RNA v 9,1–24,6 %, med leti 2010–2012 pa v 12,5–22,2 % vzorcev. Stopnja identičnosti zaporedja nukleotidov odseka RdRp norovirusnega genoma v genski skupini GII je bila 78,8–81,0 % (znotraj skupine GII.P4 92,1–99,6 %), pri genski skupini GI 100 %, med genskima skupinama GI in GII pa 58,4–60,2 %. Devet (9) vzorcev školjk smo genotipizirali v skupino GII.4, dva (2) v skupino GI.P2 in enega (1) v skupino GII.P21. Ugotovljeni sevi so filogenetsko zelo podobni sevom kliničnih vzorcev ter tudi vzorcem voda, ne glede na svetovni geografski izvor. Glede na veliko podobnost nukleotidnih zaporedij naših sevov z nekaterimi sevi iz svetovne banke podatkov domnevamo, da školjke niso onesnažene le z vodo iz območij školjčišč, ampak k temu doprinesejo najverjetneje tudi odplake velikih transportnih ladij, ki priplujejo iz oddaljenih področij širom po svetu.

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Molecular characterisation of noroviruses detected in mussels (*Mytilus galloprovincialis*) from harvesting areas in Slovenia

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SUMMARY

Noroviruses are a leading cause of viral gastroenteritis in humans and are responsible for many outbreaks worldwide. Mussels are one of the most important foodstuffs connected with norovirus outbreaks, also resulting in multinational dimensions. Two hundred and thirty-eight (238) samples of mussels (*Mytilus galloprovincialis*) were collected in periods between the years 2006-2008 and 2010-2012 to study the prevalence of noroviruses (NoVs) from harvesting areas along the Adriatic coast of Slovenia. Between 2006 and 2008, 9.1% to 24.6% of mussel samples tested by specific GI and/or GII real-time RT-PCR methods were found to be positive for NoVs while between 2010 and 2012 the percentage of NoV positive samples varied from 12.5% to 22.2%. At the nucleotide level within the *RdRp* gene fragment the genetic diversity of NoVs detected in mussels ranged between 78.8-81.0% nucleotide identity among GII strains (92.1-99.6% within the GII.P4 genotype), 100% nucleotide identity among GI and 58.4-60.2% among GI and GII strains. Nine of the NoV strains detected from mussels were genotyped as GII.4, while two samples were within GI.P2 and one was a positive sample within genotype GII.P21. This study confirmed that mussels are a potential source of the NoV infection. The detected NoVs share the same topology on the phylogenetic tree within the NoV strains detected in water samples and human patients, not only from Slovenia but also from many different countries worldwide. We can assume that mussels in harvesting areas are not only contaminated from the surrounding area but also by contaminated water and sewage from large transport ships, which are regularly present in the area.

KEY WORDS: Mussels, Norovirus, RT-PCR.

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INTRODUCTION

Shellfish are a major risk factor for food-borne outbreaks and are often associated with outbreaks of viral disease (Koopmans and Duizer, 2004; Le Guyader *et al.*, 2008; Guillois-Becel *et al.*, 2009, Suffredini *et al.*, 2011). Although harvested shellfish should be in a clean environment, faecal pollution from different sources of discharges, disposal from boats, ballast water and contaminated river discharges can contaminate the areas (Santo Domingo and Edge, 2010). Contamination of shellfish with NoVs can occur when living in polluted water. As mussels feed and so ingest viruses due to their filter feeding activity, shellfish serve as a vehicle of NoV infections (Lees, 2000, Le Guyader *et*

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al., 2003). The main factors associated with an elevated incidence or persistence of NoV contamination beside winter months are also increased rainfall and low temperatures (Campos and Lees, 2014).

NoVs are classified into five genogroups (GI to GV) of which GI and GII are most commonly associated with human infections. Genogroups are subdivided into different genotypes (Kageyama *et al.,* 2004). Genotype determination and detection of variants within genotype are useful to monitor the global spread of noroviruses (Ramirez *et al.,* 2008). In Slovenia a high prevalence of enteric viruses was detected in water sources, groundwater and surface water. Among those, noroviruses (GI and GII) were also present in environmental samples (Steyer *et al.,* 2011). The epidemic data caused by noroviruses in Slovenia have been available since the year 2000 (Poljšak Prijatelj *et al.,* 2001), but the source of the infection was usually not identifiable (Zimšek *et al.,* 2003; Štrumbelj *et al.,* 2003; Grmek Košnik *et al.,* 2007). In a study conducted in Slovenia, NoVs were detected from samples of surface and groundwater with genetic characteristics reflecting those from human clinical cases (Steyer *et al.,* 2011).

The aim of our study was to examine the mussels for the presence of NoVs, to establish their prevalence from harvesting and non-harvesting area in Slovenia and to compare the NoV sequences obtained from mussels with those obtained from human and water sources in Slovenia and worldwide whether there might be a linkage.

MATERIAL AND METHODS

Two hundred and thirty-eight (238) samples of Mediterranean mussels (*Mytilus galloprovincialis*) were tested for the presence of NoV. Samples were collected in two periods from 2006 to 2008 and from 2010 to 2012 at three harvesting areas in Slovenia: Seča, Strunjan and Debeli Rtič (Figure 1). The sampling scheme from the Seča harvesting area, where 98 samples were taken, was as follows: 23, 25, 23, 14, 10 and 3 samples per year. From the Strunjan harvesting area 80 samples were taken (10, 20, 21, 12, 12, 5) and from Debeli Rtič 54 (11, 16, 13, 9, 5, 0), respectively (Table 1). Five samples from wild

FIGURE 1 - *Location of Slovenia on a map of Europe (upper part of the picture), Slovenian coastal waters with the three harvesting areas of Mediterranean mussels (Mytilus galloprovincialis) - Seča, Strunjan and Debeli Rtič and one non-harvesting area near Piran. The coastline of Slovenia is 46.6 km long.*

living mussels were collected in the area near Piran (in 2007 two, and in 2008 three samples) and one sample collected near Strunjan in 2008 were also included in our study. Mussels were collected throughout the whole year to cover all the months. For each sample, the digestive glands of eight mussels were removed from the body and homogenized with *minimum essential media* - MEM (Gibco®, Invitrogen, USA) in the ratio 1:2 to obtain an emulsified suspension. RNA was isolated from digestive gland suspensions using RNeasy® Plus Mini Kit (Qiagen, Germany) according to the manufacturer's instructions.

Real-time RT-PCR was performed with the primer pair COG1R/COG1F and probe RING1 for the specific detection of NoV from genogroup GI and primer pair COG2R/COG2F, probe RING2 for GII, targeted sequences at the ORF1-ORF2 junction region (Kageyama *et al.,* 2003), which is the most conserved region in the NoV genome (Katayama *et al.,* 2002).

One step real-time RT-PCRs were performed on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, USA) and were as follows: reverse transcription at 50°C for 15 min and denaturation at 95°C for 2 min, following 45 cycles of denaturation at 95°C for 15 s and annealing extension at 56°C for 45 s.

TABLE 1 - *Mediterranean mussels tested for NoVs in the years from 2006 to 2008 and from 2010 to 2012.*

Samples		*Detected genogroup by real-time RT-PCR*			*N. of samples positive by real-time RT-PCR (%)*	*N. of samples negative by real-time RT-PCR*	*Samples positive by real-time RT-PCR regarding sampling area*				**Number of samples positive by RT-PCR*
Year	*All*	*GI*	*GII*	*GI and GII*			*Seča*	*Strunjan*	*Debeli Rtič*	*Piran*	
2006	44	0	3	1	4 (9.1)	40	2/23	1/10	1/11	0	1
2007	63	0	4	5	9 (14.3)	54	1/25	4/20	3/16	1	6
2008	61	1	6	8	15 (24.6)	46	2/23	5/21	7/13	1	9
2010	35	0	4	2	6 (17.1)	29	0/14	4/12	2/9	0	3
2011	27	0	6	0	6 (22.2)	21	3/10	2/12	1/5	0	1
2012	8	0	1	0	1 (12.5)	7	0/3	1/5	0/0	0	0
Total	238	1	24	16	41 (17.2)	197	8/98 (8.2%)	17/80 (21.2%)	14/54 (25.9%)	2/6 (33.3%)	20

*Only samples positive by real-time RT-PCR were tested by conventional one-step RT-PCR.

The RNA extracted from samples was amplified again in a conventional one-step RT-PCR using SuperScript® Onestep RT-PCR with Platinum® *Taq* (Life Technologies, Germany) and primer pair JV12Y/JV13I (Vennema *et al.*, 2002) for targeting part of the polymerase gene (ORF1). The cycling conditions were as follows: reverse transcription at 45°C for 45 min and denaturation at 94°C for 2 min following 50 cycles of denaturation at 94°C for 30 s; annealing at 40°C for 1 min and extension at 68°C for 2 min with final extension 68°C for 7 min.

Results were interpreted on the basis of the specific size of RT-PCR products (326 bp) by 1.8% agarose gel electrophoresis and a 100 bp DNA ladder. The specificity of the RT-PCR products was confirmed by direct Sanger sequencing. The obtained sequences have been deposited in GenBank with accession numbers JN040477-JN040486 and KF953824-KF953825.

Twelve (12) NoV sequences obtained from mussels (Figure 2) in this study were compared with 18 sequences previously determined and detected from environmental and clinical human samples published by Steyer *et al.* (2011). In addition, reference sequences for specific norovirus genogroup/genotype were downloaded from GenBank and, for comparison some norovirus sequences from GenBank showing the highest identity after BLAST search for sequences obtained in this study were selected for the analysis. Multiple sequence alignment and phylogenetic analysis was performed in MEGA software version 5 with ClustalW alignment algorithm, using the default parameters. The evolutionary relationship of taxa was inferred using Neighbor-Joining method and evolutionary distances were computed using p-distance method. Taxa clusters were tested with bootstraps of 1000 replicates. Norovirus genotype for the sequences from this study was determined online with the Norovirus Automated Genotyping tool (Kroneman *et al.*, 2011).

RESULTS

Real-time RT-PCR and conventional one-step RT-PCR

In total 41 samples (17.2%) tested positive for NoVs (only GI or GII or both in the sample) by the real-time RT-PCR method. The obtained cycle threshold (Ct) values for positive samples were from Ct 33 to 41. From 41 samples positive by real-time RT-PCR, a specific RT-PCR product of the polymerase gene (ORF1) was successfully amplified from only 20 samples (Table 1). Noroviruses of GI and GII were frequently detected as a simultaneous contamination between 2006 and 2010, while in 2011 and 2012 only seven positive samples containing NoVs from genogroup GII were detected. Between 2006 and 2008, 9.1% to 24.6% of mus-

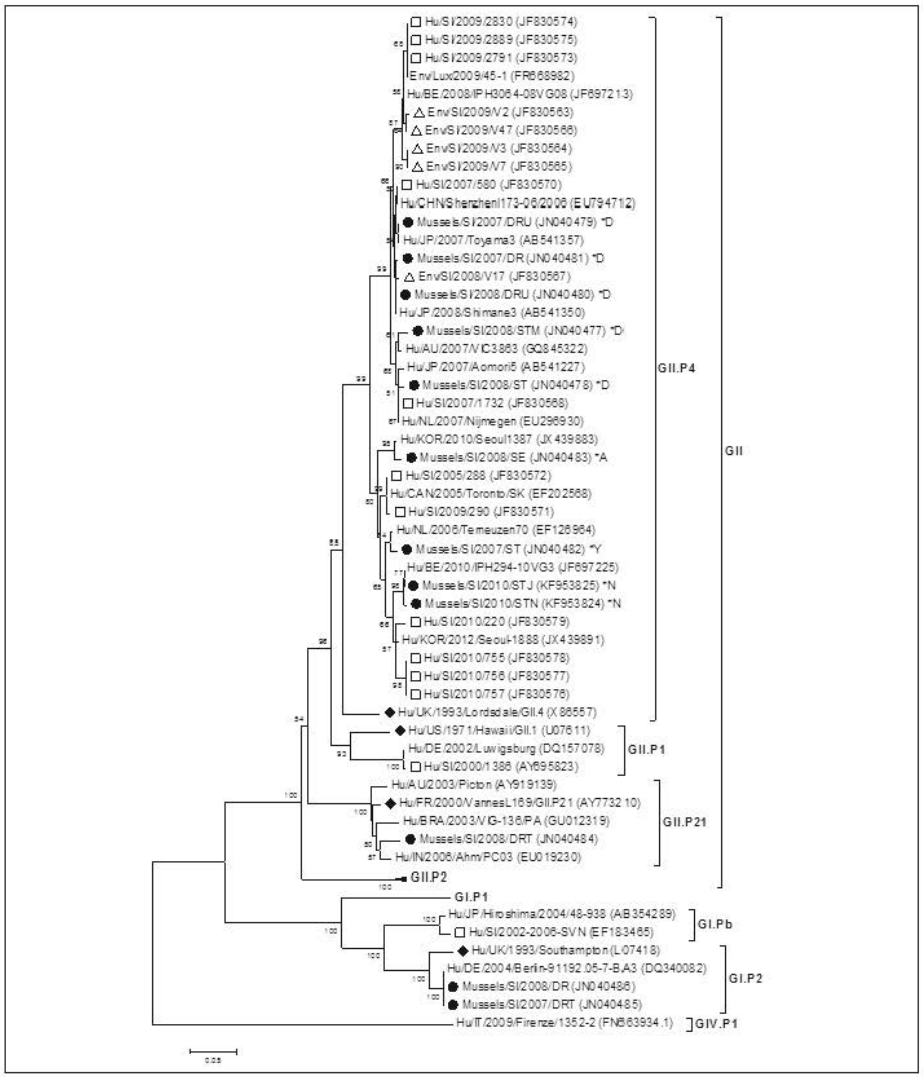

FIGURE 2 - *Phylogenetic comparison of NoV sequences detected from Mediterranean mussels (●) in Seča, Strunjan and Debeli Rtič (this study), surface water samples (Δ) and NoV sequences obtained from people suffering from gastroenteritis (□) in Slovenia (Steyer et al., 2011) and 21 closely related sequences obtained from the GenBank. Reference strains (◆) are marked with scale below (e.g. Norwalk is reference strain for I.1 genotype). The genetic relationship is based on a neighbouring joining method of the 279- nucleotide sequence of the polymerase gene. Variants of GII.4 genotypes are marked with asterisks and a letter: variant Den_Haag_2006b (*D), Apeldoorn_2007 (*A), Yerseke_2006a (*Y), New_Orleans_2009 (*N).*

sel samples tested positive, while between 2010 and 2012 the percentage of NoV positive samples was from 12.5% to 22.2% (Table 1).
In total, 98 samples from the Seča harvesting area were examined with eight of them (8.2%) found to be positive. The percentage of positive samples varied from 4.0% to 30.0% in the period from 2006 to 2011, while in 2010 and 2012 no NoV positive samples were detected. From 80 samples originating from the Strunjan harvesting area, NoVs were detected in 17 samples (21.2%) with the prevalences from 10.0% in 2006 to 33.3% in 2010. Among 54 samples from the Debeli Rtič harvesting area 14 (25.9%) samples were contaminated with NoVs. The percentage of positive samples ranged from 9.1% to 53.8% in 2008 while in 2012 no sample from that area was analysed. Wild mussels were examined from non-harvesting areas, five samples from the area near Piran and one sample from the area near Strunjan. NoVs were detected in only two samples (33.3%) from the area near Piran.

Comparison of NoV strains detected in mussels, water and clinical samples in Slovenia and globally

From 20 RT-PCR positive samples only 12 sequences of 279 nucleotides were obtained. Nine sequences (75.0%) were classified as genotype GII.4, one (8.3%) as GII.P21 and two (16.7%) as GI.P2 (Figure 1). High genetic diversity with 78.8-81.0% nucleotide identity among GII strains (92.1-99.6% within the GII.P4 genotype), 100% nucleotide identity among GI and 58.4-60.2% among GI and GII strains was detected.
The polymerase gene nucleotide sequences obtained from this study were compared with sequences already detected in Slovenia and sequences available in GenBank. The phylogenetic analysis showed close relationships among NoV sequences detected from Slovenian mussels to sequences from clinical and environmental samples in Slovenia (Steyer *et al.*, 2011) and sequences found worldwide. NoV strains detected in mussels harvested at Debeli Rtič in 2007 and 2008 (Mussels/SI/2007/DRU, Mussels/SI/2008/DRU and Mussels/SI/2007/DR) show 99.3-99.6% sequence similarity to clinical strain (Hu/SI/2007/580) and strain (Env/SI/2008/V17) detected in 2008 from surface water in strains Slovenia, whilst strains Mussels/SI/2008/DRU was 100% identical with many strains in GenBank (representative Hu/JP/2008/Shimane3) from Japan, China and Taiwan. Isolate Strain Mussels/SI/2007/DRU shares 99.3 and 99.6% sequence similarity with strains from Japan (Hu/JP/2007/Toyama3) and China (Hu/CHN/2006/Shenzhen173-06), respectively. The NoV strain s isolated from surface water in Slovenia (Env/SI/2009/V3) shares 98.9-99.3% sequence similarity with two strains from Luxembourg and Belgium (Env/Lux/2009/45-1 and Env/BE/2008/IPH3064-08VG08) and are at the amino acid level 100% identical (data not shown). Two strains from the Strunjan harvesting area (Mussels/SI/2008/STM and Mussels/SI/2008/ST) share 98.2-98.9% sequence similarity with the strain (Hu/SI/2007/1732) isolated from a patient in Slovenia and 98.2-99.3% sequence similarity with strains from Japan, Australia and Netherlands (Hu/JP/2007/Aomori5, Hu/AU/2007/VIC3863, Hu/NL/2007/Nijmegen).
The only strain detected from the Seča harvesting area (Mussels/SI/2008/SE) shares 96.4-96.8% sequence similarity with two human strains detected in 2005 in Slovenia (Hu/SI/2005/288 and Hu/SI/2005/290) and even higher (98.9%) similarity with a number of Korean strains (representative Hu/KOR/2010/Seoul1387).
Three strains from the Strunjan harvesting area (Mussels/SI/2010/STN, Mussels/SI/2010/STJ and Mussels/SI/2007/ST) share 96.1-97.1% sequence similarity with four strains detected from patients with gastroenteritis in Slovenia in 2010. The latter strain shows higher sequence similarity (98.9%) to the human (clinical) strain (Hu/NL/2006/Terneuzen70) from the Netherlands. The human strain detected in Belgium (Hu/BE/2010/IPH294-10VG3P4) and strain from the Strunjan harvesting area (Mussels/SI/2010/STN) share 99.3% nucleotide identity.
The strain (Mussels/SI/2008/DRT) from the Debeli Rtič harvesting area was not related to any NoV strain previously detected in Slovenia; it is more similar to Indian and Brazilian strains (Hu/IN/2006/Ahm/PC03/GII.P21, Hu/BRA/2003/VIG-136/PA). Two strains detected in 2007 and 2008 from the Debeli Rtič harvesting area (Mussels/SI/2007/DRT and Mussels/SI/2008/DR) are 100% identical to each other and show the closest (86%) nucleotide identi-

ty to the human strain (Hu/SI/SI-2002) detected in Slovenia and are 100% homologous with the German human strain (Hu/DE/2004/Berlin/91192.05-7-BA3) (Figure 2). The sequence analysis of the polymerase gene region of NoV strains detected in mussels harvested in Slovenian coastal waters revealed five different variants of GII.P4 strains. In 2007 the GII.P4 Yerseke_2006a variant was detected in Strunjan (Mussels/SI/2007/ST). In 2007 and 2008 five strains were classified as Den Haag 2006b variant; two of them originated from the Strunjan harvesting area (Mussels/SI/2008/STM, Mussels/SI/2008/ST) and three from the Debeli Rtič harvesting area (Mussels/SI/2007/DRU, Mussels/SI/2007/DR and Mussels/SI/2008/DRU). In 2008 the strain from the Seča harvesting area (Mussels/SI/2008/SE) was classified as the GII. P4 Apeldoorn_2007 variant. In 2010 two identical strains detected in mussels from the Strunjan harvesting area (Mussels/SI/2010/STN and Mussels/SI/2010/STJ) were classified as the GII. P4 New_Orleans_2009 variant (Figure 2).

DISCUSSION

Mussels were sampled throughout the year during a five-year period to obtain information on the NoV contamination of shellfish harvested in Slovenia, and also to determine sequence similarities among the strains isolated from shellfish with other NoV strains isolated in Slovenia. All the samples originating from Slovenian producing areas are classified as potentially contaminated (class B), meaning that mussels have to undergo a process of depuration before they can be sold on the market.

However, shellfish originating from harvesting areas in the Italian part of the Adriatic sea are mainly classified as class A (Delibera Friuli 124/2010 Allegato A). The percentage of contaminated mussels from the Slovenian harvesting areas was lower (17.2%) compared to Italian data from 2011, where contamination was 34.4% (Suffredini *et al.*, 2011). Another study in Italy carried out from 2011 to 2012 showed 6.8% samples to be positive for NoV GI and 11.9% to be positive for GII (Fusco *et al.*, 2013). Results of the study carried out in France also differ from year to year - results from a three-year study showed 35.0% of contaminated mussels (Le Guyader *et al.*, 2000). However, reports from 2010-2011 showed a surprisingly low contamination of shellfish in the market - only 9.0% (Schaeffer *et al.*, 2013).

Among mussels collected in Slovenian coastal waters, contamination with NoVs was highest at the Debeli Rtič harvesting area (25.9%; 14/54). In Strunjan, 21.2% (17/80) of mussels tested positive for NoVs whereas in Seča contamination was detected in only 8.1% (8/98) of the sampled mussels (Table 1). It is difficult to evaluate the exact degree of wild mussel contamination because only a small number of wild samples (6) were collected.

By real-time RT-PCR, on average 17.2% (41/238) of mussels were found contaminated with NoVs, 2.4% (1/41) of them were classified as GI, 58.5% (24/41) were classified as GII, while in 39.0% (16/41) both genogroups (GI and GII) were detected (Table 1). A study investigating the characteristics of infections caused by NoV in Slovenia over a period from 2000 to 2007 also showed that the majority of the outbreaks were caused by NoV GII (88.2%), while 10.9% of the outbreaks were caused by NoVs GI (Zimšek Mijovski, 2010). Among all gastroenteritis outbreaks, 89.8% were caused by NoVs and 70.3% of outbreaks occurred in kindergartens, schools, nursing homes and hospitals. The main source of infection was direct contact from person to person. Only in 8.0% of cases was food the suspected vehicle, whilst water was the suspected source in 2.2% (Zimšek Mijovski, 2010). To the authors' knowledge it has not yet been reported that shellfish are a source of outbreaks in Slovenia.

With a rate of 68.2%, genotype II.4 is the leading strain causing outbreaks, not only in Slovenia (Zimšek Mijovski, 2010) but also worldwide with a rate of more than 80.0% (Lindesmith *et al.*, 2012). Also in our study the predominant genotype detected in mussels was GII.P4 75.0% (9/12), followed by GI.P2 16.7% (1/12) and GII. P21 8.3% (1/12). Results of a large comprehensive study showed that genotype II.4 was responsible for most of the human outbreaks (52.0%), followed by genotype II.b and II.7. The proportion of genogroup I was higher in bivalve molluscs than in infected humans. The two most frequently detected genotypes in foodborne ou-

tbreaks were I.2 and I.4 (Verhoef *et al.*, 2010). The rapid evolution of GII.4 noroviruses results in the successive emergence of new variants that have been observed since 2002 (Siebenga *et al.*, 2008). In 2006 the GII.4 variants in Europe and the USA were Laurens (2006a) and Minerva (2006b) (Siebenga *et al.*, 2008). In October 2009 a new variant, GII.4.2009 New Orleans, emerged and became the predominant outbreak strain, while Minerva circulated at lower levels (Vega *et al.*, 2011). The results of this study are in agreement with the appearance of the circulating strains - variant Den Haag 2006b was circulating in the period 2007-2009 (Eden *et al.*, 2010), while New Orleans 2009 was predominant in 2009-2012 (Vega *et al.*, 2011). During 2012 it was replaced by Sydney 2012 (Eden *et al.*, 2014), which is an emergent GII.4 variant detected in Slovenia in August 2012 from a child suffering from diarrhoea (unpublished data). The authors assume that in the (near) future this strain might also be detected in mussels from Slovenian coastal waters. For a better overview of the sequences, we would have to include a higher number of the sequences.

Sequence similarity among strains detected in mussels, isolated from sources of drinking and surface water and human clinical samples from Slovenia, was 95% at the nucleotide level and 100% at the amino acid level (data not shown). On the other hand, an even greater percentage of similarity was shown within the human strains worldwide. A sequence analysis showed an up to 100% match at the nucleotide level with strains isolated from around the globe (Japan, China, Korea, India). A high variability of the sequences was probably due to the small number of sequences that were obtained over a five-year period. Surface water samples in Slovenia were contaminated in 41.3% (26/63) of cases with NoV GII, in 33.3% (21/63) with GI, while drinking water had NoV in 2.2% of samples (Steyer *et al.*, 2011). In regard to the high contamination of surface water samples, it could be expected that even in shellfish, due to their filter feeding activity, the percentage of contamination would be similar, if not even higher. It has already been reported that after the treatment process wastewaters are also the main source of surface water contamination (Simmons and Xagoraraki, 2011). In the past municipal wastewater from the towns of Koper, Piran and Izola were discharged into the sea via the Rižana river and submarine pipes located 3.5 km and 300 m from the coast, respectively (Malačič *et al.*, 2000). In 2009, two renovated wastewater treatment plants in Koper and Piran started to operate but systematic monitoring of NoVs had not been carried out. Wastewaters from Izola are now collected in the Koper treatment plant and discharged into the Rižana river. The percentage of contaminated mussels in our study varied from 9.1% to 24.6% and did not differ greatly throughout these periods (Table 1). Many other unidentified sources may contribute to the input of waste into the sea and could be one of the main sources of seawater contamination with enteric viruses. Although NoV outbreaks are reported year-round they peak during months of cold weather (Mounts *et al.*, 2000). The occurrence of NoV human infections in Slovenia is not as seasonal as in other European countries. However, half of human infections appear in the colder months of the year (Zimšek Mijovski, 2010). In this study contaminated mussel samples originated in the winter months. Only one contaminated sample dated from July (data not shown). It would be interesting to monitor enteric viruses, at least in the rivers Rižana, Drnica and Badaševica, which flow into the Adriatic Sea, to evaluate a possible correlation between enteric virus detection and concentration in rivers and consequently the detection rate of those viruses in mussels.

If such a correlation is present, it would be necessary to implement preventive measures to decrease the possibility of enteric viruses being released into rivers and consequently to the sea. Phylogenetic analysis of the present study revealed that strains isolated from mussels (harvested in Debeli Rtič from 2007 and 2008) showed the highest (99.6%) nucleotide identity with only one strain (Env/SI/2008/V17) detected from surface water in Slovenia. Similarity among GII strains isolated from mussels and human strains was lower, 96.1-98.9%, while among GI sequence similarity was 86.0%. The analysis was limited to only 12 mussel strains. In order to obtain a better overview more strains should be included in the analysis.

Genetically identical strains (Mussels/SI/2008/DR and Mussels/SI/2007/DRT) detected in dif-

ferent sampling periods were also found, reflecting the longer retention of a certain strain on the site, or contamination with identical viral strains in both sampling periods. The first hypothesis is more likely, since some studies on contaminated groundwater suggest that NoV (GI) can remain detectable for more than three years (Seitz *et al.*, 2011).

In the harvesting area near Debeli Rtič, a higher percentage of NoVs (25.9%) was detected. The reason for that could be found in the location, as this area is at the most northern point where the main Adriatic Sea current flows. The fact that the NoV strains isolated from Mediterranean mussels showed a high relatedness to the GII.4 clinical strains, strains isolated from waters in Slovenia and strains from the world, indicates that shellfish harvesting areas are contaminated with water and sewage from surrounding areas.

The authors assume that the higher levels of contamination in Debeli Rtič can also be attributed to intensive shipping in this area, discharges of wastewaters, the river estuary and the main sea current. The consumption of shellfish from Slovenian harvesting areas presents a health risk to consumers. Although the majority of the shellfish is cooked, there is a need to develop a risk assessment, taking into account all the possible factors that could have an impact on preventing possible outbreaks caused by NoV contamination.

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2.1.4 Virusno onesnaženje v proizvodni verigi školjk na slovenski obali

Ambrožič M., Henigman U., Jevšnik M., Kirbiš A., Raspor P. 2016. Viral contamination in mussel production chain on the Slovenian coastline. Slovenian Veterinary Research, sprejeto v objavo.

Izjavljam, da je verzija v doktoratu identična s sprejeto verzijo.

Med živili, ki so pogosto povezana z norovirusnimi izbruhi in izbruhi hepatitisa A svetovnih razsežnosti, so tudi školjke. V okviru uradnega nadzora se v Sloveniji od leta 2013 vzorči školjke, pri katerih se ugotavlja prisotnost norovirusov in virusov hepatitisa A. Prisotnost norovirusne RNA je bila ugotovljena med 25 % testiranimi vzorci v letu 2014 do 40 % v letu 2015. Prisotnosti virusov hepatitisa A ni bilo možno potrditi v nobenem od analiziranih vzorcev, kar povezujemo z nizkim številom okužb pri ljudeh v Sloveniji. Poleg ugotavljanja prisotnosti norovirusov in virusov hepatitisa A v školjkah smo opravili tudi pol strukturirane intervjuje s slovenskimi školjkarji. Kvalitativna analiza je razkrila, da se slovenski školjkarji zavedajo možnih tveganj na področju gojenja školjk, ampak samo v povezavi s prisotnostjo bakterije *Escherichia coli* kot indikatorskega mikroorganizma in biotoksinov. Kljub ugotovljeni prisotnosti norovirusne RNA v školjkah, prisotnih na slovenskem tržišču, lokalni školjkarji ne prepoznajo virusov kot dejavnikov tveganja, pomembnih za zagotavljanje varnosti živil. Ugotovitve kažejo, da se lokalni školjkarji zavedajo možnih mikrobioloških tveganj na področju gojenja školjk. Vendar pa kljub temu ne prepoznajo virusov kot možnih dejavnikov tveganja, kar izpostavi pomembnost kontinuiranega, rednega usposabljanja in izobraževanja pri obvladovanju virusnih okužb v proizvodni verigi školjk.

Dovoljenje za objavo članka Ambrožič in sod. (2016) v tiskani in elektronski obliki je v Prilogi D.

Viral contamination in mussel production chain on the Slovenian coastline

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Abstract

Noroviruses (NoVs) and Hepatitis A virus (HAV) are significant agents of foodborne human viral illness worldwide, both as agents of greatest concern from the consumption of shellfish. In the framework of official national monitoring shellfish samples have been collected since 2013 to determine the spread of NoVs and HAV. Contamination of shellfish samples with NoVs varied from 25% in year 2014 to 40% in year 2015. HAV was not found in any of the analyzed samples, which correlates with the low number of human infections in Slovenia. Alongside official surveillance, semi-structured interviews were carried out with local shellfish farmers regarding this subject. The qualitative analysis highlighted that Slovenian shellfish farmers are aware of food safety hazards, especially associated with hazards to primary production, but only in the context of *Escherichia coli* as an indicator microorganism, and not in the concept of viral food safety. Despite detected foodborne viruses in shellfish on the Slovenian market, local farmers are not aware of or do not recognize foodborne viruses as distinctive food safety hazards. It can be concluded that local farmers possess knowledge and information of critical points in the mussels food supply chain connected to food safety in general. However, in particular, they are not familiar with viruses that represent barrier and consequently critical point to food safety management practices. Training and education on all important aspects of viral food safety according to the current recommendations is strongly recommended for all actors within the shellfish supply chain.

Keywords: mussel farmer, Slovenia, official control, food safety, foodborne viruses, semi-structured personal interviews

1 Introduction

Mariculture is a traditional activity in the Primorska region. Mediterranean mussels (*Mytilus galloprovincialis*) are the main harvested species and, of a smaller quantity, also warty venus (*Venus verrucosa*). Mussel farming takes place in a standard manner in lines of floating buoys linked together, with longline nets hung from them. In Slovenia, within 46.6 km of coastline there are three official harvesting areas of Mediterranean mussels: Seča, Strunjan, and Debeli rtič, with 56 registered shellfish farmers on a total area of 46 ha. In 2013, 231 persons were involved in aquaculture activities in Slovenia, and only 39 persons were involved in marine fish and shellfish farming. The aquaculture sector in Slovenia is mainly characterized by small self-employed family farms, most of which have one employee, and some are assisted by unpaid family members. Total shellfish production in 2013 was 311 tonnes, and current production covers mainly the needs of the domestic market. The major cultured shellfish species, Mediterranean mussel, accounts for 83 percent of total mariculture production in Slovenia (1, 2). Next to that there were about 153 tons of imports of mussels in Slovenia but only 23 tons of export, what is a negligible quantity in alimentation compared to consumption of fresh fruit and vegetables, which are also recognized as vulnerable to viral contamination. Yet, the level of shellfish consumption by country is very different. In some countries, the mussel consumption per capita is over 3 kg per year, while it is not even part of the local diet in others (3). Per capita consumption of fresh fruit in Slovenia was in 2013 75 kg and 73 kg of fresh vegetables. However, there were no contaminated samples with NoVs or HAV of fresh produce found within Slovenian national monitoring (27). Shellfish specialties are commonly part of the culinary and gastronomic specialties only along the Slovene coast and are generally prepared and served in restaurants.

The filter-feeding nature of bivalves and the traditional way of consuming them (often raw or slightly cooked) make shellfish one of the most common vehicles of viral foodborne illness. Shellfish are filter-feeding animals, which ingest and accumulate human pathogens (5). Lees (6) reported that shellfish grown in sewage-polluted waters tend to bio-accumulate environmentally stable enteric viruses. Processing interventions such as depuration do not eliminate viral particles (7), and food consumption practices increase the health risk related to shellfish consumption. The increasing amount of data on virus detection in shellfish (8, 9, 10) and shellfish-borne viral outbreaks (11) indicates the necessity of a constant surveillance system in European countries, including Slovenia (12). The management of the harvesting areas continues to rely uniquely on bacterial standards, such as *Escherichia coli*, despite the proven fact of being unreliable tools to indicate the viral presence in harvesting areas or to control the efficiency of the process, such as depuration (13, 14). From a virological point of view, shellfish safety continues to be a sanitation challenge and to protect consumers the EU strives towards establishing legislation on this matter.

With better diagnostic technology and investigative epidemiology, it is now accepted that enteric viruses are major contributors to foodborne disease as well. Enteric viruses are transmitted through contaminated food, but also in combination with person-to-person contact or through environmental contamination. They have been increasingly recognized a significant cause of foodborne disease, despite the measures already in place, mainly targeted at reducing bacterial contamination, because of the increasing consumption of ready-to-eat foods, raw and/or minimally processed shellfish, fruits, and vegetables. This is because products are often imported from areas lacking strict hygienic measures, they are often eaten uncooked, and they often come into contact with potentially contaminated animal manure, water, ice, human hands and surfaces from the "farm-to-table" continuum (10). Most

foodborne viruses are more resistant (15) than bacteria to commonly used control measures, (e.g. refrigeration, freezing, pH, drying, UV radiation, heat, pressure, disinfection, etc.). There are currently no effective, realistic and validated risk management options to eliminate viral contamination prior to consumption without changing the normally desired characteristics of the food. Because of concerns about virus persistence during food processing, effective control strategies need to focus on the prevention of contamination. From the limited available information, foodborne viruses have a low infectious dose and are dispersed in stool or emesis in high numbers. Only a few viral/infectious particles are needed to cause an infection that may lead to illness (10, 15, 17).

Shellfish aquaculture is a marine-based industry that is affected by other land users such as tourism, recreation, forestry, agriculture, and urban development. In many cases, the public is unaware of the detrimental impact their activities have on the aquaculture sector and, consequently, also on shellfish food safety. Food safety embraces the absence or acceptable and safe levels of contaminants, adulterants, naturally occurring toxins or any other substances that can make food dangerous to human health. Microbial food safety is considered a significant public health issue but historically has focused mostly on the control of bacterial contamination; however, enteric viruses have been increasingly recognized as an important cause of foodborne disease, and control measures are being developed (16, 17). The food supply chain from stable to table includes activities such as production, processing, distribution, retail, packaging and labeling of foodstuffs, which are governed by a mass of laws, regulations, codes of practice and guidance. Nowadays, the distance that food travels from producer to consumer has increased as a result of globalization in the food trade. Moving these food products safely and efficiently from farm to fork requires a highly coordinated series of links in a long chain of trading partners. Food miles, as a term that refers to the distance food is transported from where it is grown or raised to where it is purchased by a consumer, is part of the broader issue of sustainability that deals with a large range of environmental, social and economic issues. Therefore, keeping safety and quality along the food supply chain has become a significant challenge, whereas good traceability systems, defined as the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution (18), help to minimize the production and distribution of unsafe or poor quality products.

2 Epidemiology of foodborne viruses

Although shellfish consumption can contribute to a healthy diet they are often associated with outbreaks of foodborne disease. Viral foodborne outbreaks associated with shellfish consumption have occurred in many countries (11) despite existing strategies to prevent contamination. They are often attributed to water contamination by sewage and/or during processing and serving. According to epidemiological evidence, NoVs as the predominant agents of nonbacterial gastroenteritis in humans along with Hepatitis A virus (HAV), both as agents of greatest concern from the consumption of shellfish, are important agents of foodborne human viral illness worldwide (17, 19, 20, 21, 22, 23).

Of the approximately 600 million cases of illness caused by foodborne hazards in 2010 worldwide, infectious agents that cause diarrheal diseases accounted for the vast majority (550 million), in particular noroviruses (120 million cases) and Hepatitis A virus 14 million cases (24).

A total of 5251 foodborne outbreaks were reported in 2014 in the EU (20) within the framework of member states' national monitoring. In 2014, food-borne viruses were, for the first time, identified as the most commonly detected causative agent in the reported food-borne outbreaks. 1070 food-borne outbreaks caused by viruses were reported in 2014, implicated 11740 cases, 2486 hospitalizations and 2 deaths. In strong-evidence outbreaks caused by viruses, 'crustaceans, shellfish, mollusks and products thereof' was the most commonly implicated food vehicle (44.7% of outbreaks), followed by 'buffet meals' (15.8% of outbreaks), 'mixed food' (13.2%) and 'fruit' and 'berries and juices' (both 5.3%). The place of exposure most frequently reported was 'restaurant, café, pub, bar, hotel', followed by the household. Norovirus was the most commonly reported virus implicated in the strong-evidence outbreaks and accounted for 97.6% of cases.

National statistics on foodborne viral disease are not easily available and, where present, likely to reflect significant under-reporting (17), because there is a lack of systematic surveillance for foodborne viral disease (25). Considering the scientific opinion from the EFSA, RASFF notifications and results of official controls, since 2013 the Slovenian National Zoonoses Monitoring Programme has included food sampling for the presence of NoVs and HAV in live shellfish at the retail level and distribution of local and foreign origin, which are recognized as potentially zoonotic viruses (26). In Slovenia, the Zoonoses Monitoring Programme (27) has been conducted at the national level since 1985. It is designed for the systematic collection, monitoring, analysis and communication of data on the emergence of zoonosis, zoonotic agents, and related antimicrobial resistance and comprises the recently emerging zoonotic agents, including foodborne viruses. The ultimate purpose is to capture high-quality information about infections in humans as well as in animals and the contamination of foods, providing important information that is integrated across sectors. It should provide a fundamental basis for making public health decisions with actions for reducing the risks to public health, document the impact of an intervention, track progress towards specified goals, and elucidate the epidemiology of health problems.

The results obtained within Slovenian official national monitoring have shown that live shellfish, purchased at retail stores in Slovenia during independent sampling times throughout the year were contaminated with NoVs, but HAV was not found in any of the samples analyzed (Table 1), which correlates with the low number of human infections in Slovenia (Table 2). In studies, contamination of mussels' samples varies from 16.9% (9), to 34.4% NoVs in Italy (28), to 35.0% of contaminated mussels in France (29). Henigman et al. (8) reported that mussels collected in Slovenian coastal waters were contaminated with NoVs, the highest at Debeli Rtič (25.9%), 21.2% in Strunjan, and only 8.1% in the Seča harvesting area. The difference in positive results is interlinked to the location of harvesting areas due to sea current, dense shipping, and the influx of streams and rivers.

Table 1: Presence of NoVs and HAV in live shellfish samples within Slovenian official surveillance

Year	Virus	
	NoVs	HAV
2013	5/17 (29%)	0/15
2014*	3/12 (25%)	0/12
2015*	3/10 (40%)	0/10

*Preliminary results

Table 2: Reported infections caused by NoVs and HAV in humans in Slovenia from 2007–2014 (27)

N° of cases	Year							
	2007	2008	2009	2010	2011	2012	2013	2014*
NoVs	1094	1043	1393	2012	2231	1611	2146	1316
HAV	15	17	12	9	12	11	23	10

*Preliminary results

3. Control and prevention of foodborne viruses

Virus contamination as a consequence of human handling can occur at any stage of food production, processing, and even preparation. At present, we are faced with insufficient knowledge and awareness of food safety issues among food handlers and accompanied by consumers being insufficiently informed about food safety principles in the home.

Today, we manage food safety through good practices at different levels within a food supply chain that can be described as a network of food-related businesses involved in the creation and consumption of food products that move from farm to table and are linked by information, material, and capital flows. Good practices are described in several different codes of practice designed by producers' organizations, importers and retailer's consortia and government bodies at different levels of production, processing and consumption within the food supply chain. All current active practices are segregated along the food supply chain and are not connected to a comprehensive system, resulting in the existence of exposure to potential of food hazards, especially emerging hazards, such as viruses (12, 30).

The development in different areas within the food production chain and in particularly in technological and technical means is moving very quickly. We encounter innovations in materials, and supporting measures almost daily. Consequently, the gap between knowledge and skills is widening. We are willing to accept the paradigm that drifting is the most dangerous challenge in analytical instruments. However, it is also extremely influential in technological practices. It occurs side by side with "industrial blindness", which develops as a personal characteristic of employees who do not see particular items although they are commonly present in routine operations.

Consumers play an important role in the transmission of hazards, including viruses. Implemented viral food safety guidelines (12) are not purposely designed for informing consumers, although studies in recent years have highlighted gaps in food safety knowledge and some critical safety violations regarding food handling at home (31, 32, 33, 34, 35,36). Consumer behaviour and attitudes toward food safety have shown that the levels of understanding, motivation and trust need to be further cultivated, and their training and informing due to changes in lifestyle and food consumption patterns encouraged (30, 37, 38, 39).

EFSA reported (20) that viral foodborne outbreaks most frequently occurred in 'restaurant, café, pub, bar, hotel", followed by the household. However, outbreaks of foodborne illness occurring in private homes are less likely to be reported than those in commercial and public premises, and it is believed that infections attributed to private homes are three times more frequent than those attributed to canteens (40).

Food handlers also play an important role in the transmission of enteric viruses in the shellfish supply chain (16, 20, 41, 42), especially because shellfish specialities are generally prepared and served in restaurants.

During production, harvest and packaging preparation, food can become contaminated with viruses by food handlers or after contact with virus-contaminated water and surfaces. A major contributor to the spread of disease in food production is poor hygiene practices or being in contact with faecal material or vomit (15). Food handlers are unaware of controls specific to enteric viruses (16). That is why training on all important aspects of NoVs and HAV according to the recently developed Codex Alimentarius guidelines to control viruses in food is strongly recommended. The primary purpose of the codex guidelines for the control of viruses in food is to give guidance on how to prevent or minimize the presence of human enteric viruses in food, especially NoVs and HAV, and to emphasize that management strategies regarding foodborne viruses and associated illnesses should be different from those for bacterial pathogens.

In 2011 Poklar Vatovec with co-workers (42) carried out the research to evaluate the offer of shellfish specialities in Slovene Istria restaurants and to assess food safety knowledge and behaviour of food handlers in preparing shellfish dishes. Results indicated poor food safety knowledge regardless the education of food handlers. The origin of shellfish is important in ensuring food safety; and restaurants should be convinced of good raw meat to exclude foodborne poisoning. Therefore, shellfish should be bought only at registered plants, since these are under official supervision. However they observed that shellfish were not always bought at registered plants, but supplied from the so called illegal »black market«. Next to that it was also observed that employed personnel were hardly acquainted with HACCP principles which represent major food safety hazard. Cooking (at least 90 °C for at least 90 seconds) is a critical point for ensuring food safety. The survey showed that the mid temperature was measured by only 26.8% of the interviewees with formal education and 7.3% with informal education. The remaining did not perform this procedure or it was not known whether it was performed. The results of the survey demonstrated that only 4.9% of the interviewees, regardless their education, are familiar with the correct temperature for heat treatment of shellfish. Research also pointed out that food handlers employed in Slovene Istria restaurants have insufficient knowledge on storing temperatures, storing time and the adequate methods of storing shellfish.

4. PILOT STUDY: Semi-structured interview with Slovenian mussel farmers

4.1. Pilot study illustration

In order to determine eventual connection between comprehension of viral food safety and the shellfish growing practices, the four semi-structured interviews were carried out with Slovenian mussel farmers. The interview guide covered the following topics:

- Factors responsible for food safety within the shellfish food supply chain,
- Conditions related to food safety hazards with an emphasis on foodborne viruses due to virus-commodity combination, which has been identified as one of the greatest public health concerns.

In this pilot study, an empirical grounding was important because an exploration of local farmers' viral food safety perceptions and their good hygiene practices, together with results from the National Zoonoses Monitoring Programme, outlined and gave insight into the

current situation in Slovenia. The semi-structured interviews were chosen due to the sensitivity and complexity of the subject discussed.

The semi-structured interview started with questions concerning food safety in general. The first open thematic question was: "Tell me as much as you can about the importance of the mussels production process, and about the factors that could affect its food safety." Discussions continued with the questions: "Could you please explain what food safety means from your point of view and when mussels are considered safe for a consumer?" and: "Have you ever heard about viruses that are transmitted by shellfish?" Follow-up questions were posed to complement and facilitate the dialogue. The discussions were concluded with the question: "Do you use working documents that have resulted from food safety legislation, like the HACCP plan, good practices that includes viruses?" The semi-structured interview ended with a question on whether the interviewee had something to add. Efforts were made to create trust, since issues of guilt and failure may easily arise. The results of national monitoring are supported by the responses recorded by the interviewers, which were clearly marked due to the assurance of anonymity. The letter "I" (I1) signifies "interview", while the number represents a running number of interviews.

The semi-structured interviews with the local mussel farmers were qualitatively analyzed, using the grounded theory method. This theory produces theoretical models of individuals' perspectives of a given phenomenon and the strategies they use to resolve or cope with the problem in a distinct and bounded context (43, 44, 45). Interviews were recorded using a Dictaphone and later transcribed. Transcripts of the interviews were analyzed by coding the statements of the respondents using identified notions. These notions were gathered into topic categories (44, 45, 46). The validity is justified by triangulation. Three researchers with different disciplinary basic knowledge and different experiences in research were included in the analysis and interpretation process.

4.2 Findings from semi-structured interviews

Eight topics (Scheme 1) were identified following text analysis of the respondents' answers during semi-structured interviews: compliance with the legislation, transport, traceability, employer's awareness, food handlers' awareness and competencies, microbiological contamination, communal infrastructure, and disregarding an environmental code of conduct in the sea. These topics were obtained after identifying the topics in the statements. The interviewee's answers were very guarded; consequently, more comprehensive data processing cannot be achieved.

With the intention to show comprehension within interviewee reflections, these identified topics (Scheme 1) were integrated with a specified section in Guidelines for the control of viruses in food (15) as follows: Primary production/Harvesting area; Establishment: Design and facilities; Control of operation; Establishment: Maintenance and sanitation; Establishment: Personal hygiene; Product information and consumer awareness; and Training. Specific topics (microbiological contamination, communal infrastructure, and disregarding an environmental code of conduct in the sea) represent an unrecognized threat to food safety in the mussels supply chain from viral point of view and are marked bold in the Scheme 1 due to their significance.

The identified topics are in accordance with the Codex Guidelines (15) sections, but not from the viral point of view, but in the context of bacterial contamination associated with hygiene

practices. The results indicated that the respondents rarely comprehend viral food safety separately, but view it in different combinations with already obtained knowledge and skills. This can be demonstrated by the question "Are you familiar with viruses, which are transmitted by shellfish?", which yielded no answers dealing with foodborne viruses. With other questions, dialogue was maintained, but replies always approached local farmers' familiar topics in the field of food safety as ensuring cold chain management practices; microbiological contamination connected to biotoxines and *E. coli*, but *E. coli* in conjunction with gulls' and cormorants' excrement and as obligatory indicator microorganisms; and food handlers' awareness. This aspect is most obvious in the following statement:
Citation III (I3) […] A couple of years ago, biotoxins, now viruses […] we depend on water, because mussels grow themselves […] the biggest problem is the buoys […] in summer every buoy is covered with gulls' and cormorants' faeces, and you are not able to see the color of the buoy […] I was asking if this may be an *E.coli* reservoir, but they said no […]

Local farmers link food safety to employers' awareness and food handlers' awareness and competencies, which often intersect and obstruct food safety system implementation. They associated food safety with compliance with legislation and regulations and transport practices, which can be illustrated by answers to the question "What is important for food safety within the mussels food supply chain?". This aspect is the most obvious in the following statement:
Citation I (I1) […] if anything goes wrong, it can be seen immediately due to inspection control and traceability issues, because we export all harvested mussels to wholesalers […] Anyway, I would not sell mussels, which I would not give to my children to eat […]
Citation II (I2) […] I think that the problem is not only in growing conditions but also in the awareness of employees, especially in restaurants.

Respondents also pointed out the quality of growing waters and its linkage to the communal infrastructure and sewage discharge, and failures to comply with hygiene practices on the sea was also observed. This aspect is seen in the statement:
Citation IV (I3) […] heavy rainfall and storms may flush sewage overflow or farm run-off into the growing waters […]
Recreational and economic activities in the sea were also pointed out as hazards:
Citation V (I4) […] tourists are rascals and throw garbage and discharge sewage into the sea, even though they know that it is prohibited […] next to that problems are large transport ships, which are regularly present in the area with and river estuary […] we only exploit what nature offers to us, and the quality of growing waters is not solely under our responsibility.

This overview has clearly indicated that the development of new concepts is far from sufficient to enhance implementation in real practice. It is a fact that approximately one third of the live shellfish bought on the Slovenian market are contaminated with NoVs. The qualitative analysis alongside official surveillance highlighted that Slovenian mussel farmers are aware of food safety hazards in the mussel food supply chain connected to compliance with the legislation requirements. Despite detected foodborne viruses in samples bought on the Slovenian market, local farmers are not aware of or do not recognize foodborne viruses as distinctive food safety hazards, which represent barrier and consequently critical point to food safety management practices. Despite the fact that guidelines on viruses in food (15) are enforced are mostly unknown to professionals. There is a need to disseminate current guidelines as good viral food safety practice via food safety authorities and professional associations, chambers and societies, because we have demonstrated that even professionals in the field are generally unaware of its recommendations or even existence. Generally,

despite a quite long tradition of aquaculture in Slovenia, there is no leading research institution dealing with fisheries and aquaculture. The research programmes are dispersed to different government and public institutions. Non-government institutions and farmers are only exceptionally included in research activities. Advanced level training in aquaculture is not well developed; consequently, shellfish farmers are thus more or less self-educated in accordance with the requirements of existing legislation.

5 Conclusions

The filter-feeding nature of shellfish and their tendency to concentrate any environmental or man-made contaminant present in their growing waters requires attention to these food safety issues and compliance with applicable requirements. As viruses do not grow in food, do not cause deterioration of the product, and the organoleptic properties of the food are not affected, it is questionable if control measures aiming at microbial growth inhibition are effective to reduce viral contamination. There is a need to assess whether the control measures in place for bacterial hazards require adjustments to be effective against viruses. For the time being, HACCP studies need to address prerequisite programs, such as good hygiene, agricultural and aquacultural practices, especially the origin and quality of water used in food supply chains, and adequate hand hygiene as the most effective prevention measure. Compliance with prerequisite programs, such as codex guidelines, is essential to reduce the risk of contamination. It is also beneficial to have the harmonized integration of monitoring and control to be able to routinely monitor that compliance measures are being undertaken effectively.

Food safety education is most effective when messages are targeted at changing the behaviors most likely to result in foodborne illness, such as personal hygiene, adequate cooking, avoiding cross-contamination, keeping food at safe temperatures, and avoiding foods from unsafe sources. Food safety education is most likely to be effective if the messages are targeted toward specific audiences. The results emphasize the need for tailored educational programs to improve awareness with respect to viruses and to implement innovations into good practices. Not just connected to hygiene, but even more to integrate it into comprehensive good aquaculture practice.

6 Acknowledgments
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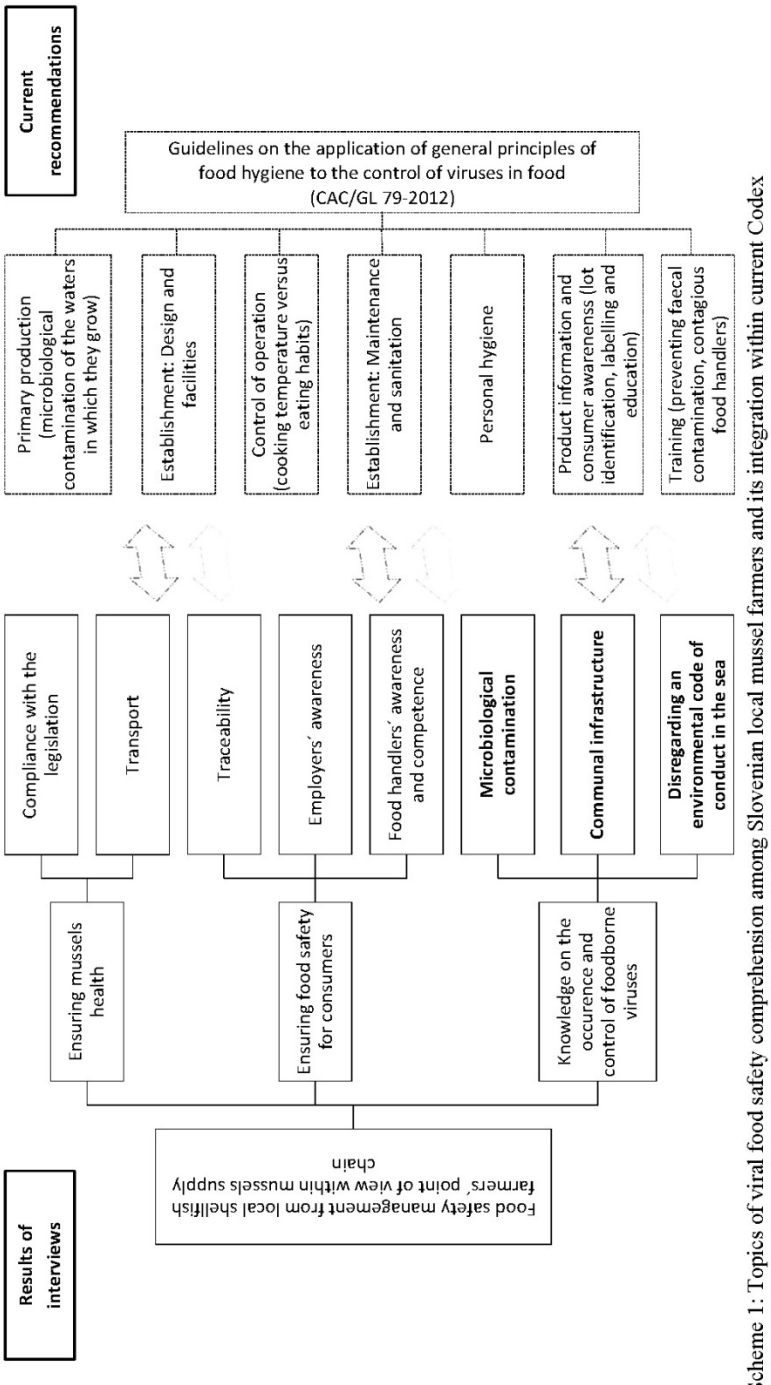

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Scheme 1: Topics of viral food safety comprehension among Slovenian local mussel farmers and its integration within current Codex Alimentarius recommendations key points (CAC/GL 79-2012)

2.1.5 Dejansko stanje obvladovanja virusnih okužb v Sloveniji med potrošniki in zaposlenimi, ki rokujejo z živili

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Izjavljam, da je verzija v doktoratu identična s sprejeto verzijo.

Glede na dostopne epidemiološke podatke so izbruhi, preneseni z virusi v hrani, v porastu. Namen kvantitativne presečne epidemiološke raziskave med 417 potrošniki in 61 zaposlenimi, ki rokujejo z živili, je bil ugotoviti njihovo poznavanje virusov kot možnega dejavnika tveganja v živilsko predelovalni oskrbovalno prehranski verigi ter vlogo potrošnikov in zaposlenih pri preprečevanju in obvladovanju virusnih okužb. Rezultati so osvetlili področja pomanjkljivega znanja in samega prepoznavanja virusov kot dejavnikov tveganja pri zagotavljanju varnosti živil tako v skupini potrošnikov kot tudi zaposlenih. Pri skupini zaposlenih smo prišli do sklepa, da z višjo doseženo stopnjo izobrazbe nivo strokovnega znanja na področju varnosti živil pada, hkrati pa z višjo doseženo stopnjo izobrazbe nivo znanja na področju obvladovanja virusnih okužb raste. V skupini potrošnikov smo prišli do zaključka, da neznanje in neozaveščenost na področju preprečevanja in obvladovanja virusnih okužb močno prevladata nad znanjem in ozaveščenostjo. Z uporabo binarnega logistično regresijskega modela smo potrdili naša predvidevanja, da izobrazba, spol in starost vplivajo na obseg znanja in ozaveščenost potrošnikov na področju obvladovanja in preprečevanja virusnih okužb. Na podlagi teh rezultatov lahko sklepamo, da anketiranci ne prepoznajo virusov kot možnih dejavnikov tveganja pri zagotavljanju varnosti živil. Rezultati so pokazali, da je usmerjeno izobraževanje ključ do izboljšanja znanja in ozaveščenosti tako za zaposlene kot tudi potrošnike.

Dovoljenje za objavo članka Ambrožič in sod. (2016) v tiskani in elektronski obliki je v Prilogi E.

Food safety expertise among professional food handlers and consumers related to foodborne viruses: Case Slovenia

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Abstract
The objective of this quantitative survey was to determine Slovenian consumers´ and food handlers' viral food safety knowledge. For this reason, interviews were conducted using structured questionnaires and included 417 consumers and 61 food handlers. Foodborne viruses were not recognisable as distinctive foodborne hazards by food handlers, which indicate on the lack of viral food safety knowledge using the chi-square test. The analysis pointed out that higher educated food handlers showed lack of food safety expertise. However, at the same time higher educated food handlers possessed more knowledge compared to less educated handlers. Multiple logistic regression analysis was performed on data restricted to consumer study group. The results pointed out that lack of awareness and knowledge strongly prevails over viral food safety knowledge and awareness. The analysis showed that consumers´ knowledge and awareness varied by consumers´ education level, age and gender. On the basis of these results, we can conclude that the profile of comprehension and consequences is not the same for food handlers and for consumers. The obtained results revealed that food safety educational initiatives should be developed to better inform consumers about safe food handling practices and habits to protect their health form foodborne disease, including viral infections.

Keywords: foodborne viruses, consumer, logistic regression analysis, food handler, food safety.

1 Introduction

During the past century, most foodborne diseases were attributed to bacterial pathogens. However, food and waterborne viral infections are becoming an increased challenge to public health (1, 2, 3, 4, 5). Of the approximately 600 million cases of illness caused by foodborne hazards in 2010 worldwide, infectious agents that cause diarrheal diseases accounted for the vast majority (550 million). Among them noroviruses (NoV) were responsible for 120 million cases and Hepatitis A virus (HAV) for 14 million cases (6).

In the EU (2), food-borne viruses were identified as the most commonly detected causative agent in the reported food-borne outbreaks (20.4% of all outbreaks) in 2014. 1,070 food-borne outbreaks were caused by viruses, implicated 11,740 cases, 2,486 hospitalizations and 2 deaths. National statistics on foodborne viral disease are not easily available and, where present, likely to reflect significant under-reporting. The burden of foodborne diseases to public health, welfare, and economy has often been underestimated due to underreporting, because they cause short-term diseases or asymptomatic infection. Outbreaks of foodborne diseases occurring in private homes are less likely to be reported than those in commercial and public premises, and it is believed that infections attributed to private homes are three times more frequent than those attributed to canteens (7). Studies in the last few years have highlighted gaps in food safety knowledge and critical safety violations regarding food handling at home (8, 9, 10) and by food handlers (11, 12, 13, 14, 15).

Consumption of ready-to-eat foods contaminated by infected food handlers remains an important risk factor for viral outbreaks (11, 16). Particularly oysters are commonly considered as the most frequently associated food vehicles in NoV outbreaks (17, 18). Other foods, especially raw materials like soft fruits and vegetables, are also recognized as relevant food vehicles of enteric viruses (19, 20, 21). The hands of food handlers may become contaminated with human enteric viruses if the handlers are shedding viruses in their faeces, changing diapers or cleaning toilet areas, and are not practising appropriate personal hygiene. These same viruses can be transmitted from human skin (hands) to foods and inanimate surfaces (12, 22, 23, 24), which serve as a secondary source of contamination if they come in contact with food. Virus contamination as a consequence of human handling can occur at any stage of the farm-to-fork continuum.

Most foodborne viruses are more resistant (25) than bacteria to commonly used control measures, (e.g. refrigeration, freezing, pH, drying, UV radiation, heat, pressure, disinfection, etc.). There are currently no effective, realistic, and validated risk management options to eliminate viral contamination prior to consumption without changing the normally desired characteristics of the food. Because the burden of viral foodborne disease, particularly NoV and HAV is high, effective control strategies need to focus on the prevention of contamination.

The vast majority of publications dealing with soft elements like culture, trust and teamwork (58) of food safety expertise are by principle focused on bacteria and hygienic issues (26, 27, 28, 29). We also contributed to those studies in the past (10, 15, 30, 31). However, there is really a shortage on soft element within food safety dealing with foodborne viruses (18), especially for consumers' studies. The preventive measures developed for bacterial infections reduction and general hygienic measures are not always efficient to reduce viral infections and contamination, we need to implement additional measures. However, with researching the root cause of infections detectable in clinical samples over time, food supply chains are

identified as the major viral transmission route, where actions directed at prevention of viral foodborne infections are being evolved and established now. Nowadays it became essential to promote improvements in food safety with strong consideration of socio-cultural factors since the main actor in food safety circle is a person, who plays a crucial role in personal food safety management.

Due to lack of satisfactory data in Slovenia as well as worldwide, a pilot study among local professional food handlers and consumers was carried out to investigate food safety handling practice and knowledge of foodborne viruses as food safety hazards. The aim of this study was to find whether professional food handlers and consumers pose viral food safety knowledge.

2 Materials and Methods

2.1 Data collection

Two self-administrable questionnaires (one for consumers and one for food handlers) were developed for this study. Both contain multiple-choice questions with already offered answers, including "do not know" and "other" for minimizing the possibility of selecting the correct answer by chance. To guarantee anonymity of respondents and enable easier identification of questionnaires, identity numbers were assigned to each questionnaire when collected. The questionnaires were pilot tested by 30 participants for questionnaires designed for consumers and 15 for food handlers during February to March of 2015 to confirm question clarity, identify response options and resulting in minor modifications of questions´ wording. Each questionnaire took approximately 5 to 10 minutes to complete. A full study was conducted from April to July of 2015.

2.1.1 Food handlers

Questionnaire for food handlers had eight questions, which were designed to assess food handlers´ knowledge and practical habits focused on viral food safety and three demographic questions (gender, year of birth; 7 level education scale). The questionnaires were delivered in two companies, which volunteer to participate in the pilot survey. In each food company, questionnaires were delivered to the responsible person for the food safety, i.e. food technologist. The number of respondents was determined regarding the number of food handlers present on the day of investigation. 93 questionnaires were distributed among food handlers and their content explained to them by the responsible person during lunch time. Completed questionnaires from two companies were mailed by the same day by responsible food technologists.

2.1.2 Consumers

A cross-sectional study of consumers´ food safety knowledge interlinked to foodborne viruses was conducted in different parts of Slovenia. Gender and age distribution were controlled to assure a balanced structure of the sample by 20 interviewers, each of whom were distributed 25 questionnaires (total of 500 questionnaires). Interviewers were trained, final year students, who visited selected households or interviewed consumers in larger shopping centres. Interviewers briefly explained the purpose and nature of the study to the potential adult respondent over 18 years of age and sought permission for inclusion of their views in the survey. As interviewers conducted interviews in their home cities, a considerable geographical distribution of data was obtained.

The questions in questionnaire dedicated to consumers were designed and structured in three groups. The first group of questions consists of questions "*Have you ever heard of foodborne viruses?", "Is vomit infectious material?", "When could you say that the food is contaminated with viruses?"* and "*Applied handwashing practices"* was designed to assess knowledge and practice habits focused on foodborne viruses and its integration into food safety practices in consumers´ home.

The second group of questions embraced questions "*In your opinion what are sources of viral contamination?"* and "*In your opinion where food can get contaminated by viruses?"* were designed to assess consumers´ awareness of foodborne viruses and its role in food safety.

The third group of questions consists of questions about demographic data (gender, year of birth, 9 level education scale).

2.2 Data preparation

2.2.1 Food handlers

For statistical analysis, we prepared demographic data groups. The question of gender had "female" and "male" option. Year of birth was recoded into three categories: ≤30 years, 31-50 years and ≥51 years. Education level was recoded from initial 7 levels into three categories: low (vocational level or less), middle (secondary school), and high (university degree or more). Recoding was used due to different level of food safety knowledge possession with intention to evaluate differences between them.

Based on the correct/incorrect answers to question, "*What is food safety?"* and "*Recognition of most common foodborne microbial hazards* in food supply chain," respectively we created variables "*have knowledge*" / "*lack of knowledge.*" When all questions were answered correctly, criteria for "*have knowledge*" was fulfilled.

2.2.2 Consumer

For the statistical analysis, we prepared demographic data groups in the same way as described for food handlers.

Based on the correct/incorrect answers to the first group of questions, which assess consumers´ knowledge, we create new set of complex variables "*have knowledge*" / "*lack of knowledge.*" When all questions were answered correctly, criteria for "*have knowledge*" was fulfilled.

Based on the correct/incorrect answers to second group of questions, which assess consumers´ awareness we created new set of complex variables "have awareness" / "lack of awareness." Formulation of complex variables from multiple questions provides more accurate information about the knowledge or lack of knowledge and awareness or lack of awareness in the field of viral food safety.

2.3 Data analysis

2.3.1 Food handlers

The relationship between food handler's demographic data (independent variable) and the food handlers´ "*knowledge" / "lack of knowledge"* (dependent variables) of food safety meaning and recognition of the most common microbial hazards in food supply chain were

analysed using the chi-square test. With chi-square test, we analysed the relationship and significance between each observed demographic data and dependent variable. In chi-square test p-value, 0.05 or less was considered significant.

2.3.2 Consumers

In the first step of the analysis, the relationship between dependent variables ("*knowledge" / "lack of knowledge*" and "*awareness" / "lack of awareness*" of viral food safety) and independent variables (demographic variables) were analysed using the chi-square test. With chi-square test, we have analysed the relationship and significance between each observed demographic data and dependent variables. In chi-square test p-value, 0.05 or less was considered significant.

In the second step of our analysis differences in complex variables "*lack of knowledge*" and "*lack of awareness*," respectively (the observed outcomes), were adjusted to the effects of observed demographic data (independent variables) using binary multiple logistic regression. With binary multiple logistic regression, we have analysed the relationship and significance between all the observed demographic data and dependent variables. Logistic regression provides a method for modelling a binary response variable, which takes values 1 and 0. In our case, we try to investigate how lack of knowledge and lack of awareness of participants can be predicted by observed demographic variable (e.g. gender). The dummy variables (reference group) were created for all independent variables considered in the model. The reference group in our analysis were (gender: female; age group: ≥51 and education: high). In the results, we report above odds ratio (OR) and 95% confidence interval (CI) with p-value.

The OR is a measure, represented the odds that an outcome would occur in the presence of a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure (32). Therefore, OR was a measure of association between an exposure and an outcome.

SPSS statistical package for Windows Version 21.0 (SPSS Inc., Chicago, IL, USA) was used for all analysis in our study.

3. Results and discussion

3.1 Food handlers

A total of 61 questionnaires (out of 93) were obtained from food business operators in Slovenia, which contact us to participate in survey. The overall response rate to the survey was 65.6 %. Of the 61 food handlers taking part in the research, half of them (50.0 %) were female and half of them are male. As seen from Table 1 8.9 % respondents were under 30 years old, 66.1 % between 31-50 and 25.0% was over 51 years old. Educational level were finished high school (40.7 %), 25.4% finished vocational level of education or less, and 33.9 % had higher education. The most significant responses for this area are presented in Fig. 1. Only quarter (21.3 %) of respondents correctly defined food safety as the assurance that food will not cause harm to the consumer, although 90.2% agreed to the question that an important part of food handlers' job responsibilities was to follow all the requirements for ensuring food safety for consumer. With this set of questions, we tested education and training efficiency, which are according to food safety management systems obligatory and necessary (33). It is no doubt that effectiveness of education and training is an important factor contributing to overall food safety. Training employees on food safety practices has been shown to be one of the most important programs that food establishments can implement (34, 35). However,

knowledge assessment not necessarily show actual knowledge and competences of food handlers´, but it is intended only for satisfying legal requirements.

Due to limited scientific research that assessed reported knowledge and behaviour related to foodborne viral transmission and prevention, we created questions, where we studied food handlers´ identification and recognition of the most common foodborne microbial hazards, including foodborne viruses in food supply chain. Knowledge (Fig. 1) was found to be age dependent, food handlers between 31-50 possessed more knowledge compared to younger or older food handlers. As expected, we found that higher educated food handlers had better knowledge, which can be illustrated by correctly identified most common foodborne microbial hazards. It was already pointed out (36, 37) that knowledge is education related. At the same time, we observed that higher educated food handlers showed lack of food safety expertise (Fig. 1) that can be illustrated by correctly identified definition of food safety meaning. This observation can be explained by the fact that higher educated food handlers are in leading positions and their job responsibilities are not strictly connected to food safety expertise.

Despite the beneficial health effects of healthy foods, there is growing awareness concerning its microbial and chemical safety (38). Increased consumption of food traditionally eaten raw and globalization of international trade have increased also the risks of viral contamination of foods, especially soft berry fruits, salad greens, shellfish and ready to eat foods. In recent years, numerous foodborne outbreaks due to consumption of berry fruit (20) and shellfish (17) contaminated by human enteric viruses have been reported. Food handlers recognized steak tartare (75.4 %), shellfish, which are eaten raw (70.4 %), and cheeses from unpasteurized milk (45.9 %) as hazardous food items, while raspberries (42.6 %) and lettuce (34.5 %) as safe and heat treated shellfish (24.6 %) and salad meal at restaurants (24.6 %) as potential safe item although the virus-commodity combinations of greatest public health concern recognised NoV and HAV in prepared (ready-to-eat) foods, bivalve molluscs, fresh produce and HEV in traditional pork dishes (18, 25, 39, 40, 41).

Food handlers play an important role in the transmission of enteric viruses due to poor hygiene practices or being in contact with faecal material or vomit (15, 25). Vomit was recognized (78.7 %) as an infectious material, less than half (40.0 %) respondents know period being infectious after recovery of gastroenteritis symptoms, and nearly half of them (45.9 %) correctly identified contamination routs with viruses.

Our study revealed that although trained, food handlers do not recognize foodborne viruses as distinctive food safety hazards (Fig. 1), which was already observed (14). It was also revealed that food handlers are not recognising ready-to-eat foods and fresh products as vehicles for virus transmission, which indicates that the study population overall has poor knowledge of viral food safety practices. As a result, food safety trainings should be developed according to the recently developed Codex Alimentarius guidelines to control viruses in food to better inform food handlers on all-important aspects of viral food safety, although food handlers possess knowledge about microbial food safety and consequences of poor hygiene practices. It was demonstrated (42, 43) that the importance of continuous and specific-goal oriented training to food handlers can lead to easily improved sanitation practices, such as health checking, adequate hand-washing, observation of proper personal hygiene, prevention of cross-contamination and correct sanitation procedures. Guidance documents on food hygiene and food transmittable diseases are essential for the training of food handlers. Foodborne viral infection remains very common in many parts of the world despite the measures already in

place, mainly targeted at reducing bacterial contamination. For this reason, the Codex Alimentarius Commission decided to develop specific guidelines for the control of viruses in food, which are available since 2012 (25). The primary purpose of the codex guidelines for the control of viruses in food is to give guidance on how to prevent or minimize the presence of human enteric viruses in food, especially NoVs and HAV, and to emphasize that management strategies regarding foodborne viruses and associated illnesses should be different from those for bacterial pathogens. Guidelines are not disseminated as legally enforceable in national guidelines or regulations and consequently remain unknown to professionals. There is a need to disseminate current guidelines as good viral food safety practice via food safety authorities and professional associations, chambers and societies to enhance awareness about foodborne viruses.

3.2 Consumers

A total of 417 questionnaires were obtained. The overall response rate to the survey was 83.4%. As seen from Table 1 60.3% of the respondents were female and 39.7% male. 43.9% respondents were under 30 years old, 24.9% between 31-50 and 31.2% was over 51 years old. Educational level of more than half of the respondents (53.0%) was finished high school, 32.6% finished vocational level of education or less, and 14.4% have higher education. Outcomes revealed (Fig. 3) that lack of knowledge strongly predominates over knowledge. This can also be illustrated by the fact that majority (94.2%) of consumers were familiar with foodborne viruses and 68.8% of consumers recognised vomit as infectious material. However, only 24.7% of respondents correctly identified that viruses do not cause organoleptic properties of the contaminated food, what deserves a special attention in education of consumers at all levels.

Table 1: Demographical characteristics of food handlers and consumers

Characteristics of survey respondents		Food handlers (%)	Consumers (%)
Gender	Female	50.0	60.3
	Male	50.0	39.7
Age	≥51	25.0	31.2
	31-50	66.1	24.9
	≤30	8.9	43.9
Education	High	33.9	14.4
	Middle	40.7	53.0
	Low	25.4	32.6

$N_{food\ handlers}=61$; $N_{consumers}=417$

Variable *"awareness"* was formulated to perceive awareness of consumers related to viral food safety in domestic food preparation. The most significant responses for this group of questions are described in Fig. 2, where outcomes revealed that also lack of awareness strongly prevails over having awareness. Female showed more viral awareness compared to men, due to evolutionary expressed patterns for caring for the home and her family. It was also observed that higher educated consumers have more awareness about viral food safety in home kitchen compared to less educated. Nearly half (48.0%) of the consumers were aware that food can become infected anywhere from farm to plate and 47.7% that human sewage and faeces; infected food handlers and animals harbouring zoonotic viruses are major sources of viral contamination.

To our knowledge, this was the first study that assessed consumers knowledge and behaviour specifically focussed to the viral food safety knowledge and awareness. There is serious lack of food safety viral studies dealing with consumers' knowledge to compare our results with. We developed suitable methodology for addressing this challenge. The approach was found relevant since it can be used specifically for focussed groups like food handler study group but also for more diverse populations and is able to estimate the relationship between observed outcomes and demographic variables.

The results of multiple logistic regression analysis of the consumers' lack of knowledge impact (Table 2) in the model pointed out that the male consumers have 1.2-times higher odds ($p = 0.458$) than female consumers for lack of viral food safety knowledge. Compared with the above 51-years-old consumers, the consumers in the age group 31-50 years have 1.1-times higher odds ($p = 0.420$) than younger or older consumers. Consumers with low level of education (vocational level or less) have 1.3-higher odds for lack of viral food safety knowledge than high-level educated consumers ($p = 0.076$). The model confirmed our assumptions that education, gender, and age influence viral food safety knowledge. Education as a solution for the above-identified lack of knowledge strongly argues for specific training on foodborne viruses also for consumers. Verhoef et al. (14) in their study proposed that for food handlers and their managers' education is crucially important for ensuring viral food safety and improve public health importance. This step can only be achieved by well available and easy to understand education given material, which is respected and followed by consumers. These results emphasize the need for tailored educational programs to improve consumers and food handlers' knowledge and awareness, as has been stressed many times in the last decade (10, 15, 29, 44, 45, 46, 47).

Table 2: Results of multiple logistic regression analysis of impact of consumers' lack of knowledge on foodborne viruses in 417 consumers adjusted on gender, age, and education level.

Independent variables		**OR**	**95% C.I. limits for OR**		**p**
			lower	upper	
Gender	Female	1.00			
	Male	1.209	0.732	1.998	0.458
Age	≥51	1.00			
	31-50	1.099	0.874	1.383	0.420
	≤30	1.016	0.832	1.241	0.874
Education	High	1.00			
	Middle	0.991	0.791	1.241	0.934
	Low	1.277	0.975	1.672	0.076

Abbreviations: OR – odds ratio; C.I. – confidence interval; *p value ≤ 0.05

The results of multiple logistic regression analysis of impact of consumers' awareness respectively (Table 3) of foodborne viruses in the model demonstrated that the male consumers have 1.8-times higher odds ($p = 0.008$) for lack of viral awareness than female consumers. Compared to the above 51-years-old consumers, the consumers under 30 years had 1.2-times higher odds ($p = 0.044$) and low-level educated consumers (vocational level or less) had 1.3-higher odds for lack of awareness than high-level educated consumers ($p = 0.002$). In addition, that model confirmed that viral food safety awareness is gender, education, and age dependent.

Table 3: Results of multiple logistic regression analysis of impact of consumers' lack of awareness on foodborne viruses in 417 consumers adjusted on gender, age, and education level

Independent variables		**OR**	**95 % C.I. limits for OR**		**P**
			lower	upper	
Gender	Female	1.00			
	Male	1.805	1.169	2.785	0.008*
Age	≥51	1.00			
	31-50	1.062	0.882	1.278	0.525
	≤30	1.196	1.005	1.424	0.044*
Education	High	1.00			
	Middle	1.131	0.928	1.378	0.223
	Low	1.303	1.042	1.628	0.020*

Abbreviations: OR – odds ratio; C.I. – confidence interval; *P value ≤ 0.05

Our conclusions raised the need to implement activities to raise awareness of key viral food safety issues. Education and training about basic food safety principles are emphasized as important factors contributing to the reduction of foodborne illnesses. However, it is of crucial importance that the message is specifically tailored and task-specific with regards to the needs of the target group. Improvement becomes more significant if substantiated with practical activity in comparison to those addressed only orally (48). For this reason, the food safety public health campaign to enhance household food safety awareness would be a suitable solution. The best worldwide good practice to enhance food safety awareness and education is Partnership for food safety education in USA (FightBAC), whose goal is to promote food safety awareness to consumers and educate them how to handle and prepare food safely. However, with this aim, European Food Information Council (EUFIC) is also established, which is poorly known to consumers.

Our study showed that there are gaps in consumers' knowledge, practices, and awareness. In addition, the earlier studies conducted on adults have indicated that food safety knowledge tends to increase with age and practice: females have higher scores than males, and younger respondents show the greatest need for additional food safety education (49, 50, 51, 52). Multiple logistic regression analysis identified education as a solution for knowledge improvement and enhanced awareness. The meta-analysis (27) has shown that food safety training increases knowledge and improves attitudes about hand hygiene practices and that refresher training and recurrent emphasis on good food handling behaviour may have ongoing positive effects on hand washing practices. It was also emphasized (53) that special training may improve knowledge of food safety but this does not always result in better and safer food handling behaviour. Unfortunately, we do not have many specific attempts to educate consumers about food safety after they finished obligatory education on primary level. However, consumers generally express their concerns on food safety issues, but only a few of them appear to be changing their food buying and consumption behaviours in view of their concerns (10, 26). Nevertheless, home kitchen is recognised as a source of verified outbreaks (2, 10). Consumer behaviour and awareness toward viral food safety have shown that the levels of understanding, motivation, and trust need to be further cultivated especially in relation to education, age, and gender. All of them are based on awareness and knowledge, which was found as limiting factor for viruses in particular as demonstrated with this research.

Consumers play an important role in the transmission of hazards, including viruses. Consumers should be aware of potential risks, proper handling, and preparation of food for a safe and balanced everyday meal (54), however they are not connected to food supply chain according to chain principles (54, 55, 56). In addition, implemented viral food safety guidelines (25) are not purposely designed for informing consumers (57). At present, we are faced with insufficient knowledge and awareness of viral food safety issues among food handlers, accompanied by consumers being insufficiently informed about food safety principles at home. The lower knowledge for viral food safety concepts indicate that food safety management failed to develop relevant and user friendly approaches to educate both food handler and consumer about implementation of recommended food safety practices associated with foodborne viruses as a response to globalisation and new consumption patterns.

Increased awareness of the importance of good food hygiene practice and training in the production and handling of foods is necessary to minimize the transmission of foodborne viral illnesses. Improving detection methods allows better monitoring of viruses in food and helps to improve the safety of those foods commonly associated with foodborne viral illnesses. The vast majority of publications dealing with soft elements of food safety expertise is by principle focused on bacteria and hygienic issues. However, there is really a shortage on soft element within food safety dealing with foodborne viruses (18), especially for consumers' studies.

4 Conclusions

Trends in food supply chain like globalization of food supply chains, health and demographic situations, social situations (increased consumption of ready-to-eat foods on one side and raw and/or minimally processed foods on the other), and environmental conditions (e.g. pollution) present new challenges for food safety. Global integrated farm to table approach and new consumption patterns enables that a contaminated food product can be consumed by a large number of people worldwide in short period of time. We are facing on one side with insufficient knowledge and awareness of food safety issues among food handlers these days. On the other side, we have insufficient informed consumers about food safety principles at home.

In our study, we came to following conclusion that food handlers, although trained, do not recognise foodborne viruses as distinctive foodborne hazards and are not sufficiently aware of the importance of foodborne viruses as transmittable pathogens for ensuring safe food for consumers. Higher educated food handlers have better knowledge for contamination agents, which indicate that education is solution for knowledge improvement and enhanced awareness. Next to that, the lack of consumers´ knowledge strongly predominates over knowledge. Models for "*viral food safety knowledge*" and "*viral food safety awareness*" confirmed that education, age, and gender influence on viral food safety, which emphasize the need for tailored educational programs to improve awareness with respect to viruses and to implement up-to date findings into good practices, which will be available and easy to understand and to handle with consumers in all ages, educational background, and living circumstances.

5 Limitations of the study

Although this research was carefully prepared, we are aware of its limitations due to lack of prior research studies in viral food safety field connected to human factor and consequently to the available data. That is the reason that the comparison based on scientific results cannot be done. Further research in this field of food safety is suggested.

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7 Conflicts of interest

The authors declare that no conflicts of interest exist.

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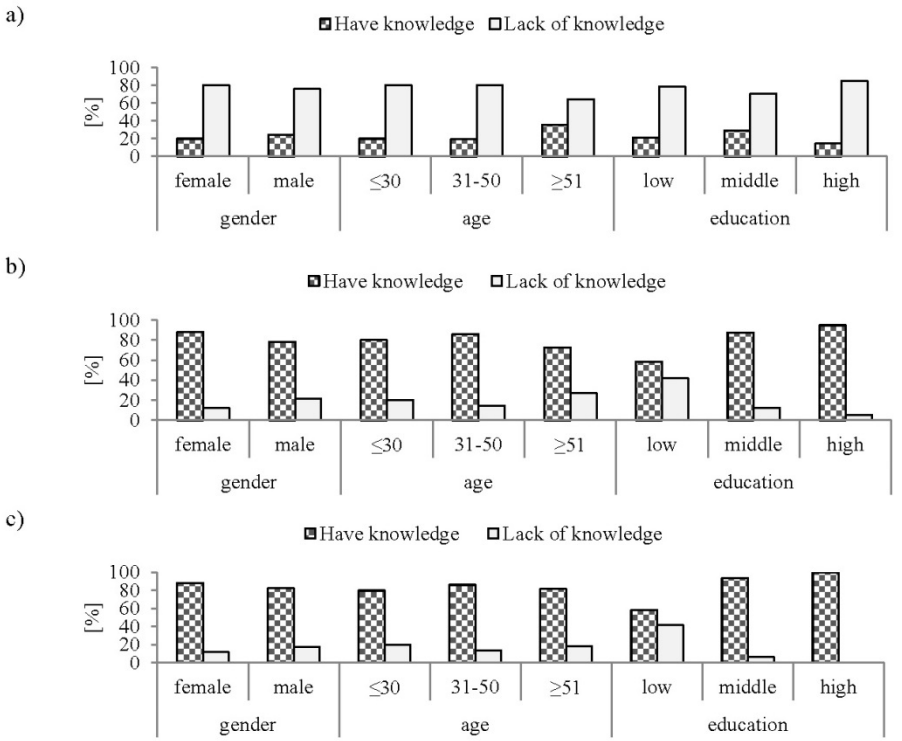


Fig 1: Understanding food safety meaning among 61 food handlers (a); identification and recognition of the most common foodborne microbial hazards, including foodborne viruses in food supply chain: b) norovirus c) hepatitis A virus

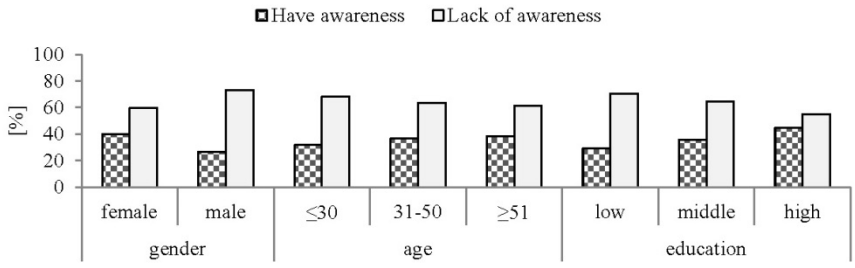


Fig. 2: Perceive awareness among 417 consumers related to viral food safety in domestic food preparation.

Fig. 3: Perceive knowledge among 417 consumers related to viral food safety in domestic food preparation.

3 RAZPRAVA IN SKLEPI

3.1 RAZPRAVA

Zaradi kompleksnosti obravnavane problematike obvladovanja virusnih okužb, prenesenih s hrano, smo razpravo razdelili v štiri sklope, od katerih vsak predstavlja zaključeno celoto in je vezan na hipotezo. Raziskovalno delo je bilo usmerjeno v ugotavljanje dejanskega stanja obvladovanja virusnih okužb v različnih stopnjah ŽPOP verige, to je od pridelovalca do uživalca, ter v izboljševanje varnosti živil z uporabo in razvojem dobrih praks, ki upoštevajo tudi viruse kot dejavnike tveganja za okužbe. Izhajajoč iz delovnih hipotez, ki so zajemale preverjanje zavedanja nevarnosti virusnih okužb vpletenih deležnikov vzdolž ŽPOP verige ter njihovega obvladovanja, smo prišli do pomembnih ugotovitev s ciljem sistemskega vrednotenja in preprečevanja virusnih okužb. Rezultati našega raziskovalnega dela lahko pomembno pripomorejo k zavedanju pomembnosti virusov kot dejavnikov tveganja v ŽPOP verigi ter posledično k vzpostavitvi sistema nadzora ter obvladovanja virusnih okužb, prenesenih z živili, saj je ena izmed temeljnih človekovih pravic pravica do kakovostne prehrane (Varuh človekovih pravic RS, 2016), katere neločljivi del je tudi varnost živil (ETP working groups, 2005).

3.1.1 Obvladovanje virusnih okužb vzdolž živilsko predelovalne oskrbovalno prehranske verige

Namen prvega sklopa disertacije je bilo preveriti, ali je obvladovanje virusnih okužb vzdolž ŽPOP doseženo z obstoječimi dobrimi praksami. Pri tem smo naleteli na uporabo raznolikega strokovnega izrazoslovja pri vseh, ki delujejo v širokem sistemu zagotavljanja potrošniku varnega živila. Osredotočili smo se na angleški jezik, saj je to danes univerzalni strokovni jezik komunikacije na tem področju. Za uspešno komuniciranje je poenotenje izrazoslovja ključnega pomena, saj je le-to predpogoj za boljše medsebojno sporazumevanje in učinkovitejše delovanje in s tem tudi za omogočanje ustreznih in usklajenih strokovnih pristopov. Prvi sklop raziskave je opisan v delu z naslovom »*Nedosledna uporaba izrazoslovja na področju varnosti živil: stalni dejavnik tveganja?*«. Kvalitativna analiza vsebine dostopne znanstvene in strokovne literature nam je omogočila določiti koncept razumevanja pojma varnosti živil. Vsebinska analiza (opisano v poglavju 2.1.1, str. 12) je raziskovalna tehnika, ki sistematično ter objektivno opisuje pomen vsebine analiziranega teksta. Prednost te metode je sposobnost sledenju razvoju in spremembam obravnavane teme skozi štirideset letno obdobje. Z metodo vsebinske analize smo analizirali vsebinske značilnosti dokumentacijskih virov. Dokumentacijske vire smo sprva

iskali s ključno besedo »varnost živil«. Zaradi preobilice zadetkov (več kot 56000 zadetkov) smo ključno besedo razširili na »varnost živil in dobra praksa«, kar je privedlo do 2294 zadetkov. Vsebinska analiza je zajemala preučevanje 2294 povzetkov (opisano v poglavju 2.1.1, str. 12) v obdobju med 1969, ko je bila izdana prva dobra higienska praksa po Codex Alimentarius do 2008. Povzetke smo vsebinsko obravnavali na podlagi dodeljenih ključnih besed.

Na podlagi rezultatov analiziranih povzetkov smo prišli do sklepa, da se je pojem varnosti skozi zgodovino spreminjal in pridobival nove dimenzije. V začetku 20. stoletja se je pojem nanašal na preprečevanje življenju ogrožajočih potvorb živil in se v obdobju industrializacije prevesil v zagotavljanje varnosti procesov kot posledice hitrega tehnološkega napredka z uporabo aditivov, razkužil in čistilnih sredstev ter začetka globalizacije poti živil in hrane. V obdobju potrošništva pa je varno živilo tisto, ki ne predstavlja nevarnosti za potrošnikovo zdravje in podaja zahtevo po zavedanju in obvladovanju možnih tveganj. Danes zagotavljamo varnost živil z različnimi dobrimi praksami, ki so posledica kulture, razvoja tehnike in tehnologije ter načina življenja. Potreba po celovitem obvladovanju posamezne stopnje in aktivnosti v proizvodni verigi je proizvajalce vodila k nastajanju dobre proizvodne prakse. Jasno postavljena načela in uspehi dobre proizvodne prakse so kmalu postavili osnovo za razvoj številnih dobrih praks (Raspor in sod., 2013), ki so specifične za posamezno branžo (Raspor in Ambrožič, 2012). Tako poznamo danes na področju varnosti živil devet dobrih praks, in sicer dobro kmetijsko prakso, dobro proizvodno prakso, dobro laboratorijsko prakso, dobro higiensko prakso, dobro transportno prakso, dobro skladiščno prakso, dobro trgovsko prakso, dobro gostinsko prakso in dobro gospodinjsko prakso (Raspor in sod., 2013; Raspor in Jevšnik, 2008).

V raziskavi sta bila opredeljena dva tipa aktivnosti, ki pomembno vplivata na zagotavljanje varnosti živil vzdolž ŽPOP verige. Temelj koncepta na eni strani predstavlja možne dejavnike tveganj, potrebne ukrepe za obvladovanje tveganj ter potrošnika. Na drugi strani pa so bila identificirana orodja, kot so zakonodaja, predpisi in izobraževanje, s katerimi se zagotavljajo visoki standardi varnosti živil in hkrati zagotavljajo zdravstveno ustreznost živil. Povezovanje vseh elementov v konceptu predstavlja temelj dobrih praks, katerih izrazoslovje na področju bi moralo biti usklajeno, vendar kažejo rezultati drugačno sliko (Baş in sod., 2007; Jevšnik in sod., 2006).

Komuniciranje oziroma povezovanje vseh členov v ŽPOP verigi je nujno zaradi zagotavljanja prepoznavanja in nadzora dejavnikov tveganja (Raspor in Ambrožič, 2012; Jevšnik in sod., 2006). Povezave omogočajo izražanje zahtev in zahtevajo stik med členi v celotni verigi. Kljub dostopnosti enotnega in enoličnega strokovnega izrazoslovja v okviru Codex Alimentarius (FAO/WHO, 2015), ki je postal internacionalno napotilo oziroma referenčni dokument, ki ga pri svojem delu uporabljajo proizvajalci, predelovalci, trgovci

in državni nadzorni organi, je neusklajeno uporabo izrazoslovja možno opaziti tako v internacionalnem kot nacionalnem prostoru. S prevajanjem strokovne literature v nacionalne jezike in obratno se izgublja opredeljeno strokovno izrazoslovje, kar vodi do nedoslednosti in neskladnosti. Skozi vsebinsko analizo dokumentacijskih virov smo ugotovili, da je pomanjkljivost sedanjih sistemov zagotavljanja varnosti živil tudi v nedosledni uporabi strokovnega izrazoslovja. Nabor različnih terminov za iste ali sorodne pojme (opisano v poglavju 2.1.1, str. 14–15) ter pomenskih razlik nakazuje na neskladje in zmedo pri uporabi strokovnega izrazoslovja in kaže potrebo po uporabi usklajenega izrazoslovja na področju varnosti živil, saj tudi neprimerno uporabljeno izrazoslovje in besedišče predstavljata tveganje pri zagotavljanju varnosti živil.

Z vsebinsko analizo smo dokazali, da so virusi redko prepoznani kot možni dejavniki tveganja (opisano v poglavju 2.1.1, str. 13) v zagotavljanju zdravstveno ustreznih živil v primerjavi z drugimi mikrobiološkimi, kemijskimi in fizikalnimi tveganji, kar se odraža tudi v številu povzetkov in naboru uporabljenih ključnih besed. Poleg tega dobre prakse ne zajemajo obvladovanja virusnih okužb v ŽPOP verigi, saj so bile razvite za potrebe obvladovanja klasičnih mikrobioloških okužb in zastrupitev. Ključni doprinos v pogledu obvladovanja virusnih okužb v ŽPOP verigi z obstoječimi dobrimi praksami se pokaže v našem pristopu vključevanja virusov v obstoječe dobre prakse.

3.1.2 Dobra higienska praksa za nadzor okužb živil z virusi

Drugi sklop disertacije je bil osredotočen na razvoj sistema zagotavljanja varnosti živil, ki bi temeljil na upoštevanju virusov kot dejavnikov tveganja in je obravnavan v delu »*Skladnost predlaganih smernic Codex Alimentarius za obvladovanje virusov v živilih z načeli dobrih praks*«. Z uporabo SWOT analize smo potrdili dobro prakso, kot jo postavlja Codex Alimentarius, kot primerno osnovo za razvoj sistema zagotavljanja varnosti živil, ki bi temeljil na upoštevanju virusov kot specifičnih dejavnikov tveganja (opisano v poglavju 2.1.2, str. 24). Codex Alimentarius (FAO/WHO, 2015) je zbirka s strani Organizacije združenih narodov za prehrano in kmetijstvo pripravljenih pravno neobvezujočih, svetovalnih internacionalnih standardov za živila, smernic, priporočil in dobrih praks, ki so namenjeni pridelovalcem in predelovalcem hrane, državnim organom ter potrošnikom. Ti standardi so uveljavljeni na področju Svetovne trgovinske organizacije kot internacionalna merila za varnost živil in predstavljajo minimalne standarde, katere morajo živila dosegati, da je zagotovljena ustrezna raven varovanja zdravja ljudi in interesov potrošnikov ob upoštevanju raznolikosti pri preskrbi s hrano na svetovnem trgu. Med cilje Komisije Codex Alimentarius prištevamo tudi večanje svetovne in nacionalne osveščenosti, povečevanje potrošnikove zaščite in vključitev širših skupnosti, saj izdelava standardov in priporočil zahteva široko strokovno posvetovanje ter sprejemanje kompromisov različnih znanstvenih

pogledov in mnenj. Codex Alimentarius je na podlagi znanstvenih ugotovitev in epidemioloških dejstev kot prvi v letu 2012 izdal Smernice dobre higienske prakse za nadzor virusov v živilih (FAO/WHO, 2012). Smernice sledijo ŽPOP verigi od primarne proizvodnje do končne potrošnje in pojasnjujejo, kako preprečiti ali zmanjšati prisotnost humanih enteričnih virusov v živilih z vidika obvladovanja virusnih okužb v vsaki stopnji vzdolž ŽPOP verige.

Z uporabo SWOT analize, ki analizira štiri vidike, in sicer prednosti (angl. Strengths), pomanjkljivosti (angl. Weaknesses), priložnosti (angl. Opportunities) ter nevarnosti (angl. Threats), kot metodološko orodje, smo skozi sam razvoj nastajanja Smernic dobre higienske prakse za nadzor virusov v živilih dokazali, da imajo le-te omejitve na področju izrazoslovja in transparentnosti (opisano v poglavju 2.1.2, str. 22–24). Codex Alimentarius predstavlja globalno referenčno točko za razvoj sistemov za zagotavljanje varnosti živil, vključno z razvojem strokovnega izrazoslovja. Kljub temu pa se v praksi razvijajo številni sistemi zagotavljanja varnosti živil s specifičnim zahtevami in izrazoslovjem (Raspor in Ambrožič, 2012). Najbolj se to vidi v razlikah ameriškega in evropskega sistema zagotavljanja varnosti živil ter uporabljenega izrazoslovja (opisano v poglavju 2.1.2, str. 24). Prednosti Smernic dobre higienske prakse za nadzor virusov v živilih (opisano v poglavju 2.1.2, str. 23) so v zagotavljanju kakovostnih, natančnih ter uporabnih informacij vsem deležnikom, vpletenim v verigo. To so prve smernice, ki so namenjene vzpostavitvi higienskih načel z namenom nadzora virusov v živilih v celotni ŽPOP verigi, saj je v okviru HACCP sistema možno identificirati samo faze procesa, kjer je možno preprečiti okužbo živil z virusi (Boxman, 2013). Sam način podajanja informacij ni vedno v pomoč uporabnikom (opisano v poglavju 2.1.2, str. 28–29), saj so kljub predpisani strukturni zasnovi v dokumentu nekatere informacije porazgubljene med različnimi poglavji, kar zmanjšuje jasnost in preglednost sporočila. Smernice same po sebi ne ponujajo rešitev (opisano v poglavju 2.1.2, str. 29), kar je možno pojasniti s pomanjkanjem rutinskih analitskih metod za odkrivanje prisotnosti večine danes aktualnih virusov. Smernice tudi ne odvračajo potrošnika od neprimernega rokovanja s kritičnimi živili, kot so školjke in jagodičevje. Smernice dobre higienske prakse za nadzor virusnih okužb (opisano v poglavju 2.1.2, str. 28) v svoje aktivnosti ne vključujejo potrošnika, kar je glavna problematika vseh dobrih praks (Raspor in sod., 2013; Raspor in Jevšnik, 2008). Smernice nalagajo odgovornost za obvladovanje virusnih okužb tako državam, živilski industriji kakor tudi potrošnikom. Države so zadolžene za določitev najboljšega načina izvajanja teh smernic, hkrati pa se je živilska industrija dolžna prilagoditi in izvajati opisana načela dobre higienske prakse. Naloga potrošnikov pa je, da se zavedajo svoje vloge in sledijo primernim navodilom in sami uporabljajo primerne higienske ukrepe. Kljub nekaterim pomanjkljivostim pa je to trenutno edina obstoječa higienskla praksa, namenjena nadzoru virusov in jo kot priporočilo povzemajo tudi avtorji v znanstvenih in strokovnih prispevkih (Boxman in sod., 2015; Verhoef, 2013; Boxman, 2013;), ki se ukvarjajo s preučevanjem vloge virusov v zagotavljanju varnosti živil. Ključni doprinos v razvoju sistema

zagotavljanja virološke varnosti je osredotočen v pregled stanja in zavedanja pomembnosti dobre higienske prakse, ki bi temeljila na upoštevanju virusov kot dejavnikov tveganja. Primerna dobra higienska praksa za nadzor virusov je osnova za izdelavo nadaljnjih strategij za učinkovito zagotavljanje varnosti živil.

3.1.3 Prepoznavanje virusov kot dejavnikov tveganja pri deležnikih vzdolž živilsko predelovalne oskrbovalne prehranske verige

Prepoznavanje virusov kot dejavnikov tveganja pri deležnikih vzdolž ŽPOP verige je predstavljeno v tretjem sklopu v delu disertacije »*Dejansko stanje obvladovanja virusnih okužb v Sloveniji med potrošniki in zaposlenimi, ki rokujejo z živili*« in obsega analizo dejanskega stanja obvladovanja virusov v verigi tako z vidika potrošnikov kot zaposlenih, ki rokujejo z živili vzdolž ŽPOP verige. V ta namen smo izvedli presečno epidemiološko raziskavo. Za zbiranje podatkov smo uporabili anketni vprašalnik med 417 potrošniki ter 61 zaposlenimi, ki rokujejo z živili. Na podlagi rezultatov smo prišli do zaključka, da tako zaposleni kot potrošniki ne prepoznajo virusnih tveganj tako jasno kot bakterijskih, ko je govora o obvladovanju varnosti živil.

Glede na dostopne epidemiološke podatke so izbruhi, povzročeni z virusi v hrani, v porastu (EFSA, 2015). S presečno epidemiološko raziskavo (opisano v poglavju 2.1.5, str. 60–62) med 417 potrošniki in 61 zaposlenimi, ki rokujejo z živili, smo preverjali njihovo poznavanje ter tudi razumevanje virusov kot možnih dejavnikov tveganja vzdolž ŽPOP verige ter njihovo vlogo pri preprečevanju okužb živil z virusi. Rezultati so osvetlili področja pomanjkljivega znanja in samega prepoznavanja virusov kot dejavnikov tveganja pri zagotavljanju varnosti živil tako v skupini potrošnikov kot tudi zaposlenih. S presečno raziskavo med zaposlenimi (opisano v poglavju 2.1.5, str. 62-64), ki rokujejo z živili, smo opazili pomanjkljivo znanje tako pri razumevanju pojma varnosti živil, s čimer smo preverjali učinkovitost izobraževanja na delovnem mestu. Pri skupini zaposlenih smo prišli do sklepa, da z izobraženostjo nivo strokovnega znanja na področju varnosti živil pada, kar lahko razložimo z dejstvom, da bolj izobraženi zasedajo vodilna delovna mesta, s tem pa izgubljajo stik s strokovnostjo področja. Samo četrtina (21,3 %) anketiranih je znala pravilno opredeliti pojem varnost živil, ki smo ga pričakovali kot zagotovilo, da živilo ni škodljivo za zdravje ljudi, čeprav skoraj vsi (90,2 %) izpostavljajo pomembnost njihove odgovornosti slediti zahtevam za zagotavljanje varnosti hrane za potrošnika. Pomanjkljivo znanje smo opazili tudi na področju poznavanja in preprečevanja virusnih okužb (opisano v poglavju 2.1.5, str. 73), kar so s primerljivimi raziskovalnimi metodami ugotovili tudi nekateri drugi avtorji (Boxman in sod., 2015, Poklar Vatovec in sod., 2014; Verhoef in sod., 2013). Poznavanje virov okužb smo preverjali z oceno (ne)varnosti posameznih živil glede na prisotnost virusov v živilih. Zaposleni so tatarski biftek (75,4 %), uživanje netoplotno obdelanih školjk (70,4 %) ter sire iz nepasteriziranega mleka (45,9 %)

prepoznali kot nevarna živila. Maline (42,6 %) in zelena glavnata solata (34,5 %) sta bili prepoznani kot varni živili ter toplotno obdelane školjke (24,6 %) in solatni obrok v restavraciji (24,6 %) kot potencialni varni živili, kljub dejstvu, da so to v literaturi (Todd in Greig, 2015; FAO/WHO, 2012; Rodriguez-Lazaro in sod., 2012; Koopmans in sod., 2004) prepoznana tvegana živila za prenos virusnih okužb, še posebej za noroviruse in viruse hepatitisa A. Kljub temu je skoraj polovica (45,9 %) zaposlenih prepoznala stik s fekalijami človeškega in/ali živalskega izvora, s človeškimi izločki, z okuženo vodo, neumivanje rok po uporabi sanitarij ter prenos preko nečistih površin kot načine prenosa virusov.

Z anketiranjem 417 potrošnikov smo ugotovili, da neznanje in neozaveščenost na področju preprečevanja in obvladovanja virusnih okužb močno prevladata nad znanjem in ozaveščenostjo (opisano v poglavju 2.1.5, str. 64-67). To lahko ponazorimo z dejstvom, da so potrošniki v večini primerov (94,2 %) prepoznali viruse, ki se prenašajo z živili in lahko povzročajo slabost, bruhanje, drisko in/ali bolečino v trebuhu kot dejavnike tveganja. Potrošniki so v več kot polovici primerov (68,8 %) prepoznali izbruhanino kot vir okužbe za tistega, ki pride v stik z njo, ampak samo 24,7 % anketirancev je pravilno odgovorilo na vprašanje, da se okuženosti z virusom ne da zagotovo ugotoviti iz zunanjega videza živila.

Z uporabo multiple logistične regresije (opisano v poglavju 2.1.5, str. 62) smo analizirali odnos med opazovanimi demografskimi skupinami (spol: moški, ženska; starostne skupine: <30; 31–50, 50<; stopnjami izobrazbe: nizka: dokončana poklicna šola ali manj; srednja: dokončna srednja šola; visoka: dokončna fakulteta ali več) in odvisnimi spremenljivkami. Za opazovani odvisni spremenljivki smo oblikovali kompleksni spremenljivki »Znanje/Neznanje«, ter »Ozaveščenost/Neozaveščenost«, s katerimi smo želeli oceniti potrošnikovo ozaveščenost ter znanje na področju obvladovanja virusnih okužb. Z oblikovanjem kompleksne spremenljivke smo z združevanjem informacij različnih vprašanj pridobili bolj natančno informacijo o neznanju in o neozaveščenosti na področju varnosti živil. Končni rezultat v multipli logistični regresiji je razmerje obetov, kjer ocenjujemo, koliko večje obete imajo izpostavljeni za opazovani izid/pojav v primerjavi z neizpostavljenimi (Szumilas, 2010).

Rezultati multiple analize so pokazale (opisano v poglavju 2.1.5, str. 65), da imajo moški potrošniki 1,2-krat večje obete ($p = 0,458$) za nepoznavanje področja obvladovanja virusnih okužb kot ženske. Potrošniki stari 31–50 let imajo v primerjavi z nad 50 let starimi potrošniki 1,1-krat večje obete ($p = 0,420$) za pomanjkanje znanja o obvladovanju virusnih okužb. Potrošniki z nižjo doseženo stopnjo izobrazbe (dokončano poklicno šolo ali manj) imajo 1,3-krat večje obete v primerjavi z visoko izobraženimi potrošniki ($p = 0,076$).

Rezultati potrošnikove ozaveščenosti (opisano v poglavju 2.1.5, str. 66) na področju obvladovanja virusnih okužb so potrdili, da imajo moški 1,8-krat večje obete (p = 0,008), da bodo neozaveščeni pri obravnavanju virusnih okužb v primerjavi z ženskami. Do 30 let stari potrošniki imajo v primerjavi z nad 50 let starimi potrošniki 1,2-krat večje obete (p = 0,044), potrošniki z nižjo izobrazbo (dokončana poklicna šola ali manj) pa imajo 1,3-krat večje obete (p = 0,002) za neozaveščenost na področju obvladovanja virusnih okužb.

Na podlagi rezultatov analize povezanosti med odvisnimi spremenljivkami in opazovanimi demografskimi skupinami smo potrdili, da anketiranci ne prepoznajo virusov kot možnih dejavnikov tveganja pri zagotavljanju varnosti živil. Ključni doprinos se pokaže v našem metodološkem pristopu k prepoznavanju virusov kot dejavnikov tveganja pri deležnikih vzdolž ŽPOP verige, saj do sedaj ni bilo izvedenih podobnih raziskav, ki bi opisovale analizo dejanskega stanja obvladovanja virusov v verigi z vidika potrošnikov. S tem smo potrdili naša predvidevanja, da izobrazba, spol in starost vplivajo na obseg znanja in ozaveščenosti na področju obvladovanja in preprečevanja virusnih okužb, prenesenih s hrano. Dobljeni rezultati so pokazali, da je usmerjeno izobraževanje in usposabljanje ključnega pomena pri boljši informiranosti in ozaveščenosti deležnikov v ŽPOP verigi. Žarišče problematike obvladovanja virusnih okužb vzdolž ŽPOP verige je potrebno obravnavati tako s stališča zaposlenega, ki rokuje z živili, kakor tudi potrošnika. Ti rezultati poudarjajo potrebo po prilagojenih izobraževalnih programih tako za potrošnike kot zaposlene, kot je bilo poudarjeno že večkrat v zadnjem desetletju (Al-Sakkaf, 2015; Byrd-Bredbenner in sod., 2013; Ergönül 2013; Sharif in sod., 2013; Milton in Mullan, 2010; Chukwuocha in sod., 2009; Jevšnik in sod., 2008a, 2008b, 2008c; Raspor in Jevšnik, 2008; Clayton in sod., 2002; Medeiros in sod., 2001). Poleg tega je potrebno izvajati aktivnosti za dvig ozaveščenosti vseh deležnikov, vpletenih v ŽPOP verigo na področju obvladovanja virusnih okužb s hrano, saj je razumevanje razlogov neobvladovanja bistvenega pomena pri njihovem odpravljanju. Podane informacije morajo biti prilagojene starosti, izobrazbi, delovnimi izkušnjami in samemu življenjskemu stilu slušateljev.

3.1.4 Ovrednotenje celovitega sistema zagotavljanja virološke varnosti živil

3.1.4.1 Preučevanje virološke varnosti školjk

Četrti sklop združuje celovit vsebinski in metodološki pristop pri preučevanju virološke varnosti živil in je združen v delih »*Molekularna karakterizacija norovirusov ugotovljenih v mediteranskih klapavicah (Mytilus galloprovincialis) iz slovenskih školjčišč*« ter »*Virusno onesnaženje v proizvodni verigi školjk na slovenski obali*«. V tem sklopu smo ugotavljali prisotnost norovirusov in virusov hepatitisa A v vzorcih mediteranskih klapavic iz vseh treh slovenskih školjčišč: Seče, Strunjana in Debelega rtiča v letih 2006–2008 ter 2010–2012, kakor tudi v vzorcih, odvzetih v okviru nacionalnega monitoringa. V okviru

nacionalnega monitoringa se je v letih 2013–2015 vzorčilo mediteranske klapavice v proizvodnji, distribucijskih centrih, skladiščih in maloprodaji domačega in tujega porekla. Z metodo RT-PCR v realnem času smo ugotovili prisotnost norovirusne RNA. Prisotnost virusov hepatitisa A ni bila ugotovljena v nobenem analiziranem vzorcu. Poleg ugotavljanja dejanske prisotnosti norovirusov in virusov hepatitisa A v slovenskem prostoru smo v ta sklop uvrstili tudi prepoznavanje potencialnih kritičnih točk, specifičnih za virusna tveganja, saj le-te niso enake oziroma nimajo enake pomembnosti, kot to velja za mikrobiološka tveganja. V ta namen smo uporabili metodo polstrukturiranih intervjujev. Z analizo smo prišli do sklepa, da se lokalni školjkarji zavedajo tveganj na področju gojenja školjk, ampak njihov nabor razmišljanj ne vključuje virusov kot možnih dejavnikov tveganja. Do podobnih zaključkov so prišli tudi avtorji Verhoef in sod. (2013) ter Boxman in sod. (2015), ko so preverjali prepoznavanje virusov pri zaposlenih v obratih javne prehrane.

V letu 2013 je bilo prvič v program uradnega nadzora Uprave Republike Slovenije za varno hrano, veterinarstvo in varstvo rastlin vključeno tudi vzorčenje školjk na prisotnost norovirusov in virusov hepatitisa A. V obdobju 2013–2015 je bilo v okviru nacionalnega monitoringa preiskanih 39 vzorcev školjk domačega in tujega porekla. Majhen obseg monitoringa nakazuje, da akterji na teh področjih ne zaznavajo problematičnosti teh okužb. Vzorčenje se je izvedlo v proizvodnji, distribucijskih centrih, skladiščih in maloprodaji. Prisotnost norovirusne RNA je bila ugotovljena med 25 % testiranimi vzorci v letu 2014 do 40 % v letu 2015 (opisano v poglavju 2.1.4, str. 46). Prisotnosti virusov hepatitisa A ni bilo mogoče najti v nobenem od analiziranih vzorcev, kar povezujemo z nizkim številom okužb pri ljudeh v Sloveniji (opisano v poglavju 2.1.4, str. 46). V državah članicah ES morajo školjke, ki so v prometu, izpolnjevati pogoje glede prisotnosti morskih biotoksinov, bakterij vrste *Salmonella* spp. in glede dovoljenega števila bakterij *Escherichia coli (E. coli)* (Regulation 2073/2005 …, 2005; Regulation 854/2004 …, 2004), ne pa tudi prisotnosti norovirusov in virusov hepatitisa A v živilih. Poleg tega smo v okviru raziskave ugotavljali prisotnost norovirusov v vzorcih mediteranskih klapavic (*Mytilus galloprovincialis*) iz vseh treh slovenskih školjčišč: Seče, Strunjana in Debelega rtiča. Z metodo RT-PCR v realnem času smo ugotavljali prisotnost norovirusov. Metode za ugotavljanje prisotnosti virusov v živilih se vse bolj razvijajo, kar prispeva k prepoznavanju virusnih okužb, prenesenih s hrano. Diagnostične metode za dokazovanje virusov v školjkah temeljijo na dokazovanju virusnih nukleinskih kislin. Pri ugotavljanju norovirusov in virusov hepatitisa A v školjkah se danes uporabljajo molekularne metode, ki temeljijo na pomnoževanju virusne RNA. Izolirano RNA se pomnožuje z verižno reakcijo s polimerazo (PCR) s predhodno transkripcijo (RT-PCR) in/ali RT-PCR v realnem času. Z molekularnimi metodami se lahko hitro in zanesljivo detektira že zelo nizke količine virusov v školjkah, zato so danes te metode nepogrešljive v vsakem diagnostičnem laboratoriju. Z omenjenimi metodami lahko v vzorcih hrane dokažemo samo virusni genom, ne pa tudi živega infektivnega virusa (Rodriguez, 2009; Cefas, 2008; FAO/WHO, 2008), kar je velika

pomanjkljivost na področju zagotavljanja varnosti živil. Zaradi vse večjih zahtev po vzpostavitvi rutinskih metod določanja virusov v živilih je leta 2013 internacionalna organizacija za standardizacijo izdala certificirano preizkusno metodo za ugotavljanje prisotnosti virusov hepatitisa A in norovirusov z uporabo PCR v realnem času tako za kvalitativno kakor tudi za kvantitativno metodo detekcije. Za izolacijo norovirusov predstavljajo školjke zelo zahteven matriks. Razlog za to je tako v prisotnosti inhibitornih snovi (Beuret in sod., 2003) v tkivih školjk kot v nizki prisotni koncentraciji virusov. Pomemben dejavnik je tudi velika genetska raznolikost sevov (Wilson, 1997). Med letoma 2006–2008 je bila ugotovljena prisotnost norovirusne RNA v 9,1–24,6 %, med letoma 2010–2012 pa v 12,5–22,2 % vzorcev (opisano v poglavju 2.1.3, str. 35). Ugotovljeni sevi so filogenetsko zelo sorodni sevom kliničnih vzorcev ter tudi vzorcem iz voda, ne glede na geografski izvor (opisano v poglavju 2.1.3, str. 36). Glede na veliko podobnost nukleotidnih zaporedij naših sevov z nekaterimi sevi iz svetovne banke podatkov domnevamo, da je gojenje školjk v veliki meri povezano z vplivi okolja. Pomembna so gibanja morskih tokov, nihanja temperature morja, ob tem pa še številni drugi dejavniki, ki so rezultat različnih človeških posegov, kot so npr. komunalne odplake in odplake velikih transportnih ladij, ki priplujejo iz oddaljenih področij širom po svetu. Zaradi precejanja morske vode školjke poleg hranil v prebavnih žlezah kopičijo biološka in kemijska onesnaževala. Tako so v školjkah lahko prisotne patogene bakterije (*Salmonela spp*., *E. coli*) in virusi (norovirusi, virusi hepatitisa A). Zdravstveno tveganje je tako povezano predvsem z načinom priprave, ki ne predvideva zadostne toplotne obdelave živila. Analiza kritičnih kontrolnih točk v HACCP sistemu v postopku gojenja školjk zajema tudi spremljanje kakovosti vode v školjčiščih, ustrezno skladiščenje ter morebitno prečiščevanje (postopek depuracije). Predpisi glede varnosti mikrobioloških kriterijev školjk za uživanje (Regulation 853/2004 …, 2004; Regulation 854/2004 …, 2004) se nanašajo le na bakteriološko onesnaženost školjk, to je število *E. coli*, ki so pokazatelj fekalnega onesnaženja. Norovirusi se na prebavne žleze školjk vežejo aktivno z ogljikovodikovimi vezmi (Le Guyader in sod., 2006). Tako vezani ostajajo lahko več tednov kljub prečiščevanju z vodo ustrezne kakovosti, medtem ko se bakterije iz školjk izločijo v 2−3 dneh (Greening in sod., 2008; Loisy in sod., 2005; Schwab in sod., 1998).

Za boljše in celovitejše razumevanje obravnavanega področja smo izvedli tudi 4 polstrukturirane intervjuje (opisano v poglavju 2.1.4, str. 48–49) s slovenskimi školjkarji. Le-to nam je omogočilo poglobljen upogled v doživljanje in pojmovanje obvladovanja virusnih okužb s strani školjkarjev. Polstrukturirani intervju je potekal v obliki sproščenega pogovora po vnaprej določenih temah in je intervjuvancu omogočal osebno izražanje mnenj in videnj. Teme so vključevale vsebine povezane z zagotavljanjnjem varnosti živil in zagotavljanje zdravstvene ustreznosti školjk s poudarkom na virusih, ki se prenašajo s školjkami. Haralambos in Holborn (1995) trdita, da vprašalniki kot standardno kvantitativno metodološko orodje ne morejo popolnoma zajeti vrednot in stališč posameznikov. Avtorji, ki uporabljajo kvalitativne pristope, pomagajo osvetliti vprašanja

iz drugačne perspektive in tako poskušajo zapolniti nastajajočo vrzel. Kvalitativne analize so v primerjavi s kvantitativnimi manj primerne za določanje vzorcev in struktur, ampak omogočajo bolj temeljito poglobljeno razumevanje vrednot in stališč posameznikov. Pred načrtovanjem strategij in rešitev za spreminjanje škodljivih in neprimernih navad je potrebno najprej določiti oblike vedenja in posameznikova prepričanja. Če primerjamo rezultate kvantitativne in kvalitativne analize v raziskavi obvladovanja virusnih okužb pri slovenskih šoljkarjih (opisano v poglavju 2.1.4, str. 49–51) in zaposlenih, ki rokujejo z živili (opisano v poglavju 2.1.5, str. 73), lahko vidimo, da se dopolnjujejo in jasno pokažejo nekatera nasprotja, kar z enostranskim metodološkim pristopom ne bi bilo možno ugotoviti. Z vprašalnikom smo ugotovili, da samo četrtina zaposlenih (21,3 %), ki rokuje z živili, prepozna pomen varnosti živil kljub obveznemu izobraževanju na delovnem mestu (opisano v poglavju 2.1.4, str. 73). Z intervjuji s šoljkarji smo dokazali, da se ti zavedajo svoje vloge v procesu zagotavljanja varnih živil potrošnikom (opisano v poglavju 2.1.4, str. 49–51). Analiza intervjujev je razkrila zavedanje pomembnosti zagotavljanja varnosti živil in tudi poistovetenja sebe kot pridelovalca šoljk s potrošnikom, ampak samo v povezavi z mikrobiološkimi dejavniki tveganja, ki pa do sedaj operativno niso vključevali virusov kot dejavnikov tveganja. Z analizo smo prišli do sklepa, da se lokalni šoljkarji zavedajo možnih tveganj na področju gojenja šoljk, saj so kot kritične točke v procesu izpostavili bakteriološko tveganje, kot je *E. coli*, sledljivost, pomembnost hladne verige in vpliv človeškega faktorja na zagotavljanje varnosti živil v celotni verigi. Velik pomen pri zagotavljanju varnih šoljk so pripisali veterinarski inšpekciji kot nadzornemu organu. Kljub temu pa smo z analizo ugotovili, da njihov nabor razmišljanj ne vključuje virusov, ki se prenašajo s šoljkami kot možnih dejavnikov tveganj v ŽPOP verigi. Slovenski šoljkarji tudi niso seznanjeni s Smernicami dobre higienske prakse za nadzor virusnih okužb po Codex Alimentarius. To privede do vprašanja izpolnjevanja odgovornosti vladnih organizacij, ki so zadolžena za izpolnjevanje pogojev, pravil in priporočil ter nudenje podpore vzdolž ŽPOP verige ter vpetosti same živilske industrije v proces nenehnega izboljševanja, kar privede do zavedanja pomembnosti kontinuiranega rednega izobraževanja in usposabljanja o vseh pomembnih vidikih zagotavljanja varnosti živil, vključno z obvladovanjem virusnih okužb.

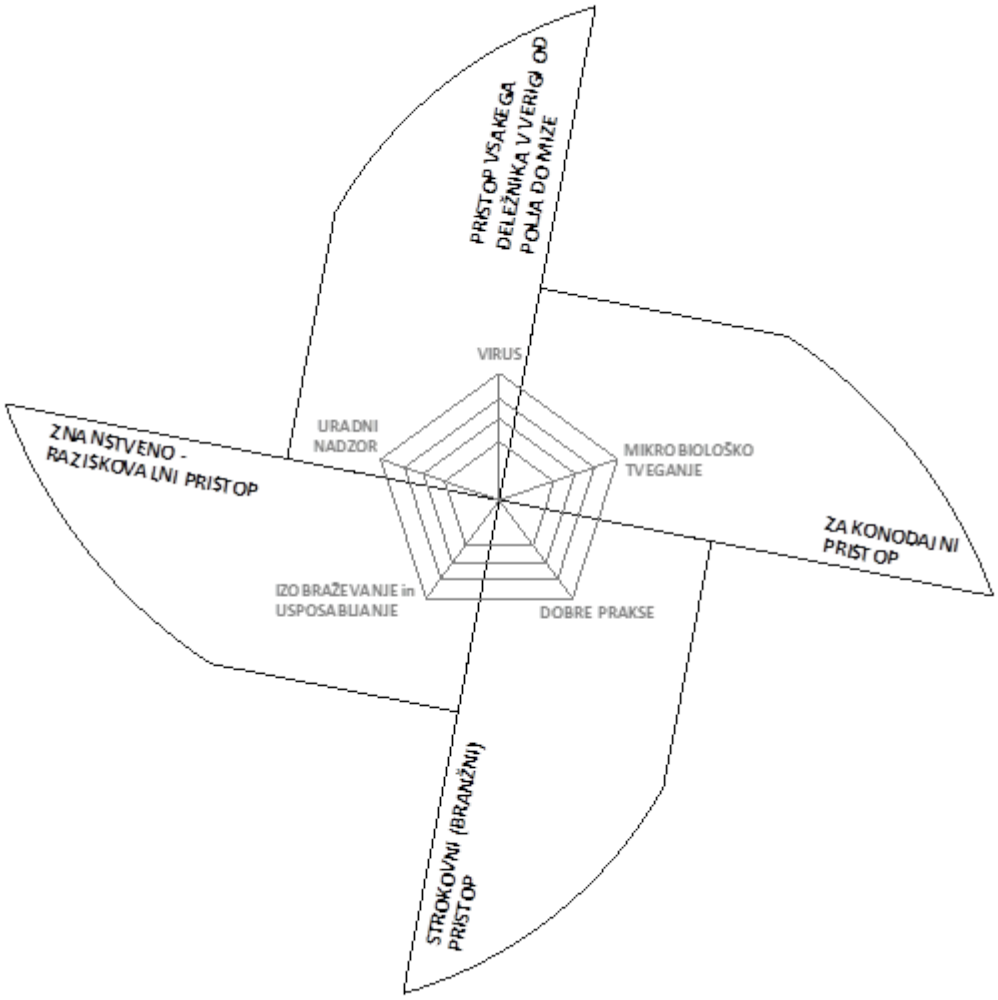
Ključni doprinos predstavlja naš vsebinski in metodološki pristop ugotavljanja zagotavljanja virološke varnosti šoljk v ŽPOP verigi šoljk. Prišli smo do jasnih ugotovitev, da zagotavljanje virološke varnosti šoljk v verigi ni doseženo z obstoječimi praksami, saj le-te operativno temeljijo na onesnaženosti šoljk s *E. coli*, ki so pokazatelj fekalnega onesnaženja. Obenem smo dokazali, da so šoljke, prisotne na slovenskem tržišču, onesnažene z norovirusi.

3.1.4.2 Konceptualni sistem zagotavljanja virološko varnega živila

Doseganje visoke ravni varstva človekovega življenja in zdravja je eden od temeljnih ciljev evropske živilske zakonodaje, določene v Uredbi (ES) št. 178/2002 (Regulation 178/2002 …, 2002). Glede na pridobljene rezultate smo oblikovali raziskovalni model (Slika 1), ki bi zagotavljal virološko varno živilo. Oblikovani konceptualni raziskovalni model v svojem središču vključuje viruse, ki morajo biti obravnavani enakovredno klasičnim dejavnikom tveganja. To pomeni, da morajo biti operativno vključeni tako v obstoječe dobre prakse, izobraževalni sistem in sistem usposabljanja kakor tudi v sistem uradnega nadzora ob skupni podpori stroke, znanstveno-raziskovalne skupnosti, zakonodaje ter vseh deležnikov v verigi od polja do mize. Virološko varno živilo lahko zagotovimo le, če so vsi elementi usklajeni in povezani v celoto, čemur pa trenutno ni tako.

EFSA med prioritetna področja spremljanja, proučevanja in priprave predlogov ukrepov na področju varnosti hrane, z vidika varovanja potrošnikov, uvršča poleg prepoznanih tudi novonastajajoče dejavnike tveganja, kot so virusi. Evropski sistem za varnost hrane temelji na predpostavki, da je treba za varovanje zdravja potrošnikov varnost zagotavljati vzdolž celotne ŽPOP. Zagotavljanje varnosti živil zahteva celostni pristop vzdolž celotne verige, kar pomeni, da mora zakonodaja postaviti minimalne zahteve na področju varnosti in kakovosti živil, uradni nadzor mora kontinuirano nadzorovati izpolnjevanje zakonskih zahtev v proizvodnji in prometu z živili, nosilci živilske dejavnosti morajo ravnati skladno z zahtevami in lastnimi programi, potrošniške organizacije morajo skrbeti za informiranje vseh nas o aktualnih spoznanjih na področju živilstva in prehrane.

Za celovito obravnavo obvladovanja virusnih okužb (Slika 1) so potrebni tako kvalitativni kot kvantitativni metodološki pristopi, saj ti omogočajo večdimenzionalno sliko o ugotavljanju vzrokov virusnih okužb in tako osvetlijo dejavnike, ki vplivajo na razumevanje stališč obvladovanja virusnih okužb. Prišli smo do sklepa, da ima sedanji način obvladovanja varnosti živil pomanjkljivosti v smislu nevključevanja ali celo zanemarjanja virusov kot dejavnikov tveganja v sistem zagotavljanja zdravstveno ustreznih živil ter da sedanje dobre prakse ne zajemajo obvladovanja virusnih okužb v ŽPOP verigi. Z opravljeno raziskavo (opisano v poglavju 2.1.4, str. 49–51; 2.1.5, str. 62–67) dejanskega stanja obvladovanja virusnih okužb smo ugotovili, da virusi med deležniki v ŽPOP verigi niso prepoznani kot dejavniki tveganja, čeprav je Codex Alimentarius na podlagi znanstvenih ugotovitev in epidemioloških dejstev kot prvi v letu 2012 izdal Smernice dobre higienske prakse za nadzor virusov v živilih (FAO/WHO, 2012). Smernice sledijo ŽPOP verigi od primarne proizvodnje do končne potrošnje in pojasnjujejo bistvo nadzora higiene živil z vidika obvladovanja virusnih okužb v stopnjah vzdolž verige.



Slika 1: Model zagotavljanja virološko varnega živila

Kompleksnost ugotovitev dodatno izpostavi potrebo po vključitvi virusov v celovito zakonodajo o varnosti hrane in krme ter higieni živil tako na nacionalni ravni kot tudi na ravni ES, saj zakonodaja omogoča razvoj dobrih praks kot orodja za izpolnjevanje zahtev zakonodaje. Še posebej to velja za mikrobiološka merila, saj predstavljajo orodje za ugotavljanje varnosti in kakovosti živil in postavljajo varnostne omejitve za mikroorganizme, njihove toksine in metabolite. Vendar vzorčenje in mikrobiološko testiranje živil samo po sebi ne predstavlja jamstva, da so proizvedena živila varna. Smiselno je, da je varnost živil zagotovljena s preventivnim pristopom, kot je ustvarjanje varnega živila, kjer so vključene dobre prakse in sistemski pristop. Odgovornost za varnost živil je porazdeljena med vse člene ŽPOP verige, vendar so potrošniki najšibkejši člen verige, zato mora država z ustreznimi predpisi in nadzorom poskrbeti za njihovo zaščito in hkrati osveščenost. Verodostojnost informacij, način informiranja ter kontinuiteta le-tega je temeljnega pomena za dvig osveščenosti vseh, ki smo v ŽPOP verigi. Vsi, ki delujemo v

ŽPOP verigi, nosimo del odgovornosti za varno hrano in se moramo tudi zavedati svoje odgovornosti pri zagotavljanju varne hrane vzdolž verige. Varnost živil mora biti usmerjena v znanje, neprestano izobraževanje in izmenjavo informacij. Tako stroka kot izobraževalni proces na področju živilstva morata slediti napredku tehnologije in znanosti za dosego dobrega zdravja posameznika in družbe. Le poučen in informiran posameznik pozna morebitna tveganja, do katerih lahko pride zaradi neustreznega ravnanja z živili. Glede na to, da aktualni sistemi zagotavljanja varnosti živil temeljijo na človeku (Čebular in sod., 2014; Raspor in Jevšnik, 2008; Jevšnik in sod., 2006), je človeka, ki vstopa v delovne procese na različnih stopnjah in ravneh v verigi od polja do mize, treba permanentno izobraževati.

V cilju sistemskega vrednotenja in preprečitve virusne okužbe vzdolž ŽPOP verige je potreben celosten pristop, ki bi gradil na zaupanju in kredibilnosti med deležniki v verigi in bil sposoben učinkovitejšega odzivanja na nastajajoča in na novo ugotovljena zdravstvena tveganja, kot so virusi v živilih, v procesu zagotavljanja varnosti živil. Na eni strani je potrebno viruse kot dejavnike tveganja vključiti v zakonodajno področje in hkrati vzpostaviti nadzor nad kontinuiranem izpolnjevanju zakonskih zahtev, saj glavne elemente sistema zagotavljanja varnega živila predstavlja regulatorni in nadzorni sistem, sistem odločanja, ki vsebuje oceno tveganja ter mehanizmi vključevanja javnosti. Virusi so redko prepoznani kot možna tveganja v trenutno uporabljenih sistemih zagotavljanja zdravstveno ustreznih živil v primerjavi z drugimi tveganji, kar nakazuje potrebo po vzpostavitvi interaktivne izmenjave informacij in mnenj med tistimi, ki ocenjujejo tveganja, tistimi, ki skrbijo za obvladovanje tveganj, znanstveniki, živilsko industrijo, objekte javne prehrane in prometa z živili in ne na koncu tudi potrošniki. Integralni zaščitni ukrepi za zmanjševanje virološkega tveganja v ŽPOP verigi morajo v žarišče postaviti posameznika, saj je zagotavljanje varnosti živil v današnjem svetu uspešno le ob skupnem naporu vseh deležnikov vzdolž verige. Ugotovitve nakazujejo potrebo po pripravi in permanentni izvedbi izobraževalnih vsebin in usposabljanj, ki bi celovito obravnavali varnost živil, vključno z virusi. Vzpostaviti bi bilo potrebno učinkovitejši način primarnega izobraževanja, ki bi gradil odnos posameznika in posledično tudi celotne družbe do živila in njegove kakovosti in varnosti, saj so kakovostna in varna živila naša temeljna pravica. Posameznikov odnos do hrane se gradi že v najzgodnejši mladosti in zahteva ustrezne pristope ter dobre zglede.

3.2 SKLEPI

Na osnovi rezultatov dokumentacijskih virov in analize dejanskega stanja obvladovanja virusnih okužb na različnih stopnjah ŽPOP verige lahko povzamemo pridobljena spoznanja:

- Zagotavljanje virološke varnosti živil v ŽPOP verigi ni doseženo z obstoječimi dobrimi praksami kljub obstoju Smernic dobre higienske prakse za nadzor virusov.
- Codex Alimentarius predstavlja primerno osnovo za razvoj sistema zagotavljanja varnosti živil, ki temelji na upoštevanju virusov kot dejavnikov tveganja.
- Deležniki v ŽPOP verigi ne prepoznajo virusov, prenesenih s živili, kot dejavnikov tveganja na področju zagotavljanja varnosti živil.
- Interaktivna komunikacija (vertikalno in horizontalno po verigi), sistemski pristop (sistem varnosti živil je voden, nadziran, pregledovan in posodobljen), vključitev virusov v celovito zakonodajo o varnosti hrane in krme ter razvoj dobrih praks kot orodja za izpolnjevanje zahtev zakonodaje in izobraževanje so kritične točke v zagotavljanju virološke varnosti živil, saj je preventivni pristop trenutno edina rešitev v zagotavljanju virološko varnega živila.

4 POVZETEK (SUMMARY)

4.1 POVZETEK

V preteklosti je bila mikrobiološka varnost živil osredotočena predvsem na nadzor bakterijskih okužb. V zadnjih desetletjih so virusne okužbe prebivalstva prepoznane kot veliko javnozdravstveno breme. Z iskanjem vzrokov za porast virusnih okužb v kliničnih vzorcih je bila ŽPOP veriga opredeljena kot glavna pot prenosa, kjer se ukrepi, usmerjeni v preprečevanje virusnih okužb, šele razvijajo in uveljavljajo v sistemih zagotavljanja varnosti živil. Za zdravstveno ustreznost živil, vključno z obvladovanjem virusnih okužb, je bistven celosten pristop, ki upošteva dejstvo, da je oskrba z živili vezana na celotno ŽPOP verigo, ampak hkrati izključuje potrošnika. Komuniciranje oziroma povezovanje z namenom izražanja zahtev vseh deležnikov vzdolž verige je nujno zaradi obvladovanja virusnih okužb, prenesenih s hrano. Virusi so redko prepoznani kot možna tveganja v trenutno uporabljenih sistemih zagotavljanja zdravstveno ustreznih živil v primerjavi z drugimi mikrobiološkimi, kemijskimi in fizikalnimi tveganji, kar se odraža tudi v naboru uporabljenih ključnih besed in posledično tudi v neusklajeni uporabi izrazoslovja. Z raziskavo smo potrdili, da je dobra praksa, kot jo postavlja Codex Alimentarius, primerna osnova za razvoj sistema zagotavljanja varnosti živil, ki bi temeljil na upoštevanju virusov kot specifičnih dejavnikov tveganja, saj predstavlja globalni referenčni dokument, ki je priznan s strani vseh deležnikov. Zaradi kompleksnosti pri reševanju problematike smo pristopili iz različnih smeri, saj smo le tako lahko osvetlili dejavnike, ki pomembno vplivajo na razumevanje stališč obvladovanja virusnih okužb. Z metodami smo podprli odločitve o kritičnih točkah, za katere menimo, da so bistvenega pomena pri zagotavljanju obvladovanja varnosti živil. Živila se z virusi lahko okužijo že v fazi pridelave, lahko pa do okužbe pride naknadno pri obdelavi, predelavi, distribuciji, kakor tudi v domači kuhinji. Vendar na področju preprečevanja in obvladovanja virusnih okužb s hrano tako zaposleni, ki rokujejo z živili, kot potrošniki še vedno niso dovolj ozaveščeni. Poleg tega ne kažejo interesa za obvladovanje virusnih okužb, saj jim primanjkuje znanja in praktičnih veščin, kar priča o pomembnosti kontinuiranega rednega usposabljanja in izobraževanja o vseh pomembnih vidikih varnosti živil. Z rezultati analize dejanskega stanja obvladovanja virusnih okužb, prenesenih s hrano vzdolž ŽPOP verige, smo potrdili postavljene hipoteze. Ugotovitve nakazujejo potrebo po vzpostavitvi učinkovitejšega sistemskega pristopa, ki bi zagotavljal nadzor, interaktivno komunikacijo in izvajanje izobraževalnih vsebin, ki celovito obravnavajo varnost živil, vključno z virusi in tako pripomorejo k celostni strategiji zagotavljanja varnosti živil. Postavljeni model zagotavljanja virološko varnega živila, poudarja pomembnost enakovrednega obravnavanja virusov s klasičnimi dejavniki tveganja ter aktivnega sodelovanja vseh deležnikov ŽPOP verige.

4.2 SUMMARY

In the past, the microbiological food safety management systems focused primarily on the control of bacterial infections. The diseases caused by foodborne viruses fall into three main types: gastroenteritis, enterically transmitted hepatitis, and illnesses that causes illness after they migrate to other organs. In the last decades, several enteric viruses have been identified as etiological agents of viral gastroenteritis in healthcare as epidemic and sporadic gastroenteritis is a growing public health burden. With researching the root cause of infections detectable in clinical samples over time, food supply chains are identified as the major viral transmission route, where actions directed at prevention of viral foodborne infections are being evolved and established now. Food safety is best assured by an integrated, multidisciplinary approach »from farm to fork«, but at the same time puts consumer out of its circle. In order to ensure viral food safety, it is essential to create links between the actors in food supply chain. Foodborne viruses are not recognised as food safety hazards in comparison with other microbiological, chemical, and physical hazards in food safety management systems, which also reflects in used food safety terminology. The results pointed out that Codex Alimentarius guidelines to the control of viruses in food serves as firm foundation for ensuring viral food safety due to its role in national legislation and food safety management system development and implementation. Due to the complexity in the field of controlling foodborne viral infections, different methodological approaches were used to point out the factors and viewpoints and support decisions influencing understanding and management of viral food safety practices. Food may become contaminated by foodborne viruses anywhere in food supply chain, including consumer´s home kitchen. On the basis of our results, both consumers and food handlers do not recognize foodborne viruses as distinctive food safety hazards and consequently are not aware their role in viral food safety management practices, which indicates on the importance of training and education of all up-to-date relevant aspects. With the results of actual level viral food safety management practices within food supply chain, we confirmed all postulated hypotheses. Actors in food supply chain are not familiar with foodborne viruses that represent barrier and consequently critical point in food safety management practices. The findings suggest the need to establish controlled system approach, where communication and education along food supply chain are essentials to ensure viral food safety and thus contribute to the overall food safety. The presented concept of ensuring viral food safety is focused on equal integration of foodborne viruses as classical food safety hazards in systems for ensuring food safety as well as the active participation of all stakeholders in food supply chain.

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