

## **RICERCHE EFFETTUATE**

### **IGIENE DEGLI ALIMENTI AD USO UMANO**

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#### **A microbial subtyping approach for the identification of food potentially involved in listeriosis in Northern Italy**

EFSA J. - Vol. Suppl ( 2015). - p 99 [Nr. Estr. 7145]

EFSA scientific conference "Shaping the future of food safety, together" (2nd : Milan, Italy : 14-16 October 2015)

Appicciafuoco B, Dragone R, Frazzoli C, Bolzoni<sup>°</sup>G, Mantovani A, Ferrini AM

#### **Microbial screening for quinolones residues in cow milk by bio-optical method**

J Pharm Biomed Anal. - Vol. 106 ( 2015). - p 179-185. - 30 bib ref [Nr. Estr. 6038]

The use of antibiotics on lactating cows should be monitored for the possible risk of milk contamination with residues. Accordingly, Maximum Residue Levels (MRLs) are established by the European Commission to guarantee consumers safety. As pointed out by Dec 2002/657/EC, screening is the first step in the strategy for antibiotic residue control, thus playing a key role in the whole control procedure. However, current routine screening methods applied in milk chain still fail to detect residues of quinolones at concentrations of interest. This paper reports the findings of a new bio-optical method for the screening of quinolones residues in bovine milk, based on *E. coli* ATCC 11303 growth inhibition. The effect of blank and spiked cow milk samples (aliquots equivalents to 0.8%, v/v) is evaluated in Mueller Hinton Broth (MHb) and MHb enriched with MgSO<sub>4</sub> 2% (MHb-Mg) inoculated with the test strain at the concentration of 10<sup>4</sup> CFU/mL. The presence of quinolones inhibits the cellular growth in MHb, while this effect is neutralized in MHb-Mg allowing both detection and presumptive identification of quinolones. Growth of the test strain is monitored at 37 °C in a Bioscreen C automated system, and Optical Density (OD) at 600 nm is recorded every 10 min after shaking for 10 s. Growth curves (OD vs. time) of *E. coli* ATCC 11303 are assessed in milk samples, with and without quinolones, and their differences in terms of OD (OD<sub>600nm</sub> = ODMHb-Mg - ODMHb) are calculated. The presence of quinolones is detected by the cellular growth inhibition (OD vs time, none increase in the value OD) and presumptively identified through the increase of the slope of OD<sub>600nm</sub> curve (OD vs. time), after about 3 h of incubation. The detection limit for ciprofloxacin and enrofloxacin is at the level of MRL, for marbofloxacin is at 2-fold the MRL whereas for danofloxacin is at 4-fold the MRL. Although the sensitivity of the method could be further improved and the procedure automated, it is a promising step forward to integrate screening assays into the control process and, in particular, to fill in the gap for quinolones; moreover, these technological developments contribute to the One Health perspective through the monitoring of safe and correct use of veterinary antibiotics.

Bardasi<sup>°</sup>L, Taddei<sup>°</sup>R, Nocera L, Ricchi<sup>°</sup>M, Meriald i<sup>°</sup>G

#### **Shiga toxin-producing Escherichia coli in meat and vegetable products in Emilia Romagna Region, years 2012-2013**

Ital J Food Safety. - Vol. 4 no 1 ( 2015). - no 4511 (p 33-35). - 11 bib ref ( ultimo accesso 23/07/2015 <http://www.pagepressjournals.org/index.php/ijfs/article/view/ijfs.2015.4511> ) [Nr. Estr. 6084]

In 2012-2013 Emilia-Romagna Region introduced a monitoring plan for Shiga toxin-producing *Escherichia coli* (STEC) in foodstuff. Six hundred eighty-nine meat samples and 273 fruit and vegetable products were analyzed according to ISO TS 13136. Pre-enriched samples were tested by multiplex real time PCR targeting the virulence genes *eae*, *stx1* and *stx2*. *Stx2* positive samples were investigated for the presence of serogroup O104 associated gene. O103, O111, O145, O157,

026 associated genes were tested on samples positive for stx in association with eae gene. Isolation of E. coli strains was attempted from samples positive for serogroup-associated genes. Thirtyfour meat products (4.9%) resulted positive for stx1 and/or stx2 genes and 46 (6.7%) for stx1 and/or stx2 genes in association with eae gene. Forty-five (6.5%) samples resulted positive at least at one serogroup. Serogroup O103, O104, O111, O145, O157 and O26 genes were detected respectively in 1.3, 0.3, 0.1, 3.9, 2.9 and 2.5% samples; 0.6% samples resulted positive for STEC isolation (2 E. coli O103 and 2 E. coli O157). It is worth noting that STEC virulence genes were detected at high frequency (19%) in fresh pork meat sausages. Four (1.5%) vegetable samples were positive for stx1 and/or stx2 genes and 1 (0.4%) for stx1 and/or stx2 genes in association with eae gene; none resulted positive for the tested serogroups. Only a low number of samples positive by molecular methods were confirmed by cultural isolation. It is therefore of the uttermost importance for appropriate risk management, to be fully aware of the meaning of the analytical result.

Bernini V, Dalzini<sup>o</sup>E, Lazzi C, Bottari B, Bisotti S, Fontana M, Neviani E

**A multi-sampling approach to evaluate an infrared surface treatment for reducing Listeria monocytogenes contamination on whole Gorgonzola cheese rinds**

Food Control. - Vol. 55 ( 2015). - p 75-81. - 32 bib ref [Nr. Estr. 6097]

The microbial ecology of Gorgonzola cheese rind is the focus of many studies because the surface can be contaminated by pathogenic microorganisms. Among food-borne pathogens, particular attention is focused on the behaviour of Listeria monocytogenes that is able to grow at refrigeration temperatures and it could also grow during ripening. The Consortium for the Protection of Gorgonzola Cheese declares the rind not edible but the pathogen may also be transferred during cutting and portioning. Therefore, the decontamination of rinds is important to increasing cheese safety. To achieve this goal, many different strategies have been proposed. In this study, the application of an infrared surface treatment to decontaminate cheese rinds is proposed. The presence of L. monocytogenes, which was artificially inoculated in cheese rinds together with cheese rind microflora, and the cheese rind microflora were monitored before and after the treatment of 32 samples of Gorgonzola cheese rinds. The infrared surface treatment provided good reduction of the rind microflora, and L. monocytogenes was particularly affected by this. The treatment, applied to cheeses at the end of ripening, does not interfere with the ripening process and offers the advantages of short time exposures and easy installation of the equipment in cheese plants. Moreover, this study demonstrated that the sampling method affects the detection of cheese rind microflora. In fact, a non-destructive sampling method, based on a sponge and often used for surface sampling but never before applied to ready to eat food sampling, was compared with a traditional but destructive method, based on rind scraping. Regarding L. monocytogenes, the sponge method allowed to estimate even only  $5.71 \pm 0.79 \log \text{ cfu g}_{-1}$  of cells reduction after the treatment while the higher reduction when considering the rind scraping method was  $4.06 \pm 3.38 \log \text{ cfu g}_{-1}$ . The sponge method, combined with the classic scraping one, besides offering the great advantage of not being destructive, allowed to differentiate the effect that the treatment has on the microflora located on the surface from those in deeper layers..

Bertasi<sup>o</sup>B, Zanardini<sup>o</sup>N, Tilola<sup>o</sup>M, Bottega<sup>o</sup>E, Ca stagnola<sup>o</sup>N, Losio<sup>o</sup>MN

**Sviluppo di una metodica molecolare per la ricerca di specie ittiche in prodotti alimentari**

XVI Congresso Nazionale SIDiLV : 30 Settembre - 2 Ottobre 2015 Montesilvano (PE) : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2015]. - p 272-273. - 5 bib ref [Nr. Estr. 7053]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (16. : Montesilvano (PE) : 30 Settembre - 2 Ottobre 2015)

*The introduction on of several new species of fish from developing countries is a prerequisite for*

*commercial fraud being perpetrated, as well as health, difficult to detect either on the sole basis of morphological identification on both in the case of products based on fish more complex as processed products and/or canned. It is therefore necessary to have effective methods of identification on, is applicable to the whole fish that prepared foods. It proceeded then the refinement and application on of the technique of barcoding both of processed products (commercial and with treatment conditions reproduced in the laboratory), both on a large variety of fish samples.*

Bertocchi°L, Ghidini S, Fedrizzi°G, Lorenzi°V

### **Case-study and risk management of dioxins and PCBs bovine milk contaminations in a high industrialized area in Northern Italy**

Environ Sci Pollut Res. - Vol. 22 no 13 ( 2015). - p 9775-9785 - 37 bib ref [Nr. Estr. 5980]

Milk supplied to a dairy plant in Brescia City (Northern Italy) was found to be contaminated by dioxin like PCBs at levels above the European (EU) action limit (2 pg WHO-TEQ/g fat). As a consequence, 14 dairy farms were sampled individually, in order to identify and possibly eliminate the source of contamination. All the farms were located in Brescia or just nearby, an area that is characterized by a strong industrialization. Four out of the 14 farms showed contamination levels above the legal maximum limit set by European Commission at 5.5 pg WHO-TEQ/g fat for the sum of dioxins and DL-PCBs. Concentrations of 8.16, 6.83, 5.71 and 5.65 pg WHO-TEQ/g fat were detected. In the three most polluted farms, cow ration was substituted with feed coming from uncontaminated areas and the time needed to reduce milk pollution was evaluated. In all the three farms, contamination levels dropped below the EU legal limit after only 1 month from the removal of the pollution source. In each sampled farm, DLPCBs were the major contributors to the total WHO-TEQ level, with percentages up to 87 % in the most contaminated one. PCB 126 WHO-TEQ value explained by itself large part of this contamination, and its decrease was fundamental for the reduction of milk contamination levels. This study provides an example of an on-field successful emergency intervention that succeeded in decontamination of dairy cows, allowing a fast restart of their production activity.

Biancardi°A, Dall'Asta C

### **Presenza di ocratossina A (OTA) in campioni commerciali di formaggio grattugiato**

V Congresso nazionale "Le micotossine nella filiera agro-alimentare" : Istituto Superiore di Sanità Roma, Roma 28-30 Settembre 2015 : riassunti / a cura di Carlo Brera... [et al.]. - Roma : Istituto Superiore di Sanità, 2015. - (ISTISAN Congressi ; 15/C4) p 44 [Nr. Estr. 7117]

Congresso nazionale "Le micotossine nella filiera agro-alimentare" (5. : Roma : 28-30 Settembre 2015)

L'ocratossina A è una micotossina, principalmente prodotta come metabolita secondario da *Penicillium* e *Aspergillus*. Il Regolamento (CE) 1881/2006 ha stabilito diversi limiti di tolleranza per svariate tipologie di matrici; non esiste attualmente un limite per salumi e formaggi. Per quanto riguarda espressamente la matrice formaggio, dalle evidenze della letteratura scientifica è noto che la contaminazione da OTA non riguarda la materia prima, ovvero il latte. Nondimeno la contaminazione della superficie della forma in fase di stagionatura è un evento possibile. Considerando che il disciplinare di produzione dei grattugiati prevede l'uso della crosta fino ad un massimo del 18%, lo scopo di questo studio è di valutare la presenza di OTA in grattugiati commerciali. Sono stati effettuati due set di campionamenti di buste commerciali "formaggio fresco grattugiato": un primo set di 40 campioni e un secondo set di 71 campioni, fatti in periodi diversi. Sono stati analizzati con metodo LC-MS/MS messo a punto e validato in house nell'ambito del presente studio. In sintesi il recupero medio è 94% (N=24, 4 livelli di drogaggio, 6 repliche/livello), il LOQ è pari a 1 ppb (S/N=10). L'incertezza estesa relativa è pari a 25% (gradi di libertà n=52, fattore di copertura k=2,01). Nel I set i valori trovati variano da un minimo di <1 mg/Kg ad un massimo di 54,07 mg/Kg. Nel I set la % di presenza media di OTA è 15%. Nel II set i valori trovati variano da un minimo di <1 mg/Kg ad un massimo di 51,10 mg/Kg. Nel II set la % di presenza media di OTA è 20%.

Complessivamente tra I e II set di dati, la presenza media di OTA è pari al 18%. In conclusione, la percentuale media di negatività (OTA-free) è del tutto rassicurante (82%). La presenza di OTA nel restante 18% è sicuramente da attribuire all'uso di croste contaminate. La soluzione del problema può semplicemente svilupparsi attraverso due approcci complementari: 1. approccio tecnologico: si realizza sia con un'adeguata cura dell'igiene degli ambienti di stagionatura sia con opportuna spazzolatura delle forme; 2. approccio regolatorio attraverso: - l'emanazione di limiti di tolleranza di OTA non solo sulle croste ma anche sul prodotto finito (in linea con quelli già esistenti su altre matrici come da Reg. 1881/2006); - l'introduzione di piani di monitoraggio..

Biancardi°A, Dall'\_Asta C

#### **Determination of sterigmatocystin in feed by LC-MS/MS**

Food Addit Contam Part A. - Vol. 32 no 12 ( 2015). - p 2093-2100. - 29 bib ref [Nr. Estr. 7143]

An LC-MS/MS method is proposed for the analysis of sterigmatocystin in cereals and feed. The method is based on a solid-liquid extraction and a dilute-and-shoot approach. Accuracy and precision were established at the LOQ (1 µg kg<sup>-1</sup>); the mean overall recovery (n = 6) was 98%, with a confidence interval of 3.8% and a CV% of 3.7%. Accuracy and precision were also assessed at three other concentration levels (2.03, 5.07 and 10.14 µg kg<sup>-1</sup>; six replicates per level). The mean overall recovery (n = 24, LOQ included) was 99% with a confidence interval of 0.8% and a CV% of 1.9%. The method was then applied to 14 naturally incurred feed samples. Aflatoxin B1 was present in the range 28.7–240.1 µg kg<sup>-1</sup>, while lower concentrations of sterigmatocystin were found (0.7–2.2 µg kg<sup>-1</sup>). This method may represent a valuable choice, ensuring a high level of accuracy and precision, as well as high-throughput performance. Therefore, it meets the recent EFSA opinion recommendation in terms of availability of fast and sensitive methods (recommended LOQ = 1.5 µg kg<sup>-1</sup>) in order to increase data collection to allow for the assessment of dietary exposure.

Bianchini°V, Luini°M, Jonas R, Kittl S, Kuhnert P

#### **Genotipizzazione e antibiotico resistenza di Campylobacter jejuni isolati da bovini e piccioni in aziende di bovine da latte del Nord Italia**

XVI Congresso Nazionale SIDiLV : 30 Settembre - 2 Ottobre 2015 Montesilvano (PE) : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2015]. - p 79-80. - 5 bib ref [Nr. Estr. 7044]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (16. : Montesilvano (PE) : 30 Settembre - 2 Ottobre 2015)

*To get an overview of genotypes and antibiotic resistances in C. jejuni isolated from milk, cattle feces, and pigeons in dairy herds of Northern Italy, flaB-typing was applied to 78 isolates previously characterized by MLST, and genotypic resistances towards macrolides and quinolones based on point mutations in the 23S rRNA and gyrA genes were determined. flaB-typing revealed 22 different types and was useful to further differentiate strains with an identical ST and to identify a pigeon-specific clone. Macrolide resistance was not found; quinolone resistance was detected in 23.3% of isolates. A relationship between specific genotypes and antibiotic resistance was observed. Our data confirm that pigeons do not play a role in the spread of C. jejuni among cattle and are not responsible for milk contamination. A relevant number of milk samples were contaminated by C. jejuni resistant to quinolones, representing a possible source of human resistant strains.*

Bianchini°V, Zugnoni A, Morabito S, Rossetti C, Ar dissino G, Luini°M

#### **La RAPD-PCR nello screening molecolare di Escherichia coli verocitotossici (VTEC) isolati da fonti animali, alimentari e umane**

XVI Congresso Nazionale SIDiLV : 30 Settembre - 2 Ottobre 2015 Montesilvano (PE) : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2015]. - p 336-337. - 6 bib ref [Nr. Estr. 7063]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (16. : Montesilvano (PE) : 30 Settembre - 2 Ottobre 2015)

*Molecular typing of bacterial isolates is effective for epidemiological surveillance. Here, a collection of 133 verocitotoxic Escherichia coli (VTEC) strains isolated from bovine, ovine, human and pigeon feces and foods, with a known virulence profile (stx1, stx2, eae) were investigated to: i) identify VTEC belonging to the 13 serogroups mainly associated to human disease; ii) evaluate the genetic relatedness among the strains using random amplified polymorphic DNA (RAPD)-PCR; iii) define the antibiotic susceptibility patterns. The majority of the strains didn't belong to any of the serogroups searched. Various antimicrobial resistance patterns were found. RAPD-PCR analysis allowed us to detect variation in VTEC strains of different origin. We observed a recurrence of RAPD profiles between different farms, and a prevalence of one profile per farm. RAPD-PCR is an easy and low-cost analysis which would be suitable for epidemiological studies.*

Bolzoni°G, Asmussen B

#### **National TBC-conversion in Italy : on the road to more uniform bacteria settlement!**

National TBC-conversion equation in Italy : 14 September 2015 / written by Berte Asmussen. - [s.l. : rawmilkconnect.dk, 2015]. - 5 p ( ultimo accesso 14/06/2016  
[http://www.iss.it/binary/latte/cont/Extracts\\_and\\_comments\\_to\\_the\\_Italian\\_conversion\\_report\\_september\\_2015\\_.pdf](http://www.iss.it/binary/latte/cont/Extracts_and_comments_to_the_Italian_conversion_report_september_2015_.pdf) ) [Nr. Estr. 7185]

Bolzoni°G, Baiguera°C, Zanardi°G, Buffoli°E

#### **Caratterizzazione della qualità del latte italiano: un primo passo**

XVI Congresso Nazionale SIDiLV : 30 Settembre - 2 Ottobre 2015 Montesilvano (PE) : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2015]. - p 274-275 [Nr. Estr. 7054]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (16. : Montesilvano (PE) : 30 Settembre - 2 Ottobre 2015)

*Every day different Italian laboratories test milk for quality, safety and technological characteristics. The number of data is massive, but currently there is not a standardized system to frame the national situation. To reach this aim we started with the collection and the statistical evaluation of monthly mean data of the most common analytical parameters, coming from 16 Italian laboratories (IIZZSS, regional breeding association and private labs), during 2013. The data derived from more than 600.000 bulk tank milk samples, collected twice a month, from about 20.000 dairy farms. The results give a picture of the Italian milk quality and its dynamic during the year. The increasing the number of labs, data and analytical parameters are the next step of the project; the real final goal will be the unification of analytical results of tank bulk milk sample with milk production of each herd, for more representative and realistic evaluation of national mean value.*

Bolzoni°G, Buffoli°E, Marcolini°A

#### **Pastorizzazione del latte e trattamento equivalente**

Latte. - Vol. 89 no 7 ( 2015). - p 18-21 [Nr. Estr. 7184]

Al fine di consentire la verifica e la dimostrazione dell'equivalenza dei trattamenti termici del latte (prevista dal reg. 2074/2005) è stato sviluppato un foglio di calcolo utilizzabile sia nell'ambito dei controlli interni di processo da parte di caseifici o industrie lattiero-casearie sia nel corso di ispezioni e verifiche da parte delle autorità sanitarie.

Bolzoni°G, Marcolini°A, Buffoli°E

**End of the derogations to Regulation (EC) 853/2004 for cow's milk in Italy**

Ital J Food Sci. - Vol. 27 ( 2015). - p 1-8. - 12 bib ref [Nr. Estr. 6018]

Derogations for somatic cell and total bacterial count limits had allowed non-compliant milk to be used for cheesemaking in Italy. Commercial and health considerations prompted a decision to implement a program to gradually repeal the derogations. In this study, we report the statistical evaluation of the situation in 2007-2008, the outcomes of the program to dose the derogation and observations of its effects during its implementation from 2010-2013 in the Lombardy region. The introduction of a progressive decrease of the limit allowed regulators to minimize the negative impact on production levels by focusing on the management of the most non-compliant farms first.

Bolzoni°G, Marcolini°A, Delle\_Donne°G, Appicciaf uoco B, Ferrini AM

**New national conversion line for Bactoscan FC in Italy : a step forward**

Ital J Food Sci. - Vol. 27 ( 2015). - p 191-197. - 13 bib ref [Nr. Estr. 6072]

To improve the reproducibility of flow cytometry technique for total bacterial count in milk, a conversion from instrumental results (impulseshtL) to the reference method resultes (cfu/mL) is needed. In 2008 in Italy, a project for a common conversion line for Bactoscan FC was initiated. In this paper we report on the second phase of the project focusing on the statistical procedure used to evaluate the validity of the data. The new conversion line, representative of national milk (2,732 valid samples from 29 labs) obtained from both rounds of the study is:  $\text{Logi}^\circ(\text{cfu mL}^{-1}) = \text{Logi}, (\text{IBC} \times 0.939 + 2.559, \text{ with } S_{y..} = 0.282 \text{ with an application range up to } 70,000 \text{ IBC}.$

Bolzoni°G, Marcolini°A, Delle\_Donne°G, Appiccia fuoco, Ferrini AM

**New Italian conversion line for the enumeration of total bacteria in raw milk with Bactoscan FC**

4th FOODSEG Symposium : 23th-24th of April 2015, Roma / [s.l. : s.n., 2015]. - 1 p [Nr. Estr. 7279]

FOODSEG Symposium (4th : Roma : 23th-24th of April 2015)

Bonardi S, Brindani F, Morganti°M, Alpigiani I, Ca vallini P, Barilli E, Bolzoni°L, Pongolini°S

**Isolation of Salmonella enterica in pigs at slaughter and genetic identity between isolates of porcine and human origin in Northern Italy**

ESPHM 2015 : 7th European Symposium of Porcine Health Management : 22-24 April, 2015 Nantes, France : proceedings / [s.l. : s.n., 2015]. - p 103 (Oral Presentation 034) [Nr. Estr. 7088]

European Symposium of Porcine Health Management (ESPHM) (7th : Nantes, France : 22-24 April, 2015)

Introduction: Salmonella spp. is a zoonotic microorganism responsible for food-borne disease worldwide. Even if the EU-trend is decreasing, salmonellosis is still the second most common food-borne disease in the European Union and swine are considered an important reservoir of microorganisms for humans. Materials and Methods: From June 2013 to October 2014, 201 pigs at slaughter were tested for Salmonella spp. in mesenteric lymph nodes. Pigs were reared in 61 farms

of four Italian regions (Lombardy, Emilia-Romagna, Veneto and Piedmont) and slaughtered in one abattoir in Emilia-Romagna region. A total of 67 batches of pigs were tested, and 3 animals per batch were randomly selected along the slaughter line. Prior to slaughter, 66 faecal samples were collected at lairage (two per sampling day). Salmonella spp. was detected following the ISO 6579:2002 method for lymph nodes and the ISO 6579:2002/Amd.1:2007 for faecal samples. Serotyping of isolates was performed following the Kauffmann White - Le Minoir scheme. Genotyping was carried out by PFGE after digestion of DNA with the restriction enzyme XbaI according to the Pulse Net protocol. A total of around 3.000 Salmonella isolates from hospitalized human patients of Emilia-Romagna region were typed from October 2011 to October 2014. They were linked to several salmonellosis outbreaks or single cases. Results: Overall, 39 lymph nodes (19.4%) and 33 faecal samples (50.0%) were found positive for Salmonella. The following Salmonella serovars were detected, listed in order of frequency: S. Derby, S. enterica 1, 4, [5], 12:i:-, S. Rissen, S. Brandenburg, S. Manhattan, S. London, S. Livingstone, S. Muenchen, S. Stanley, S. Give. All serovars except S. Stanley were isolated from human cases of infection. Comparing PFGE profiles between the porcine and human compartments, the largest proportion of pulsotypes that were present in both compartments was observed among the isolates of S. enterica 1, 4, [5], 12:i:- (7 out of the 10 porcine pulsotypes were present in the human isolates) whereas the smallest proportion of shared pulsotypes was observed among the isolates of S. Derby (6 out of the 15 porcine pulsotypes were present in the human isolates). Interestingly, pulsotypes of S. enterica 1, 4, [5], 12:i:- isolated from pigs did not always correspond to S. enterica 1, 4, [5], 12:i:- isolates in the human compartment, instead, in some cases they corresponded to the biphasic S. Typhimurium. Conclusion: This study confirms the role of pigs as reservoir of pathogenic Salmonella strains for humans. Future studies will be necessary to acquire better knowledge on the ecology of S. Typhimurium and its monophasic variant between the porcine and human compartments, considering the importance of these serotypes in human salmonellosis.

Buffoli°E, Gramaglia M, Ferrini AM, Martinelli°N, Bolzoni°G

**Determinazione della fosfatasi alcalina nel latte : stabilita' a lungo termine dei campioni** = Alkaline phosphatase determination in milk : long term stability of samples

Sci Tec Latt Casearia. - Vol. 66 no 3-4 ( 2015). - p 97-105. - 4 bib ref [Nr. Estr. 7280]

Con il presente lavoro si fornisce una indicazione circa la stabilità nel tempo dell'attività fosfatasica in campioni di latte crudo e pastorizzato conservati in congelatore a  $-18^{\circ}\text{C}$ . La conservazione è stata protratta per sei mesi, periodo considerato più che sufficiente a simulare le condizioni che si possono verificare nello svolgimento della procedura di revisione di analisi di campioni legali, non conformi, da parte del Laboratorio Nazionale di Riferimento dell' I.S.S. in applicazione della Legge 283/1962 e 441/1963, in tema di sicurezza alimentare. A partire dal 146° giorno, l'attività fosfatasica, eseguita con il metodo fluorimetrico (ISO 11816-1:2013), ha evidenziato un decremento statisticamente significativo, quantificabile tra il 10 e il 20%, rispetto al valore iniziale osservato sul latte refrigerato di partenza. Il calo è risultato più evidente nel latte crudo rispetto a quello pastorizzato. Sulla base dei risultati ottenuti, è stato possibile definire, prudenzialmente, come appropriato un limite massimo di conservazione in congelatore di 90 giorni per garantire risultati analitici affidabili in ambito di controllo ufficiale. Le nostre osservazioni dimostrano che, anche in caso di periodi di conservazione più prolungati, la parziale riduzione dell'attività fosfatasica permette di ottenere risultati analitici relativamente affidabili seppur con opportune interpretazioni in funzione del tipo di campione e dello scopo dell'analisi.

*The stability of alkaline phosphatase (ALP) activity in raw and pasteurized milk samples stored at  $-18^{\circ}\text{C}$  was tested over a period of six months to widely cover the maximum time allocated by the Italian Law n°283/1962 (modified by law n. 441/1963) to National Reference Lab (Istituto Superiore di Sanità), in case of "revisione analisi" (confirmation of results by the NRL on legal samples in case of not compliance with food legislation). Scope of the study was to evaluate the correct extent of time to perform the ALP determination according to ISO 11816-1 in frozen milk samples. The ALP activity decrease in frozen milk samples resulted significant starting from day 146 and was quantified under 10-20% of the initial values (more evident in raw milk). On the basis of these results, a conservative storage determination in the frame of the official control. Nevertheless, our*

*observations demonstrate that even more prolonged storage periods, regardless of the type of samples analyzed, produced only a partial decrease of ALP activity, allowing in any case the interpretation of the result.*

Cammi°G, Cosciani\_Cunico°E, Dalzini°E, Russo°S, Daminelli°P, Garbarino°C, Ricchi°M, Arrigoni°N

**Crescita di *Listeria monocytogenes* in tre diverse tipologie di frutta di IV gamma (melone, papaya e mela) durante il periodo di conservabilità**

Ricerca, sinergie e prospettive nel controllo degli alimenti : XXV Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI) : Sorrento, 28-30 Ottobre 2015 : abstract book / [s.l. : s.n., 2015]. - p 18-19 (C38) [Nr. Estr. 7166]

Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI) (25. : Sorrento : 28-30 Ottobre 2015)

Lo scopo del lavoro è stato quello di valutare il comportamento di *Listeria monocytogenes* in tre differenti tipologie di prodotti vegetali di IV gamma, rappresentati da papaya, melone e mela freschi, commercializzati pre-tagliati in vaschette, da conservarsi a temperatura di frigorifero per un periodo di 7 giorni (compreso il giorno di confezionamento). In relazione al valore di acidità della frutta, sono stati effettuati tre challenge test per verificare il potenziale di crescita (8) (meta a fette) e il tasso di crescita massimo ( $u_{max}$ ) (papaya e melone a pezzi) di *L. monocytogenes*, nei prodotti artificialmente contaminati; protocolli applicati sono stati quelli indicati dal Documento tecnico di orientamento per gli studi sulla vita commerciale degli alimenti pronti al consumo inerenti *Listeria monocytogenes* AFSSA/ EU Community Reference Laboratory For *Listeria monocytogenes* 2008. Il tasso massimo di crescita ( $wax$ ) di *L. monocytogenes*, calcolato grazie al software DMfit, sulla base del modello primario Baranyi, è risultato essere di 0.0445  $10g^{-1} 10 UFC/h$  (tempo di duplicazione 6 ore e 45 minuti) nella papaya a pezzi e di 0,0288  $log_{10} UFC/h$  (tempo di duplicazione 10 ore e 45 minuti) nel melone cubettato, conservati rispettivamente alla temperatura statica di 10 e 8°C. Inoltre, utilizzando il modello secondario Ratkowsky, è stato possibile stimare, per questi due prodotti, il tasso massimo di crescita a diverse temperature di conservazione (4, 6 e 12°C) per valutare in quanto tempo (ore e giorni), partendo da livelli ipotetici di contaminazione, viene raggiunto il limite massimo di 100 ufc/g di *L. monocytogenes*, ammesso dal Regolamento (CE) 2073/2005 per gli alimenti pronti per il consumo. Sia nel caso della papaya che del melone, solamente partendo da un livello di contaminazione iniziale estremamente contenuto (1 colonia di *L. monocytogenes*/25 g) e rispettando temperature di conservazione non superiori ai 6°C, si raggiunge il limite normato dal Reg. (CE) 2073/2005, in un tempo superiore a quello della shelf-life indicata per questi prodotti. Nella mela a fette il potenziale di crescita massimo (8) osservato è risultato essere di 0,93  $10g^{-1} 10 UFC/g$ , quindi superiore al limite di 0.5  $log_{10} 10 UFC/g$ , che caratterizza gli alimenti che non supportano lo sviluppo di *L. monocytogenes*. Anche la stima della crescita di *L. monocytogenes* effettuata mediante l'utilizzo di modelli predittivi, partendo da condizioni simili a quelle presenti nello studio sperimentale, ha evidenziato tassi di crescita superiori a quelli attestati. La papaya ed il melone si confermano essere substrati favorevoli allo sviluppo di *L. monocytogenes* che può raggiungere, nelle previste condizioni di conservazione di questi prodotti, livelli di contaminazione superiori a quelli consentiti a livello legislativo. Nella mela a fette, la crescita di *L. monocytogenes* è stata superiore a quella attesa per un alimento che, sulla base del valore di acidità, non viene considerato supportante lo sviluppo del microrganismo. I risultati dello studio confermano l'importanza dell'utilizzo del challenge test microbiologici per la valutazione della shelf-life dei prodotti ortofrutticoli freschi pronti per il consumo, al fine di una corretta gestione del rischio alimentare.

Casadio°M, Tosi°G, Massi°P, Mazzotti E, Fiorentini°L

**Valutazione del rischio d'inquinamento da aflatoxina M1, nel latte bovino destinato al consumo umano in Romagna** = Evaluation of the risk of Aflatoxin M1 contamination in bovine milk for human consumption in Romagna, Italy

Large Anim Rev. - Vol. 21 no 5 ( 2015). - p 187-190. -15 bib ref [Nr. Estr. 7098]



Le aflatossine sono micotossine prodotte da miceti del genere *Aspergillus*, in particolare *A. flavus* e *A. parasiticus*, più raro *A. nomius*. L'aflatossina M, (AM,) è un metabolita idrossilato di aflatossina B, (AB,) a livello epatico. AM, si può rilevare nel latte e suoi derivati, prodotti da animali che hanno consumato mangimi contaminati da AB,. Il Reg. (CE) n°1881/2006 stabilisce il tenore massimo per AM, nel latte 0,050 µg/kg (50 ppt). Scopo del presente lavoro è stato quello di valutare la presenza di AM, nel latte bovino di massa, prelevato da 68 allevamenti in Romagna (province Rimini, Forlì-Cesena, Ravenna) da gennaio 2011 a dicembre 2014 (4 anni) e destinato al consumo umano. Per determinare AM, nel latte è stato impiegato un test ELISA "I'SCREEN AFLA MI" (TECNA S.r.l., Trieste, Italy). Sono stati analizzati in totale 1728 campioni. La classe di valori maggiormente rappresentata è stata quella tra 5-25 ppt (62%), quindi al di sotto dei limiti di legge, seguita in ordine decrescente dalle classi: <5 ppt (32%), 26-50 ppt (5%) e >50 ppt, circa l'1%, corrispondente a 15 campioni. Tra questi ultimi, dieci sono stati rilevati nel secondo semestre degli anni presi in considerazione (da luglio a dicembre) e cinque nel primo (da gennaio a giugno). Solamente l'1% ha superato il limite di legge. Grazie al sistema di monitoraggio si può garantire un buon livello di sicurezza per il consumatore finale, compresi bambini e anziani.

*Aflatoxins are mycotoxins produced by Aspergillus species, especially A. flavus, A. parasiticus and A. nomius, which are abundant in areas where there is a hot humid climate. Aflatoxin M, (AM,) is a hydroxylated metabolite of Aflatoxin B, (AB,) that can occur in milk and milk products from animals consuming feed contaminated with B Aflatoxins. Human exposure to AM, at levels of nanograms per day occurs mainly through consumption of aflatoxin-contaminated milk. Aflatoxins are carcinogenic. The Commission Regulation (EC) n°1881 of 19 December 2006 sets maximum levels for certain contaminants in foodstuffs. The annex shows that the maximum level for AM, in raw milk, heat treated milk as well as milk for the manufacture of milk based products is 0,050 µg/kg (50 ppt). The aim of this paper is to present the results of AM, detection in tank cow milk samples collected from farms based in Romagna (Italy). This milk is destined for human consumption. From January 2011 to December 2014 (four years), 1728 raw cow milk samples were collected from 68 farms in Romagna (Rimini, Forlì-Cesena, Ravenna). In order to analyze these milk samples at IZSLER laboratory, a commercial ELISA kit "I'SCREEN AFLA M," was used for screening AM, concentration in milk. Data referred to that period show that the range of values mainly represented is 5-25 ppt (62%), under law maximum level. In decreasing order, it is followed by the range <5 ppt (32%), then 26-50 ppt (5%) and lastly >50 ppt (about 1%). Over the four-year period, the 15 samples (about 10/0) exceeding law maximum level were detected in the second half of the year (10 samples from July to December), while 5 were found in the first half of the year. These data show a good level of health safety for the consumer. Continuous monitoring is necessary to limit positive cases. Continuous sampling can monitor the concentration of AM,, thus helping to manage any emergency that might arise.*

Chiesa L, Pavlovic R, Dusi°G, Pasquale E, Casati A , Panseri S, Arioli F

**Determination of α- and β-boldenone sulfate, glucuronide and free forms, and androstadienedione in bovine urine using immunoaffinity columns clean-up and liquid chromatography tandem mass spectrometry analysis**

Talanta. - Vol. 131 ( 2015). - p 163-169. - 22 bib ref [Nr. Estr. 5859]

The debate about the origins of boldenone in bovine urine is ongoing for two decades in Europe. Despite the fact that its use as a growth promoter has been banned in the European Union (EU) since 1981, its detection in bovine urine, in the form of α-boldenone conjugate, is considered fully compliant up to 2 ng mL<sup>-1</sup>. The conjugated form of β-boldenone must be absent. In recent years, the literature about boldenone has focused on the identification of biomarkers that can indicate an illicit treatment. β-boldenone sulfate is a candidate molecule, even if the only studies currently available have taken place in small populations. In this study, a method for the determination of sulfate and glucuronate conjugates of β-boldenone was developed and validated according to the European Commission Decision 2002/657/EC and applied to α-boldenone sulfate and glucuronide, α- and β-boldenone free forms and androstadienedione (ADD), too. The clean-up with immunoaffinity columns enabled the direct determination of the conjugates and free forms and allowed specific and sensitive analyses of urine samples randomly selected to verify this method. The decision limits (CCα) ranged between 0.07 and 0.08 ng mL<sup>-1</sup>, the detection capabilities (CCβ)

between 0.08 and 0.1 ng mL<sup>-1</sup>. Recovery was higher than 92% for all the analytes. Intra-day repeatability was between 5.8% and 17.2%, and inter-day repeatability was between 6.0% and 21.8% for the studied free and conjugated forms. This method has been developed as a powerful tool with the aim to study the origin of boldenone in a trial on a significant number of animals.

Cosciani\_Cunico E, Dalzini°E, Ducoli°S, Sfamini° C, Bertasi°B, Losio°MN, Daminelli°P, Varisco°G

#### **Behaviour of *Listeria monocytogenes* and *Escherichia coli* O157:H7 during the cheese making of traditional raw-milk cheeses from Italian Alps**

Ital J Food Safety. - Vol. 4 no 3 ( 2015). - no 4585 (p 88-91). - 24 bib ref (ultimo accesso 10/06/2016 <http://www.pagepressjournals.org/index.php/ijfs/article/view/ijfs.2015.4585> ) [Nr. Estr. 7031]

The behaviour of *Listeria monocytogenes* and *Escherichia coli* O157:H7 was studied during the manufacture and ripening of two traditional Italian Alps cheeses. Each cheese type was manufactured in a pilot plant from raw cow milk (without the addition of starter cultures) artificially inoculated with *L. monocytogenes* and *E. coli* O157:H7 to a final concentration of about 4 log CFU/mL. The pathogens were enumerated throughout the cheese making and ripening processes to study their behaviour. When the milk was inoculated with 4 Log CFU/mL, the pathogens counts increased in the first time during the manufacturing process and then remained constant, until the end of ripening, or decreased significantly. Results indicate that the environment and nature of food borne pathogens affected the concentration of the bacteria during the manufacturing and ripening process. Thus, the presence of low cells numbers of *L. monocytogenes* and *E. coli* O157:H7 in milk destined for the production of raw milk cheeses characterized by a cooking of the curd less than 48°C can constitute a hazard for the consumer.

Dalipi R, Borgese L, Zacco A, Tsuji K, Sangiorgi°E , Piro R, Bontempi E, Depero LE

#### **Determination of trace elements in Italian wines by means of total reflection X-ray fluorescence spectroscopy**

Int J Environ Anal Chem. - Vol. 95 no 13 ( 2015). - p 1208-1218. - 28 bib ref ( ultimo accesso 05/07/2016 <http://www.tandfonline.com/doi/abs/10.1080/03067319.2015.1036861> ) [Nr. Estr. 6041]

International Symposium on Environmental Analytical Chemistry (38th: Lausanne : June 2014)  
Symposium on Chemistry and Fate of Modern Pesticides (14th : Ioannina : September 2014)

This work was performed to highlight the advantages of total reflection X-ray fluorescence spectroscopy (TXRF) for multi-elemental qualitative and quantitative analysis of wine. Indeed the International Organization of Vine and Wine (OIV) selected some potentially toxic elements and proposed limit values for their concentration in wines. Direct TXRF analysis of nine wine samples from Emilia Romagna region of Italy was performed in two different laboratories: Italy and Japan. Wine dehydration was also evaluated as sample conservation mean. Traces of Fe, Cu, Zn and Pb are present in all the analysed samples, with concentrations lower than the limits established by the OIV. The target hazard quotients (THQs) were also calculated for seven elements (Cd, Mn, Fe, Ni, Cu, Zn and Sr) to determine their potential detrimental effects. The results show that TXRF is a fast, simple and accurate analytical technique for trace element analysis of wine. Moreover, dehydration is an effective way to store wine samples for further elemental analysis.

Dalzini°E

**Sicurezza dei formaggi a latte crudo**

Setting a model for a sustainable dairy chain : 22 luglio 2015, EXPO2015, Milano : book of abstract / a cura del Gruppo operativo del progetto "Spazi espositivi per la Ricerca - Padiglione Italia EXPO 2015". - [Milano : s.n., 2015]. - p 49-52 [Nr. Estr. 6089]

Setting a model for a sustainable dairy chain : Milano : July 22nd, 2015)

Dalzini°E, Cosciani-Cunico°E, Bernini V, Bertasi° B, Losio°MN, Daminelli°P, Varisco°G

**Behaviour of Escherichia coli O157 (VTEC), Salmonella Typhimurium and Listeria monocytogenes during the manufacture, ripening and shelf life of low fat salami**

Food Control. - Vol. 47 ( 2015). - p 306-311. - 44 bib ref [Nr. Estr. 5786]

The aim of this study was to evaluate the behaviour of Escherichia coli, Salmonella Typhimurium and Listeria monocytogenes in an innovative semi-dry reduced fat Italian salami. The product is made from pork meat and it is characterized by less than 20% fat, lactose-, gluten- and milk protein-free. It is developed in Italy according to EC Regulation No. 1924/2006 "on nutrition and health claims made on foods". Multi-strain cocktails of each pathogen were used to inoculate (5 log cfu g<sup>-1</sup>) separately the salami batter. During the manufacture and ripening, E. coli, S. Typhimurium, and L. monocytogenes decreased by 2.5, 1.65 and 0.5 log cfu g<sup>-1</sup> respectively from the initial inoculated levels. Experimental data indicated that, during the shelf life in a condition of moderate thermal abuse (8–12 °C), the portioned and vacuum packed low fat salami are not able to support the growth of L. monocytogenes.

Dalzini°E, Cosciani-Cunico°E, Monastero°P, Varisco°G, Losio°MN, Daminelli°P

**Microbial interactions in Blue-veined Cheese : effect on Listeria monocytogenes behavior and shelf-life prediction**

European Symposium on Food Safety : 20-22 April 2015, Cardiff, Wales / [s.l. : s.n., 2015]. - p 81-82 [Nr. Estr. 6034]

European Symposium on Food Safety (IAFP) : Cardiff, Wales : 20-22 April 2015)

Introduction: Among dairy products, blue-veined cheeses are known to be the most frequently contaminated with Listeria spp. This can be due to process contamination (during the piercing at the beginning of the ripening or during the cut at the beginning of the shelf life). The development of a complex microbial community (lactic acid bacteria, yeasts and molds) which metabolize different compounds, modulate the pH values that can affect the pathogen behavior. Purpose: The objectives of this study were to i) investigate the effect of the physicochemical changes on the L. monocytogenes behavior during the cheesemaking, ii) study the pathogen behavior during the shelf life assuming different time contamination: at the beginning of the ripening and at the beginning of the shelf life, and iii) predict the shelf life with different hypothetical scenarios. Methods: Two challenge tests were carried out with L. monocytogenes: (i) by cow milk contamination to evaluate the pathogen behavior during the process, and (ii) by contamination of portioned cheese to evaluate the growth pathogen during the shelf life. For microbiological analysis, counts were estimated by plate count. The growth curves of L. monocytogenes were fitted using DMFit web edition, in order to predict the shelf life at different storage temperature. Results: The pH value decreases by  $6.65 \pm 0.02$  in milk to  $4.93 \pm 0.01$  in cheese (1 d); during the ripening the molds growth (from  $2.77 \pm 0.01$  to  $6.45 \pm 0.39$  log CFU/g) causes an increase of the pH until  $6.64 \pm 0.14$ , thus aiding development of L. monocytogenes. During the shelf life, the pathogen was able to grow in cheese in both contamination time, but with difference growth rates. Significance: The present data can be a useful tool for the quantitative risk assessment for Listeria in blue-veined cheese and to study alternative ways to reduce the Listeria contamination in this type cheese.

Daminelli°P, Bontempi°G, Dalzini°E, Todeschi°S, Marina\_Nadia L [i.e. Losio°MN], Varisco°G

**La rete degli II.ZZ.SS. oltre EXPO 2015 : nuove proposte per la sicurezza degli alimenti**

XVI Congresso Nazionale SIDiLV : 30 Settembre - 2 Ottobre 2015 Montesilvano (PE) : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2015]. - p 288-289. - 5 bib ref [Nr. Estr. 7065]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (16. : Montesilvano (PE) : 30 Settembre - 2 Ottobre 2015)

*Expo 2015 with the theme "Feeding the Planet, Energy for Life" is an essential showcase through which the National Health System, and in particular II.ZZ.SS network wants to present itself as a reference point for consumers and food business operators in the management and definition of the risk analysis principles. The collaboration between II.ZZ.SS. and ISS will benefit from updated databases that allow processing of data scientifically expendable: objective, as indicated by the Reg. EC 178/2002, achievable through updating an information system, which is Ars Alimentaria. The creation of a network of II. ZZ. SS. and ISS is a key tool to ensure a uniform approach in the risk analysis strategy, wanting to provide a path able to respond to major in the field of food safety, professionalism, objectivity, transparency, independence, responsiveness, meliness*

Di\_Bartolo I, Pavoni°E, Tofani S, Consoli°M, Galu ppini°E, Losio°MN, Ruggeri FM, Varisco°G

**Waterborne norovirus outbreak during a summer excursion in Northern Italy**

New microbiol. - Vol. 38 ( 2015). - p 109-112. - 18 bib ref [Nr. Estr. 6001]

In September 2011, an acute gastroenteritis outbreak affected 33 children in Northern Italy. Patients had drunk river water during an excursion. Identical GI.4 norovirus genomes were detected from one patient's stools and from the river water. Improper discharge of human sewage into the river may have caused this waterborne outbreak.

Fedrizzi°G, Caprai°E, Accurso°D, Caschetto°MG, Padovani A, Calò L, Menotta°S

**Mycotoxins liquid chromatographic-tandem mass spectrometric analysis in food matrices : 3-years of surveillance**

4th MS-Food Day : October 07-09, 2015, Foggia : book of abstracts / [s.l. : s.n, 2015]. - p 161-163 - 5 bib ref [Nr. Estr. 7125]

Mass Spectrometry Food Day (4th : Foggia : October 07-09, 2015)

Ferri R, Hashim D, Smith DR, Guazzetti S, Donna F, Ferretti°E, Curatolo°M, Moneta°C, Beone GM, Lucchini RG

**Metal contamination of home garden soils and cultivated vegetables in the province of Brescia, Italy : implications for human exposure**

Sci Total Environ. - Vol. 518-519 ( 2015). - p 507-517. - 64 bib ref [Nr. Estr. 6056]

Background: For the past century, ferroalloy industries in Brescia province, Italy produced particulate emissions enriched in manganese (Mn), lead (Pb), zinc (Zn), copper (Cu), cadmium(Cd), chromium(Cr), iron (Fe), and aluminum (Al). This study assessed metal concentrations in soil and vegetables of regions with varying ferroalloy industrial activity levels. Methods: Home gardens

(n=63) were selected in three regions of varying ferroalloy plant activity durations in Brescia province. Total soil metal concentration and extractability were measured by X-Ray Fluorescence (XRF), aqua regia extraction, and modified Community Bureau of Reference (BCR) sequential extraction. Unwashed and washed spinach and turnips cultivated in the same gardens were analyzed for metal concentrations by flame atomic absorption spectrometry. Results: Median soil Al, Cd, Fe, Mn, Pb, and Zn concentrations were significantly higher in home gardens near ferroalloy plants compared to reference home gardens. The BCR method yielded the most mobile soil fraction (the sum of extractable metals in Fractions 1 and 2) and all metal concentrations were higher in ferroalloy plant areas. Unwashed spinach showed higher metal concentrations compared to washed spinach. However, some metals in washed spinach were higher in the reference area likely due to history of agricultural product use. Over 60% of spinach samples exceeded the 2- to 4-fold Commission of European Communities and Codex Alimentarius Commission maximum Pb concentrations, and 10% of the same spinach samples exceeded 2- to 3-fold maximum Cd concentrations set by both organizations. Turnip metal concentrations were below maximum standard reference values. Conclusions: Prolonged industrial emissions increase median metal concentrations and most soluble fractions (BCR F1 + F2) in home garden soils near ferroalloy plants. Areas near ferroalloy plant sites had spinach Cd and Pb metal concentrations several-fold above maximum standard references. We recommend thorough washing of vegetables to minimize metal exposure.

Formenti N, Trogu T, Gaffuri<sup>o</sup>N, Viganò R, Besozzi M, Ferrari N, Lanfranchi PI

**Seroprevalence of *Toxoplasma gondii* in game ungulates as indicator of foodborne zoonoses risk**

EFSA J. - Vol. Suppl ( 2015). - p 119-120 (Poster n. 217) [Nr. Estr. 7188]

EFSA scientific conference "Shaping the future of food safety, together" (2nd : Milan, Italy : 14-16 October 2015)

Objectives: The protozoan *Toxoplasma gondii* is the most spread among parasitic zoonoses, and the consumption of raw or undercooked meat have been shown to be one of the main risk factor for human infection. *T.gondii* can infect many animal species and, among intermediate hosts, several ungulates are reported and may be source of infection for consumers, hunters and slaughterers (manipulation and handling of carcasses). Therefore, we performed a serological analysis in chamois (*Rupicapra r. rupicapra*), roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and mouflon (*Ovis musimon*), which represent the most consumed game meat in the Alps, in order to evaluate the potential foodborne zoonotic risk. Material and methods: Sera of game ungulates were gathered from two areas of Central-West Italian Alps. Overall 91 chamois, 74 roe deer and 63 red deer sera were sampled from area 1 (VB) during three hunting seasons (2011-2013) while 66 chamois, 44 roe deer, 25 red deer and 13 mouflon were sampled in area 2 (VC) during two hunting seasons (2013-2014). For each subject age, gender and the shooting localities were registered. Sera were tested by a commercial ELISA kit and data were analysed through Generalized Linear Models. Results: Prevalence of area 1 were 3.3% in chamois, 24.3% in roe deer and 17.4% in red deer. Deer resulted significantly more infected than chamois. No significant effects of gender, age class, shooting localities and year were recorded on the probability of being positive. Prevalence of area 2 were 4.5% in chamois, 13.6% in roe deer, 8% in red deer and 46% in mouflon. Mouflon resulted significantly more infested than chamois. No other significant effects were recorded. Conclusions: The emerged seropositivities proves the presence of *T gondii* in both study areas, in all the host species. Thus a wide spread of the protozoan in the Alpine ecosystem appears. As the contamination of pastures by cats' oocysts is the more likely transmission route, even if the transplacental one can not be excluded, the remote habitat-use of chamois could explain its lower infection than both deer and mouflon. Concerning zoonotic risk, mouflon and deer appear a more likely source of *T gondii* infection for humans than chamois, although the risk associated with the consumption of its raw or undercooked meat or handling of its infected carcasses can not be completely excluded.

Franco A, Leekitcharoenphon P, Feltrin F, Alba P, Cordaro G, Iurescia M, Tolli R, D'Incau M, Staffolani M, Di\_Giannatale E, Hendriks en RS, Battisti A

**Emergence of a clonal lineage of multidrug-resistant ESBL-producing salmonella infantis transmitted from broilers and broiler meat to humans in Italy between 2011 and 2014**

PLoS One. - Vol. 10 no 12 ( 2015). - p e0144802 (15 p). - 36 bib ref (ultimo accesso 06/07/2016 <http://www.plosone.org/article/fetchObject.action?uri=info:doi/10.1371/journal.pone.0144802&representation=PDF>) [Nr. Estr. 7200]

We report the spread of a clone of multidrug-resistant (MDR), ESBL-producing (blaCTX-M-1) *Salmonella enterica* subsp. *enterica* serovar *Infantis*, in the Italian broiler chicken industry and along the food-chain. This was first detected in Italy in 2011 and led to human infection in Italy in 2013–2014. A set (n = 49) of extended-spectrum cephalosporin (ESC)-resistant (R) isolates of *S. Infantis* (2011–2014) from humans, food-producing animals and meat thereof, were studied along with a selected set of earlier and more recent ESC-susceptible (ESC-S) isolates (n = 42, 2001–2014). They were characterized by macrorestriction-PFGE analysis and genetic environment of ESC-resistance. Isolates representative of PFGE-patterns and origin were submitted to Whole Genome Sequencing. The emerging ESC-R clone, detected mainly from broiler chickens, broiler meat and humans, showed a minimum pattern of clinical resistance to cefotaxime, tetracycline, sulfonamides, and trimethoprim, beside ciprofloxacin microbiological resistance (MIC 0.25 mg/L). All isolates of this clone harbored a conjugative megaplasmid (~ 280–320 Kb), similar to that described in ESC-susceptible *S. Infantis* in Israel (pESI-like) in 2014. This megaplasmid carried the ESBL gene blaCTX-M-1, and additional genes [tet(A), sul1, dfrA1 and dfrA14] mediating cefotaxime, tetracycline, sulfonamide, and trimethoprim resistance. It also contained genes conferring enhanced colonization capability, virulence (fimbriae, yersiniabactin), resistance and fitness (qacE1, mer) in the intensive-farming environment. This emerging clone of *S. Infantis* has been causing infections in humans, most likely through the broiler industry. Since *S. Infantis* is among major serovars causing human infections in Europe and is an emerging non-typhoidal *Salmonella* globally, further spread of this lineage in primary productions deserves quick and thorough risk-management strategies.

Franzini G, Carra E, Baldi D, Naldi S, Morganti M, Merialdi G, Bergamini F, Losio N, Pongolini S, Gattuso A, Rugna G

**Caratterizzazione di *Listeria monocytogenes* in due macelli suini : prevalenza, profilo molecolare (PFGE) e pattern di contaminazione**

Ricerca, sinergie e prospettive nel controllo degli alimenti : XXV Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI) : Sorrento, 28-30 Ottobre 2015 : abstract book / [s.l. : s.n., 2015]. - p 37-38 (Poster P36) [Nr. Estr. 7277]

Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI) (25. : Sorrento : 28-30 Ottobre 2015)

Galarini R, Dusi G, Pellicciotti S, Giusepponi D, Romanelli S, Rossi R, Saluti G, Moretti S

**Innovative approach to detect residues of antibiotics in food**

Atti del LXIX Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : XII Convegno AIPVet, Il Convegno RNIV, XV Convegno SICV, XIII Convegno SIRA, XI Convegno So.Fi.Vet : Perugia, 15-17 Giugno 2015 / [s.l. : s.n., 2015]. - p 224-225. - 2 bib ref [Nr. Estr. 7015]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 69 Convegno AIPVet : 12 Convegno RNIV : 2 Convegno SICV : 15 Convegno SIRA : 13 Convegno So.Fi.Vet : 11 : Perugia : 15-17 Giugno 2015)

For the first time in Italy an instrumental multiclass method was developed and validated for the

simultaneous determination of 62 veterinary drugs belonging to ten different families in meat and milk. Samples were extracted and analysed by liquid chromatography coupled to a hybrid high resolution mass spectrometry. The results of a preliminary survey carried out on meat samples collected from local markets demonstrated the fundamental role of multiclass methods in the residue control of veterinary drugs. Several classes of antibiotics are normally used in farm to treat or prevent diseases, but they can also be illegally used posing a risk of residues occurrence in products. Therefore, control laboratories have to manage a considerable number of samples and analyse a large number of analytes. In this context, multiclass approaches are of great interest and in the last decade they have become reality thanks to the widespread diffusion of instruments based on mass-spectrometry technology. Aim is to develop and validate a multiclass method for antibiotic determination in food covering screening, confirmatory and quantification functions. Aim is to develop and validate a multiclass method for antibiotic determination in food covering screening, confirmatory and quantification functions. Sample preparation (muscle and milk) consisted in the extraction with a mixture of acetonitrile and water. The redissolved extracts were injected in a Thermo Ultimate 3000 Ultra High Performance Liquid Chromatography system coupled to a Thermo high resolution Q-Exactive mass analyzer (Thermo Scientific, Bremen, Germany). The acquisition (positive ionization) was achieved in full scan mode (screening) and in data depending scan for confirmatory purposes. The chromatographic separation was performed in gradient mode within 30 minutes. Sixty-two antibiotics belonging to ten different drug families (amphenicols, beta-lactams, diamino- pyrimidine, lincosamides, macrolides, pleuromutilins, quinolones, rifamycins, sulphonamides and tetracyclines) have been successfully included in the scope of the method. The list of analytes has been preliminary selected considering both veterinary practices and EU Regulation 37/2010 [1]. Its performance characteristics were compliant with the European criteria [2]. Seventy-one bovine meat samples have been collected at retail and analysed. The bad news is that, regardless their origin, the calf meats ( $\leq$  8 months of age) were largely contaminated (30%). The good news is that all positive samples were compliant, i.e. containing concentrations lower than the fixed Maximum Residue Limits, MRLs [1]. Tetracycline was the most found antibiotic class. The proposed method largely improves the control of antibiotic residues in food and it could replace the combination of microbiological tests and instrumental single-class procedures currently used. This approach can finally provide an analytical support based on risk assessment principles improving the cost-effectiveness of food safety policies. Studies are in progress to evaluate the method applicability to other food of animal origin.

Gallina S, Bianchi DM, Ru G, Maurella C, Barzanti P, Baioni E, Virgilio S, Mioni R, Lanni L, Migliazzo A, Losio MN, Bove D, Scuota S, Goffredo E, Decastelli L

**Microbiological recovery from bovine, swine, equine, and ovine carcasses : comparison of excision, sponge and swab sampling methods**

Food Control. - Vol. 50 ( 2015). - p 919-924. - 25 bib ref [Nr. Estr. 5908]

Legislation introduced under European Commission Regulation (EC) n° 2073/2005 and later amendments (Reg. 1441/2007/EC, Reg. 365/2010/EC, Reg. 1089/2011/EC, Reg. 209/2013/EC) mandates that food business operators carry out microbiological analyses on meat carcass surfaces after slaughter procedures as part of hygiene monitoring of production. Besides setting forth general rules for sampling and sample preparation, Regulation EC 2073/2005 requires that operators comply with ISO 17604, which lists destructive and non-destructive sampling methods, selection of sampling sites, and rules for sample storage and transport. This study compares the effectiveness of destructive (excision) and non-destructive (sponge and wetdry swabbing) methods for the recovery of total viable count (TVC) and Enterobacteriaceae on carcass surfaces. To do this, we pooled samples collected from carcasses of four animal species (cattle, n° 120; pigs, n° 130; horses, n° 84; and small ruminants [sheep and goats], n° 121). TVC and Enterobacteriaceae were enumerated and compared for each sampling method. Microbiological analyses were performed according to ISO 4833:2003 for TVC and ISO 21528:2004 for Enterobacteriaceae. The effectiveness of the sampling methods was analyzed by comparing the differences between the median of colony forming units per square centimeter (CFU/cm<sup>2</sup>) for TVC and Enterobacteriaceae recovered by each method. Non-parametric analysis of variance for repeated measures was applied for each species separately. Excision was the most effective method. The relationship between the CFU recovered by

swabbing, by sponge, and by excision, for all species, was generally better than 1:5. This is in contrast with the Italian Ministry of Health Memorandum (23 December 2002), which states that non-destructive methods recover 20% of the destructive method.

Galuppini°E, Suffredini E, Bertasi°B, Mangeri°L, Meletti°F, Di\_Pasquale S, Delibato E, De\_Medici D, Losio°MN

**Confronto di metodi alternativi alla ISO 15216-2:2013 per la concentrazione di epatite A da matrici vegetali**

XVI Congresso Nazionale SIDiLV : 30 Settembre - 2 Ottobre 2015 Montesilvano (PE) : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2015]. - p 68-69. - 3 bib ref [Nr. Estr. 7066]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (16. : Montesilvano (PE) : 30 Settembre - 2 Ottobre 2015)

*Hepatitis A is an important problem of public health all over the world. Outbreaks of HAV are mostly associated with three categories of food: shellfish and seafood eaten raw, soft fruits and vegetables contaminated by irrigation water or by infected people, and prepared foods. Analysis are commonly performed by ISO/TS 15216-2:2013 that, particularly for the preparation of vegetables samples, requires large volumes for virus elution and several analytical steps for its concentration. The aim of this work was to evaluate different sample preparation methods, compared with the ISO method.*

Giacometti F, Bonilauri°P, Albonetti S, Amatiste S, Arrigoni°N, Bianchi M, Bertasi°B, Bilei S, Bolzoni°G, Cascone G, Comin D, Daminel li°P, Decastelli L, Meriardi°G, Mioni R, Peli A, Petruzzelli A, Tonucci°F, Bonerba E, Serraino A

**Quantitative risk assessment of human salmonellosis and listeriosis related to the consumption of raw milk in Italy**

J Food Prot. - Vol. 78 no 1 ( 2015). - p 13-21. - 30 bib ref [Nr. Estr. 6000]

Two quantitative risk assessment (RA) models were developed to describe the risk of salmonellosis and listeriosis linked to consumption of raw milk sold in vending machines in Italy. Exposure assessment considered the official microbiological records monitoring raw milk samples from vending machines performed by the regional veterinary authorities from 2008 to 2011, microbial growth during storage, destruction experiments, consumption frequency of raw milk, serving size, and consumption preference. Two separate RA models were developed: one for the consumption of boiled milk and the other for the consumption of raw milk. The RA models predicted no human listeriosis cases per year either in the best or worst storage conditions and with or without boiling raw milk, whereas the annual estimated cases of salmonellosis depend on the dose-response relationships used in the model, the milk storage conditions, and consumer behavior in relation to boiling raw milk or not. For example, the estimated salmonellosis cases ranged from no expected cases, assuming that the entire population boiled milk before consumption, to a maximum of 980,128 cases, assuming that the entire population drank raw milk without boiling, in the worst milk storage conditions, and with the lowest dose-response model. The findings of this study clearly show how consumer behavior could affect the probability and number of salmonellosis cases and in general, the risk of illness. Hence, the proposed RA models emphasize yet again that boiling milk before drinking is a simple yet effective tool to protect consumers against the risk of illness inherent in the consumption of raw milk. The models may also offer risk managers a useful tool to identify or implement appropriate measures to control the risk of acquiring foodborne pathogens. Quantification of the risks associated with raw milk consumption is necessary from a public health perspective.



Giacometti F, Bonilauri<sup>°</sup>P, Amatiste S, Arrigoni<sup>°</sup>N, Bianchi M, Losio<sup>°</sup>MN, Bilei S, Cascone G, Comin D, Daminelli<sup>°</sup>P, Decastelli L, Merialdi<sup>°</sup>G, Mioni R, Peli A, Petruzzelli A, Tonucci F, Piva S, Serraino A

**Human campylobacteriosis related to the consumption of raw milk sold by vending machines in Italy : quantitative risk assessment based on official controls over four years**

Prev Vet Med. - Vol. 121 ( 2015). - p 151-158. - 39 bib ref [Nr. Estr. 6083]

A quantitative risk assessment (RA) model was developed to describe the risk of campylobacteriosis linked to consumption of raw milk sold in vending machines in Italy. Exposure assessment was based on the official microbiological records of raw milk samples from vending machines monitored by the regional Veterinary Authorities from 2008 to 2011, microbial growth during storage, destruction experiments, consumption frequency of raw milk, serving size, consumption preference and age of consumers. The differential risk considered milk handled under regulation conditions (4 °C throughout all phases) and the worst time-temperature field handling conditions detected. Two separate RA models were developed, one for the consumption of boiled milk and the other for the consumption of raw milk, and two different dose response (D–R) relationships were considered. The RA model predicted no human campylobacteriosis cases per year either in the best (4 °C) storage conditions or in the case of thermal abuse in case of boiling raw milk, whereas in case of raw milk consumption the annual estimated campylobacteriosis cases depend on the dose-response relationships used in the model (D–R I or D–R II), the milk time-temperature storage conditions, consumer behaviour and age of consumers, namely young (with two cut-off values of =5 or =6 years old for the sensitive population) versus adult consumers. The annual estimated cases for young consumers using D–R II for the sensitive population (=5 years old) ranged between 1013.7/100,000 population and 8110.3/100,000 population and for adult consumers using D–R I between 79.4/100,000 population and 333.1/100,000 population. Quantification of the risks associated with raw milk consumption is necessary from a public health perspective and the proposed RA model represents a useful and flexible tool to perform future RAs based on local consumer habits to support decision-making on safety policies. Further educational programmes for raw milk consumers or potential raw milk consumers are required to encourage consumers to boil milk to reduce the associated risk of illness.

2.

Giacometti F, Losio<sup>°</sup>MN, Daminelli<sup>°</sup>P, Cosciani-Cunzio<sup>°</sup>E, Dalzini<sup>°</sup>E, Serraino A

**Arcobacter butzleri and Arcobacter cryaerophilus survival and growth in artisanal and industrial ricotta cheese**

J Dairy Sci. - Vol. 98 no 10 ( 2015). - p 6776-6781. - 38 bib ref [Nr. Estr. 7079]

Ricotta cheese is a ready-to-eat product with properties (pH >6.0, aw >0.98–0.99) and moisture content (75–80%) that may pose a risk to public health due to postprocess contamination by several bacterial pathogens, including Arcobacters. The objective of the study was to evaluate the behavior of Arcobacter butzleri and Arcobacter cryaerophilus in ricotta cheese during its shelf life assuming postprocessing contamination. Two types of ricotta cheese, artisanal water buffalo (WB) and industrial cow milk ricotta cheese, were experimentally contaminated with A. butzleri and A. cryaerophilus and the count was monitored at 2 different temperatures (6°C and 12°C) during shelf life of 5 d for WB cheese and 22 d for industrial ricotta cheese. In WB ricotta cheese the A. butzleri count remained stable during the 5 d of storage at 6°C, whereas a moderate but significant decrease was observed in A. cryaerophilus count. The counts of both species increased when WB ricotta cheese was stored at 12°C. In industrial ricotta cheese stored at 6°C, a significant reduction was observed both in A. butzleri and A. cryaerophilus counts during the 22-d storage period; at 12°C storage, a count increase was observed for both Arcobacter species up to d 14 of storage after which the log cfu/g count resulted constant until d 22 of storage. The ability of A. butzleri and A. cryaerophilus to survive at 6°C and to grow at 12°C in ricotta cheese has significant food safety implications.

Giacometti F, Lucchi A, Di\_Francesco A, Delogu M, Grilli E, Guarniero I, Stancampiano L, Manfreda G, Meriardi°G, Serraino A

**Arcobacter butzleri, Arcobacter cryaerophilus, and Arcobacter skirrowii circulation in a dairy farm and sources of milk contamination**

Appl Environ Microbiol. - Vol. 81 no 15 ( 2015). - p 5055-5063. - 40 bib ref [Nr. Estr. 6092]

Even though dairy cows are known carriers of Arcobacter species and raw or minimally processed foods are recognized as the main sources of human Arcobacter infections in industrialized countries, data on Arcobacter excretion patterns in cows and in milk are scant. This study aimed to identify potentially pathogenic Arcobacter species in a dairy herd and to investigate the routes of Arcobacter transmission among animals and the potential sources of cattle infection and milk contamination. A strategy of sampling the same 50 dairy animals, feed, water, and milk every month for a 10-month period, as well as the sampling of quarter milk, animal teats, the milking environment, and animals living on the farm (pigeons and cats), was used to evaluate, by pulsed-field gel electrophoresis (PFGE), the characteristic patterns in animals, their living environment, and the raw milk they produced. Of the 463 samples collected, 105 (22.6%) were positive for Arcobacter spp. by culture examination. All the matrices except quarter milk and pigeon gut samples were positive, with prevalences ranging from 15 to 83% depending on the sample. Only three Arcobacter species, Arcobacter cryaerophilus (54.2%), A. butzleri (34.2%), and A. skirrowii (32.3%), were detected. PFGE analysis of 370 isolates from positive samples provided strong evidence of Arcobacter circulation in the herd: cattle likely acquire the microorganisms by orofecal transmission, either by direct contact or from the environment, or both. Water appears to be a major source of animal infection. Raw milk produced by the farm and collected from a bulk tank was frequently contaminated (80%) by A. butzleri; our PFGE findings excluded primary contamination of milk, whereas teats and milking machine surfaces could be sources of Arcobacter milk contamination.

Grilli E, Foresti F, Fustini M, Zanoni°MG, Pasqual i P, Callaway TR, Piva A , Alborali°GL

**Microencapsulated sorbic acid and pure botanicals affect Salmonella typhimurium shedding in pigs : a close-up look from weaning to slaughter in controlled and field conditions**

Foodborne Pathog Dis. - Vol. 12 no 10 ( 2015). - p 813-819. - 25 bib ref [Nr. Estr. 7128]

The aim of this study was to assess the efficacy of a combination of sorbic acid, thymol, and carvacrol in reducing the prevalence and shedding level of Salmonella Typhimurium in pigs either in a controlled challenge environment or in a production setting. In the first study, 24 weaned piglets were separated in 4 isolation units (6 piglets/isolation unit). Each unit received either a basal diet (no treatment) or a microencapsulated mixture of sorbic acid, thymol, and carvacrol at 1, 2, or 5 g/kg of feed. After 21 d, pigs were orally challenged with 6 log<sub>10</sub> colony-forming units of Salmonella Typhimurium. Blood samples and feces from rectal ampullae were collected every week. On d56 of the study, pigs were euthanized and necropsied to collect intestinal contents (jejunum through colon) and ileocecal lymph nodes. Samples were analyzed for Salmonella Typhimurium and serological analysis was also conducted. In the second study, an all-in-all-out multisite pig farm that was positive for monophasic Salmonella Typhimurium was followed throughout a production cycle from weaning to slaughter. Pigs received either a basal diet or the basal diet including 5 g/kg of the microencapsulated additive. Environmental, fecal, and blood samples were collected monthly, and cecal contents and ileocecal lymph nodes were collected at slaughter to isolate and enumerate Salmonella. The results indicate that the additive at 5 g/kg tended to reduce Salmonella fecal prevalence in both a controlled challenge ( p = 0.07) and in production conditions ( p = 0.03). Nevertheless, the additive did not reduce the number of pigs seropositive for Salmonella, nor it reduced the Salmonella prevalence at slaughter. The data indicate that these additives are not effective alone but must be used in conjunction with appropriate containment measures at lairage in order to prevent reinfection in pigs and to reduce the number of pigs carrying Salmonella entering

the food chain.

Losio<sup>°</sup>MN, Bozzo G, Galuppini<sup>°</sup>E, Vito M, Bertasi<sup>°</sup> B, Pavoni<sup>°</sup>E, Finazzi<sup>°</sup>G  
**Silter cheese, a traditional Italian dairy product : a source of feasible probiotic strains**  
Int J Food Properties. - Vol. 18 ( 2015). - p 492-498. - 29 bib ref [Nr. Estr. 5931]

Silter cheese is a traditional hard cheese, produced in Valcamonica, Brescia, Italy. A total of 426 lactic strains isolated from Silter were analysed to determine their probiotic characteristics. 274 strains out of 426 were found to produce bacteriocins against at least one of eight different pathogens (Salmonella enterica, Listeria monocytogenes, Salmonella derby, Salmonella thymimurium, Salmonella napoli, Staphylococcus aureus, E. coli O157:H7, Salmonella enteritidis). In addition, 211 of 274 bacteriocin-producer strains adhered to Caco-2 cells and were characterized by RiboPrinter, revealing predominance of Enterococcus faecalis (26%) and Enterococcus durans-faecium (22%). These findings suggest that Silter may qualify as an important source of feasible probiotic strains.

Losio<sup>°</sup>MN, Pavoni<sup>°</sup>E, Bilei S, Bertasi<sup>°</sup>B, Bove D, Capuano F, Farneti S, Blasi G, Comin D, Cardamone C, Decastelli L, Delibato E, De\_Santis P, Di\_Pasquale S, Gattuso A, Goffredo E, Fadda A, Pisanu M, De\_Medici D  
**Microbiological survey of raw and ready-to-eat leafy green vegetables marketed in Italy**  
Int J Food Microbiol. - Vol. 210 ( 2015). - p 88-91. - 20 bib ref [Nr. Estr. 6075]

The presence of foodborne pathogens (Salmonella spp., Listeria monocytogenes, Escherichia coli O157:H7, thermotolerant Campylobacter, Yersinia enterocolitica and norovirus) in fresh leafy (FL) and ready-to-eat (RTE) vegetable products, sampled at random on the Italian market, was investigated to evaluate the level of risk to consumers. Nine regional laboratories, representing 18 of the 20 regions of Italy and in which 97.7% of the country's population resides, were involved in this study. All laboratories used the same sampling procedures and analytical methods. The vegetable samples were screened using validated real-time PCR (RT-PCR) methods and standardized reference ISO culturing methods. The results show that 3.7% of 1372 fresh leafy vegetable products and 1.8% of 1160 "fresh-cut" or "ready-to-eat" (RTE) vegetable retailed in supermarkets or farm markets, were contaminated with one or more foodborne pathogens harmful to human health.

Menotta<sup>°</sup>S, Abbaleo<sup>°</sup>S, Zerbini<sup>°</sup>S, Padovani A, Garofalo Q, Cuciniello G, Fedrizzi<sup>°</sup>G

**Migration of melamine and formaldehyde from tableware**

4th MS-Food Day : October 07-09, 2015, Foggia : book of abstracts / [s.l. : s.n., 2015]. - p 124-127 - 8 bib ref [Nr. Estr. 7126]

Mass Spectrometry Food Day (4th : Foggia : October 07-09, 2015)

Menotta<sup>°</sup>S, Cannavacciuolo<sup>°</sup>A, Accurso<sup>°</sup>D, Piana L, Naldi G, Fedrizzi<sup>°</sup>G  
**Contaminanti ambientali e neonicotinoidi nel miele e nel polline**

Ricerca, sinergie e prospettive nel controllo degli alimenti : XXV Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI), Sorrento, 28-30 Ottobre : abstract book / [s.l. : s.n., 2015]. - p 17

(C34) [Nr. Estr. 7085]

Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI) (25. : Sorrento : 28- 30 Ottobre 2015)

Lo scopo dello studio era quello di verificare il grado di contaminazione di campioni di miele e polline di provenienza nazionale, considerando la diversa origine botanica e regionale. Sono stati analizzati 71 campioni di miele e 54 campioni di polline prodotti nel 2012; il miele di produzione nazionale proveniva da 18 regioni (15 differenti origini botaniche) mentre il polline proveniva da 6 diverse regioni. I campioni sono stati forniti dall'Osservatorio Nazionale Miele. Sui campioni di miele pronto per la commercializzazione e sui campioni di polline sono state eseguite le determinazioni di residui di alcuni fitofarmaci neonicotinoidi: Imidacloprid, Thiametoxan, Acetamiprid, Clothianidin, Thiocloprid; le analisi sono state eseguite mediante tecnica LC-MS/MS. Inoltre su tutti i campioni sono state eseguite le ricerche di Arsenico (As), Cadmio (Cd), Cromo (Cr), Ferro (Fe), Manganese (Mn), Mercurio (Hg), Nichel (Ni), Piombo (Pb), Rame (Cu), Stagno (Sn), Tallio (Tl) e Zinco (Zn); le analisi sono state eseguite con tecnica ICP-MS. In 28 campioni di miele scelti casualmente sono state effettuate le determinazioni delle sostanze perfluoroalchiliche (PFAS) mediante LC-MS/MS. Infine 10 campioni di miele sono stati analizzati per la ricerca di Policlorodibenzodiossine (PCDD), Policlorodibenzofurani (PCDF) e Policlorobifenili diossina like (PCB-DL) e non diossina-like (PCB-NDL) mediante tecnica HRGC-HRMS. In 11 campioni di miele e 17 campioni di polline è stata rilevata la presenza di neonicotinoidi. In alcuni campioni è stata trovata la contemporanea presenza di 2 o più neonicotinoidi. Thiametoxan e Clothianidin non sono stati rinvenuti nei campioni di miele, mentre nel polline sono stati evidenziati tutti e 5 i composti della famiglia dei neonicotinoidi. Nessun campione presenta concentrazioni superiori ai limiti massimi indicati dal Reg. CE 396/2005 e s.m.i. Il miele di agrumi è risultato essere il più contaminato. Nessun campione di miele e di polline è risultato contaminato da PCDD/F, PCB e PFAS. Le concentrazioni dei singoli elementi rilevate sono risultate diverse in relazione all'origine botanica e regionale del campione. Le concentrazioni di Cd, Fe, Ni, Mn, Zn e Cu erano significativamente più alte ( $p < 0,05$ ) nel polline rispetto al miele; al contrario la concentrazione di Cr era più alta ( $p < 0,05$ ) nel miele. Tra le varie tipologie di miele quello di melata è risultato essere quello in cui sono stati osservati campioni con composizione minerale che, per proporzione dei diversi metalli, si differenziavano dalla norma. In base al recente Regolamento comunitario (UE) n°2015/1005 del 25/06/2015, che definisce i tenori massimi di Pb applicabili dal 01/01/2016, 3 campioni di miele risulterebbero non conformi per le concentrazioni di Pb rilevate. Lo studio ha evidenziato una relazione tra le origini regionali e botaniche e la contaminazione di miele e polline. Diverse variabili potrebbero influenzare l'origine della contaminazione del miele e del polline, come la pratica del nomadismo, lo scambio di materiale apistico, le pratiche di stoccaggio e di confezionamento.

Meriardi°G, Ramini°M, Ravanetti E, Gherri G, Boni lauri°P

#### **Reduction of *Listeria innocua* contamination in vacuum-packaged dry-cured Italian pork products after high hydrostatic pressure treatment**

Ital J Food Safety. - Vol. 4 no 2 ( 2015). - no 4515 (p 101-103). - 24 bib ref ( ultimo accesso 23/07/2015 <http://www.pagepressjournals.org/index.php/ijfs/article/view/ijfs.2015.4515> ) [Nr. Estr. 6079]

The present work aims to present the results of the application of a treatment with high hydrostatic pressure (HHP) on Italian fermented and dry-cured pork products. The products used in this study were portioned cured ham, portioned bacon and salami, vacuum packaged and produced by a single processing company. Two studies were conducted on a single batch of the three products by means of an artificial contamination with *Listeria innocua* as a surrogate of *L. monocytogenes*. In the first trial a superficial contamination was obtained by immersion for 3 min in the culture broth with a concentration of approximately 9 log cfu/mL. At the end of the inoculum step, the pieces were dried at room temperature and vacuum packaged. In the second trial 50 kg of minced pork meat were contaminated before production of salami. In both cases the inoculum contained 5 strains of *L. innocua*. Subsequently, in both trials, 10 samples were randomly divided into two groups of 5 pieces each: i) TH group, samples treated with HHP; ii) group C, control samples, not subjected to any

treatment. All samples were stored at refrigeration temperature at the end of HHP treatments (if applied), and analyzed for the determination of the surface (P trial) and deep (2" trial) quantitative contamination of *L. innocua*. pH and aw were also determined on 3 pieces of each products belonging to group C. The difference between the medians of the log cfu/cm<sup>2</sup> or g established between controls and treated were compared using the non-parametric test (Kruskal-Wallis test) with  $P < 0.01$ . In all products and in both trials the level of contamination detected in treatment groups was always significantly lower than in controls ( $P < 0.01$ ). In particular, in vacuum-packaged ham, bacon and salami viability logarithmic viability reductions equal to -2.29, -2.54 and -2.51 were observed, respectively. This study aimed to evaluate a not thermal treatment on Italian cured or fermented pork products. The results of this study need to be confirmed in different products and in a greater number of lots, but they appear promising, also because of the considerable literature available for different categories of products (cheese, vegetables and fruit).

Merigo<sup>°</sup>D, Dalzini<sup>°</sup>E, Galuppini<sup>°</sup>E, Monastero<sup>°</sup>P, Pavoni<sup>°</sup>E, Losio<sup>°</sup>MN

### **Ozone use in industrial vegetable washing : a critical review of in-factory trials and a shelf-life prediction study**

European Symposium on Food Safety : 20-22 April 2015, Cardiff, Wales / [s.l. : s.n., 2015]. - p 99 [Nr. Estr. 6035]

European Symposium on Food Safety (IAFP) : Cardiff, Wales : 20-22 April 2015)

Introduction: Ozone (O<sub>3</sub>) is efficient in reducing pathogens. Its application to produce at the post-harvest stage could be efficient in inactivating bacteria and viruses and can cause destruction of pesticides and chemical residues, On the other hand the use of ozone has disadvantages such as instability and reactivity with organic materials, thus the effective elimination of microorganisms may require high concentrations which may cause sensory flaws in fresh produce or corrosion damage in plants. Purpose: The aims of this study were to investigate the effect of different O<sub>3</sub> washing treatments (at 0.5, 1.5 and 2.5 ppm for 10 min) on i) vegetables decontamination, ii) water decontamination shelf life. Methods: The salad (*Lactuca sativa*) used for the challenge test was contaminated with *Listeria innocua* and subjected separately to washing for 10 min with different O<sub>3</sub> concentrations. Each min the samples were collected to verify the bacterial inactivation. After 3, 5 and 10 min of each treatment others samples were packed in modified atmosphere and stored at 12°C for 10 days. The growth curves of *L. innocua* were fitted using DMFit web edition, in order to calculate the growth rates. The effect of the same treatments on the washing water contaminated with *L. innocua* and feline calicivirus (FCV) was also evaluated. Results: Only during the salad washing with 2.5 ppm of O<sub>3</sub> a significant reduction of *L. innocua* was observed (from  $5.79 \pm 0.01$  to  $5.12 \pm 0.02$  log CFU/g) after 1 minute. No correlation was found between the growth rates calculated during the storage of salad after different treatment. In water, log inactivations (range from 2.22 to 6.56) of *L. innocua* and FCV were observed after 1 minute. Significance: O<sub>3</sub> treatments could be a viable decontamination method for the food industry.

Merla<sup>°</sup>C, Andreoli<sup>°</sup>G, Vicari<sup>°</sup>N, Dalla\_Valle C, Cavanna C, Guglielminetti ML, Biancardi<sup>°</sup>A, Fabbi<sup>°</sup>M

### **Specie fungine produttrici di Ocratossina A nei salami lombardi prodotti negli anni 2011-2014**

17° Congresso Nazionale Società Italiana di Tossicologia : "Sicurezza, salute e sviluppo sostenibile: la tossicologia al servizio della società" / [s.l. : s.n., 2015]. - 6019]

Congresso Nazionale Società Italiana di Tossicologia (17. : Milano : 17-20 Marzo 2015)

Le muffe appartenenti ai generi *Aspergillus* e *Penicillium* spesso presenti sulla superficie dei salami, regolano il pH e il grado di umidità per la corretta stagionatura del prodotto. Sebbene la legge italiana consenta l'utilizzo di colture starter di *Penicillium chrysogenum* e di *P. nalgiovense* nella produzione degli insaccati crudi (D.M. 28/12/1994 G.U. n. 89), i produttori di salame tradizionale non

ne fanno utilizzo permettendo la crescita dei funghi naturalmente presenti nell'aria degli ambienti di stagionatura. Alcuni ceppi di *Aspergillus* (soprattutto *A. ochraceus*, *A. westerdijkiae*, *A. carbonarius* e *A. niger*) e di *Penicillium* (*P. verrucosum* e *P. nordicum*) possono produrre e rilasciare nel salame ocratossina A (OTA). Lo scopo di questo lavoro è il monitoraggio della flora fungina colonizzante i salami tradizionali prodotti in Lombardia negli anni 2011-2014 con valutazione delle tecniche per la rilevazione di ceppi ocratossinogeni. La flora fungina di 123 salami con stagionatura variabile (1 settimana - 7 mesi) è stata ricercata secondo il metodo ISO 21527:2008 per la numerazione di lieviti e muffe. Sono stati isolati 235 ceppi di cui 155 appartenenti al genere *Penicillium* e 15 appartenenti al genere *Aspergillus*. L'identificazione delle specie è stata condotta sfruttando le caratteristiche colturali e morfologiche come previsto dalle chiavi di identificazione di Samson (2010). L'identificazione dei 37 ceppi più rappresentati è stata confermata sequenziando il frammento comprendente le regioni ITS1 (Internal Transcribed Spacer 1) e ITS2 del rDNA e per 15 di questi, 14 *Penicillium* e 1 *Aspergillus*, è stata inoltre sequenziata una porzione del gene della  $\beta$  tubulina come ulteriore conferma. Le sequenze ottenute da ciascun campione sono state confrontate in banca dati NCBI mediante il programma BLAST. Per 4 salami (2 provenienti dalla provincia di Pavia, uno da Milano e uno da Monza e Brianza) in cui sono stati isolate specie potenzialmente ocratossinogene (3 *A. westerdijkiae* e 1 *P. nordicum*) la ricerca dell'ocratossina A è stata condotta attraverso cromatografia liquida accoppiata in tandem alla spettrometria di massa. L'identificazione a livello morfologico delle specie fungine a causa dell'esistenza di specie morfologicamente affini tra loro, ha fornito solamente un'indicazione a livello di gruppo e perciò ha reso necessaria una conferma molecolare. L'identificazione delle specie ottenuta mediante il sequenziamento del frammento ITS ha confermato l'identificazione a livello di gruppo. Il sequenziamento della porzione del gene della  $\beta$  tubulina ha permesso l'identificazione fino al livello di specie per tutti i ceppi. Per verificare l'attendibilità delle tipizzazioni e in particolare modo quelle riguardanti i ceppi potenzialmente micotossinogenici si è ricercata l'OTA nei 4 salami colonizzati. In 2 salami la quantità di OTA è risultata ben superiore alla quantità massima suggerita per legge per carni suine e prodotti derivati (1  $\mu\text{g}/\text{kg}$ ) (Circ. Min. San. n. 10 09/06/1999 G.U. n.135). In uno di questi si è riscontrato un livello di OTA 600 volte superiore al limite, mentre nell'altro di 7 volte. Nei rimanenti due salami la quantità di micotossina superava di poco i limiti previsti. In un salame colonizzato da *A. westerdijkiae* e positivo ad OTA (1,4  $\mu\text{g}/\text{kg}$ ), ricreando le condizioni ideali per la micotossinogenesi ( $a_w < 0,79$  e  $T=20-30\text{ }^\circ\text{C}$ ) si è ottenuta una massiccia sintesi di tossina nell'arco di 10 giorni (3,7  $\mu\text{g}/\text{kg}$ ). Attualmente stiamo procedendo con la messa a punto di una metodica di PCR Real Time mirata a riscontrare la presenza di funghi ocratossinogeni amplificando geni coinvolti nella biosintesi della tossina.

Merla<sup>°</sup>C, Merla C, Andreoli<sup>°</sup>G, Guglielminetti ML, Biancardi<sup>°</sup>A, Rovida E, Pozzi C, Gennari<sup>°</sup>L, Scotto<sup>°</sup>Di\_Fasano P, Fabbi<sup>°</sup>M

#### **Indagine sull'origine di una colonizzazione da parte di muffa ocratossinogena in un salumificio dell'Oltrepo' Pavese**

XVI Congresso Nazionale SIDiLV : 30 Settembre - 2 Ottobre 2015 Montesilvano (PE) : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2015]. - p 312. - 6 bib ref [Nr. Estr. 7056]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (16. : Montesilvano (PE) : 30 Settembre - 2 Ottobre 2015)

*Ochratoxin A (OTA) is a mycotoxin synthesized in cereals, spices and in fermented meat products by several Aspergillus and Penicillium species. Even if moulds are essential for the correct ripening of salami, the presence of ochratoxigenic moulds and OTA whose toxic effect is known has to be monitored. In two salami manufactured by the same producer in Oltrepo' area - Pavia province in different periods of 2014 we have isolated Aspergillus westerdijkiae, a strong OTA-producer and we have found OTA over the limits permitted by Italian regulation. Subsequently we sampled the air, the surfaces and some probable sources of contamination in this family-run factory. We have found A. westerdijkiae from the air of all rooms sampled, from six surface points in the ripening rooms and from the surfaces of two different salami batches produced in 2015. The same strain was also isolated from the soil collected just outside the factory and from outdoor air.*

Pavoni<sup>°</sup>E, Arcangeli G, Dalzini<sup>°</sup>E, Bertasi<sup>°</sup>B, Terreggino C, Montesi F, Manfrin A, Bertoli E, Brutti A, Varisco<sup>°</sup>G, Losio<sup>°</sup>MN

**Synergistic effect of high hydrostatic pressure (HHP) and marination treatment on the inactivation of Hepatitis A virus in mussels (*Mytilus galloprovincialis*)**

Food Environ Virol. - Vol. 7 (2015). - p 76-85. - 39 bib ref [Nr. Estr. 5993]

Consumption of raw or insufficiently cooked mussels contaminated with hepatitis A virus (HAV) is a major cause of infection to humans. The origin of mussels commonly used for the preparation of marinated seafood salads is often unknown, since different producers worldwide undergo a pre-cooking treatment at the original collection site with methods and parameters not always indicated. These treatments could be insufficient for the inactivation of HAV, which is characterized by a high temperature resistance. Both high hydrostatic pressure (HHP) and marinade treatments have been shown to affect HAV vitality. In this study, two treatments (HHP and marinating) were combined in order to assess a potential synergistic effect on the virus vitality. A kinetic test was conducted by subjecting the experimentally-contaminated mussels (HAV titre: 10<sup>6</sup>/ml TCID<sub>50</sub>) to marinating, and to different HHP treatment (4,000; 5,000; and 6,000 bar for 1, 5, and 9 min). Virus post-treatment vitality was assessed by its ability to grow on cell cultures and by quantitative real-time RT-PCR to evaluate virus resistance under such conditions. Marinating treatment alone (final pH 4.3, and NaCl 2 %) did not inactivate the virus. On the other hand, the use of HHP treatment alone on non-marinated HAV-contaminated mussels was effective only above 5,000 bar for 5 min. The results of the present study elucidate the synergistic effect of a combination between marination and HHP treatments on the inactivation of the virus.

Pavoni<sup>°</sup>E, Barbieri<sup>°</sup>I, Bertasi<sup>°</sup>B, Lombardi<sup>°</sup>G, Corradioli<sup>°</sup>P, Losio<sup>°</sup>MN

**Detection and molecular characterisation of swine Hepatitis E virus in Brescia province, Italy**

Ital J Food Safety. - Vol. 4 no 2 (2015). - no 4587 (p 69-74). - 43 bib ref (ultimo accesso 10/06/2016 <http://www.pagepressjournals.org/index.php/ijfs/article/view/ijfs.2015.4587>) [Nr. Estr. 6055]

Hepatitis E virus (HEV) is an important public health concern in many developing countries and it occurs in sporadic forms in industrialized areas. With the discovery of swine HEV in pigs, which is genetically closely related to human HEV, hepatitis E is considered to be a zoonotic disease. To investigate the circulation of HEV within a distinct area of Lombardy region (Northern Italy), 17 pig farms were subjected to monitoring study by collection of fresh stool samples each represented by ground-pooled specimens. In particular, three distinct types of breeding farms were focused, represented by farrow to weaning, farrow to finish and fattening farms, respectively. Epidemiological data confirm that in Europe the seroprevalence in pigs, more than 9 months of age, ranges from 51.4 to 75%, while in 3-9 months fatteners is about 38%. In France and Italy, the positivity among farms is respectively 30 and 97.4% and the seroprevalence in Italy is 50.2%. Since HEV viremia was typically observed in the early period of life in swine, faeces were collected in boxes containing weaning pigs. For the study, 183 stool samples were collected and amplifications were performed with universal primers specific for the ORF2 region of genome. Twenty-eight samples resulted positive to HEV RNA and genotyping demonstrated that they were closely related to HEV strains belonging to genotype 3 and circulating in Europe. Comparison with reference strains from GenBank excluded their similarity to genotype 1, 2 or 4 confirming that genotype 3 strains are circulating in Europe. Since it was demonstrated that swine act as a reservoir for HEV, and since many strains into HEV genotype 3 share a strong molecular similarity to human HEV, it was important to detect the presence of HEV in a restricted area with a very high density of pigs.

Pellicciotti<sup>°</sup>S, Moretti S, Galarini R, Gamba<sup>°</sup>V, Dusì<sup>°</sup>G

**A new approach to detect antibiotic residues in muscle tissues : development of a high**

### **resolution mass spectrometry screening method**

Atti del XXV Congresso della Divisione di Chimica Analitica della Società Chimica Italiana : 13-17 Settembre 2015 Trieste / editore, Antonella Rossi. - Trieste : Università degli Studi di Firenze, 2015. - p 234. - 2 bib ref [Nr. Estr. 7030]

Congresso della Divisione di Chimica Analitica della Società Chimica Italiana (25. : Trieste : 13-17 Settembre 2015)

A very common practice to screen antibiotic residues in sample of animal origin is based on microbiological assays using plate test bacterial growth inhibition techniques. These methods are able to cover many antibiotic classes, offering lowcost analysis. However, due to their detection mode microbiological assays do not permit to discriminate one antibiotic from another one and, for several compounds, do not reach the maximum residue limits (MRLs) set by European Commission regulation (EU) 37/2010/EC [1]. Due to these drawbacks, we developed a LC-HRMS procedure for the screening of more than 70 antimicrobial compounds belonging to the following veterinary drugs families: amphenicols, beta-lactams, diamino-pyrimidine, lincosamides, macrolides, pleuromutilins, quinolones, rifamycins, sulphonamides and tetracyclines. To be able to analyze at the same time all these compounds with different physical and chemical properties, generic and non-selective sample preparation procedure has been optimized. Muscle samples were extracted twice: at first with a acetonitrile/water mixture and then with acetonitrile. The extract was evaporated to dryness and the residue was dissolved in ammonium acetate buffer. Mass spectrometric determination was carried out on LTQ-Orbitrap mass spectrometer XL operating in full scan acquisition mode at a resolving power of 60.000 full width at half maximum (FWHM). The high resolving power combined with high mass accuracy 5 ppm allow to detect a specific analyte of interest just knowing the exact mass of the molecular ion and the corresponding LC retention time. The proposed method has been successfully validated demonstrating a detection capability (CC(3) equal to 10 µg kg' for all the investigated compounds [2]. Therefore it is applicable to routine official control of antibiotic residues in muscle samples replacing the traditional screening test currently used in Italy.

Pellicciotti°S, Moretti S, Gamba°V, Galarini R, D usi°G

### **Confirmatory method for the analysis of (beta)-lactam antibiotic residues in muscle by liquid chromatography coupled to high resolution mass spectrometry**

MASSA 2015 : Alghero, June 10-12, 2015 / Italian Chemical Society Division of Mass Spectrometry, Istituto Zooprofilattico Sperimentale della Sardegna "G. Pegreffii". - [Sassari : Unisversità di Sassari, 2015]. - p 87-88. - 2 bib ref [Nr. Estr. 6081]

MASSA : Alghero : June 10-12, 2015)

A confirmatory method for the residue determination, in muscle samples, of 21 veterinary drugs belonging to the f3-lactams family has been developed and validated. The proposed procedure is simple and rapid involving only an extraction followed by liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS) determination. EU Commission Decision 2002/657/EC was used as guideline for the validation.

Piro R, Sangiorgi°E, Gazziero M

### **Rapid screening analysis of olive oil based on fingerprinting holistic approach**

Fats, Oils and Lipids : new challenges in technology, quality control and health : 13th Euro Fed Lipid Congress, 27-30 September 2015, Florence, Italy : book of abstracts / [s.l. : s.n., 2015]. - p 350 [Nr. Estr. 7081]

Euro Fed Lipid Congress (13th : Florence, Italy : 27-30 September 2015)

Traditional strategies for the food fraud control have relied on the detection of specific marker



compounds and the comparison to genuine reference data. Furthermore, an adulterant can be detected only if it is known beforehand and specifically searched by the analyst. Traditional quality control strategies are not designed to look for a huge number of potential contaminants, but a new adulterants will not be never unveiled if it is not known. For those reasons an holistic approach is needed, that is based on several measurements and many evaluations at once, i.e. a fingerprinting approach. The fingerprinting approach may provide rapid and screening high-throughput analyses. The characteristic spectrum of a food material can be related to its properties and thus to its authenticity. The combination of different instrumentation as NIR for general composition, TXRF for metals and other elements and DART/HRMS for small molecules identification seem to be an excellent analytical approach. Examples of rapid non-targeted screening using different techniques (NIR, TXRF and DART/HRMS) both for qualitative and quantitative tests for olive oil are presented.

Riva A, Borghi E, Cirasola D, Colmegna°S, Borgo F, Amato E, Pontello MM, Morace G

**Methicillin-resistant *Staphylococcus aureus* in raw milk : prevalence, SCCmec typing, enterotoxin characterization, and antimicrobial resistance patterns**

J Food Prot. - Vol. 78 no 6 ( 2015). - p 1142-1146. - 41 bib ref [Nr. Estr. 7101]

*Staphylococcus aureus* is a known major cause of foodborne illnesses, and raw milk and dairy products are often contaminated by enterotoxigenic and antimicrobial-resistant *S. aureus* strains. In the present study, 35 *S. aureus* strains were isolated from 383 raw milk samples collected from various dairy herds in the province of Milan (northern Italy). The isolates were characterized based on their antimicrobial susceptibility patterns and the presence of genes encoding staphylococcal enterotoxins (sea, seb, sec, sed, and see). About half (45.7%) of the strains were enterotoxigenic, and 37.1% were resistant to at least one of the antimicrobial drugs tested. Seven (20%) of 35 isolates were identified as methicillin-resistant *S. aureus* (MRSA), and SCCmec typing performed with a multiplex PCR assay revealed the presence of gene cassettes IV and V, typical of community-acquired MRSA, and I and II, characteristic of health care-associated MRSA. The MRSA strains were evaluated for the presence of the Pantone-Valentine leukocidin gene, but this gene was not found. The results of the present study revealed the presence of toxin-producing *S. aureus* and MRSA strains in raw milk. MRSA and enterotoxigenic *S. aureus* in dairy farms are an important risk factor for the spread of staphylococcal infections; therefore, further studies are needed to find strategies for monitoring and controlling the presence of *S. aureus*, especially MRSA, in dairy products.

Rubini°S, Barbieri S, Pavoni°E, Bertasi°B, Cozzi L, Bergamini M, Suffredini E

**Risk associated to *Vibrio parahaemolyticus* in shellfish in Ferrara (Emilia Romagna)**

Eur J Public Health. - Vol. 25 suppl 3 ( 2015). - p 416-417 [Nr. Estr. 7075]

European Public Health Conference (8th : Milan, Italy : 14-17 October 2015)

*Vibrio parahaemolyticus* is an autochthonous microorganism of the marine environments, frequently isolated from seafood, including bivalve mollusks, and a human pathogen responsible for gastroenteritis outbreaks and sporadic cases. The mechanism of pathogenicity has yet to be comprehensively determined, but two haemolysins (TDH and TRH) have been recognized as virulence factors. The aims of this work were: a) the evaluation of the prevalence of *V. parahaemolyticus* in mollusks harvested in Ferrara; b) the evaluation of the presence of pathogenic strains; c) the assessment of a possible effect on the public health. A total of 859 m011usc samples (601 Manila clams and 258 mussels) were collected and analyzed from January 2011 to March 2015 by the Local Health Service (AUSL) of Ferrara as a part of the regional Bivalve Mollusks Monitoring Plan. Analyses were performed according to ISO/TS 21872-1:2007 and isolates were characterized for species-specific (toxR) and pathogenicity genes (tdh and trh) by PCR. *V. parahaemolyticus* was

detected in 288 samples (33.5%), 251 Manila clams and 37 mussels, with a statistically significant difference (Fisher's exact test  $p < 0.0001$ ) between the prevalence in the two species (41.8% in clams vs. 14.3% in mussels). The molecular characterization showed the presence of the *toxR* gene in 276 isolates (95.8%), while the *tdh* and *trh* genes were detected respectively in 21 and 15 isolates; one more strain was characterized by the simultaneous presence of both pathogenicity markers. Overall, the prevalence of potentially pathogenic *V. parahaemolyticus* strains was 13.4% and, significantly, almost all (36 out of 37) of them were isolated from Manila clams. The data provided in this study on the prevalence of potentially pathogenic *V. parahaemolyticus* in different shellfish species harvested in the Ferrara district, will help defining guidelines for the management of the associated risk to this microorganism.

Rubini°S, Fedrizzi°G, Menotta°S, Boschetti L, Cagnini M, Pigozzi S, Riccardi E, Pompei M, Milandri A

**Presenza di acido okadaico nelle vongole veraci : ricerca e gestione di un equilibrio tra produzione e salute del consumatore**

17° Congresso Nazionale Società Italiana di Tossicologia : "Sicurezza, salute e sviluppo sostenibile: la tossicologia al servizio della società" / [s.l. : s.n., 2015]. - 1 p [Nr. Estr. 7149]

Congresso Nazionale Società Italiana di Tossicologia (17. : Milano : 17-20 Marzo 2015)

In Italia l'acido okadaico (AO) e i suoi derivati dinofisitossina-1 (DTX-1), dinofisitossina-2 (DTX-2) e dinofisitossina-3 (DTX-3) sono i composti maggiormente responsabili della sindrome diarroica, un tempo denominata DSP (Diarrhetic Shellfish Poisoning). Si tratta di principi attivi prodotti da alghe appartenenti ai generi *Dinophysis* e *Prorocentrum* responsabili della contaminazione di molluschi bivalvi in diverse aree del mondo (Hallegraeff, 2004). La presenza di queste tossine nei molluschi eduli comporta rischi per la salute umana in quanto provoca una sintomatologia gastroenterica caratterizzata da diarrea, nausea, vomito e dolori addominali che inizia 3-12 ore dopo l'assunzione di molluschi contaminati (Yasumoto et al., 1978). L'Unione Europea ha emanato numerosi provvedimenti legislativi atti a tutelare la salute pubblica nei confronti delle biotossine marine. Nel 2012 in un'area della Sacca di Goro (Ferrara), è stata riscontrata la presenza di AO e suoi derivati nelle vongole veraci (*Venerupis philippinartun*), solitamente mai contaminate. Il presente lavoro intende descrivere l'inatteso fenomeno e le azioni intraprese per la sua gestione. Il Piano di Monitoraggio dei molluschi bivalvi viene applicato regolarmente, in Regione Emilia Romagna, dal 1997. I campioni destinati all'esame biotossicologico vengono prelevati con frequenza diversa a seconda della specie di mollusco da esaminare, del luogo di allevamento e della concentrazione di biotossine rilevate negli esami precedenti. Dal 1997 al 2012 i casi di positività per tossine lipofile sono stati -rilevati esclusivamente nei mitili (*Mytilus galloprovincialis*). La comparsa dei primi risultati positivi nelle vongole veraci della Sacca di Goro verificatasi nel maggio 2012 ha imposto un innalzamento della soglia di attenzione, nonché un aumento della frequenza dei prelievi e dell'area sottoposta al campionamento. Contestualmente ai campioni di *Venerupis philippinartun* sono stati prelevati anche campioni di acqua e di sedimento. Dei 147 campioni di vongole veraci analizzati dalla comparsa del primo caso di positività fino al dicembre 2014, i campioni risultati positivi per presenza di acido okadaico e suoi derivati sono stati 54: 15 di questi contenevano tali tossine in concentrazioni superiori al limite di legge (160 mg AOeq./kg di parte edibile), determinandone il divieto di raccolta da parte dell'Autorità Competente. Tale divieto viene revocato dopo 2 esami con esito negativo, eseguiti a distanza di non meno di 48 ore l'uno dall'altro. Visto che la distribuzione delle biotossine non era uniforme all'interno dell'area monitorata, si è ritenuto necessario ridefinire la stessa, creando due distinte stazioni di campionamento in base ai risultati ottenuti. Questo fenomeno di contaminazione da tossine lipofile del gruppo dell'acido okadaico è da imputarsi alla presenza di elevate concentrazioni di *Prorocentrum lima*, microalga epifita che ha trovato un ambiente idoneo alla proliferazione sulla macroalga *Gracilaria veniculophylla*.

Rubini°S, Losio°MN, Boschetti L, Pavoni°E, Galuppi°E, Suffredini E, Melloni°

R, , Bolognesi°E, Barbieri S, Arcangeli G

**Vibrio vulnificus nei molluschi bivalvi : aspetti sanitari e normativi**

Atti del IV°Convegno Nazionale Società Italiana di Ricerca Applicata alla Molluschicoltura (SIRAM) : Chioggia, 06 Novembre 2015 / [s.l. : s.n., 2015]. - p 65-66. - 6 bib ref [Nr. Estr. 7110]

Convegno Nazionale Società Italiana di Ricerca Applicata alla Molluschicoltura (SIRAM) (4. : Chioggia : 06 Novembre 2015)

*Vibrio vulnificus is a Gram-negative, halophilic bacillus that is a natural inhabitant of estuarine and coastal waters. It has been isolated in different marine species, including invertebrates. It can cause gastroenteritis, serious skin infections and septicemia in human. The infection is linked to a high mortality grade (50% - 70% of cases). The aim of this work were to determine the occurrence of V. vulnificus in mollusc (clams) harvested in the Ferrara coastal area after a reported human case of vibriosis, and to evaluate the risk for public health.*

Sangiorgi°E, Simoni°M, Berneri°R, Bragolusi M, Piro R

**Rapid method to evaluate metals concentration in olive oil**

Fats, Oils and Lipids : new challenges in technology, quality control and health : 13th Euro Fed Lipid Congress, 27-30 September 2015, Florence, Italy : book of abstracts / [s.l. : s.n., 2015]. - p 349 [Nr. Estr. 7080]

Euro Fed Lipid Congress (13th : Florence, Italy : 27-30 September 2015)

Inorganic profile of oil determination requires normally time consuming steps like acidic mineralization/destruction of organic matter followed by AAS or ICP determination. Aim of this work was to determine the concentration of metals and elements in olive oil with a simple and time-saving Method: metals were extracted from the oil with water, yttrium and gallium was added as internal standard and TXRF was used for the determination of elements present in the aqueous phase. 40 olive oils were analyzed (extra virgin olive oil, virgin olive oil, refined oil) from the market and from local producers. Mg, P, S, K, Ca, Ba, Cl, Br, Mn, Fe, Ni, Cr, Cu, Al, Zn, As and Pb presence was determined. The analytical possibilities of TXRF technique are discussed. These results, along with other simple and rapid analysis, like DART/MS analysis for triglycerides and NIR analysis for elementary composition, could offer a valuable aid to olive oil fraud detection, oil characterization and authenticity.

Sangiorgi°E, Simoni°M, Berneri°R, Piro°R

**Water soluble element analysis of olive oil using TXRF**

7th International symposium on recent advances in food analysis : November 3-6, 2015, Prague, Czech Republic : book of abstracts / editors, Jana Pulkrabova ...[et al.]. - [Praha : University of Chemistry and Technology, 2015]. - p 317 [Nr. Estr. 7114]

International symposium on recent advances in food analysis (7th : Prague, Czech Republic : November 3-6, 2015)

The determination of the inorganic profile of edible oils is important for the metabolic role of same elements and to verify possible contamination or adulteration. Element analysis of oil normally requires some time consuming steps like acidic mineralization/destruction of organic matter followed by AAS or ICP determination. Aim of this work was detect some water soluble elements in olive oil using a simple and time-saving Total X Ray Reflection method: metals were extracted from the oil with water, Yttrium or Gallium was added as internal standard and a TXRF spectrometer was used for the determination of the elements that are present in the aqueous phase. About 100 olive oils (extra virgin olive oil, virgin olive oil, refined oil), from the market and from local PDO producers, were analyzed. Mg, P, S, K, Ca, Ba, Cl, Br, Mn, Fe, Ni, Cr, Cu, Al, Zn, As, Ti and Pb were determined and their presence in olive oil, along with the analytical possibilities of TXRF technique

are discussed. The dataset were computed using multicomponent statistica) analysis trying to enhance possible differences for genuineness or geographical origin assessment purpose.

Savi°R, Ricchi°M, Cammi°G, Garbarino°C, Leo°S, Pongolini°S, Arrigoni°N  
**Survey on the presence of Mycobacterium avium subsp. paratuberculosis in ground beef from an industrial meat plant**

Vet Microbiol. - Vol. 177 ( 2015). - p 403-408 . - 25 bib ref [Nr. Estr. 6076]

Paratuberculosis of ruminants is characterised by chronic enteritis but, at advanced stages of the disease, a systemic dissemination of Mycobacterium avium subsp. paratuberculosis (MAP) in tissues and organs can occur. MAP has been recovered from lymph nodes and muscles of clinical and sub-clinical cows. In most countries, dairy and beef cattle infected with paratuberculosis are routinely sent to slaughter and the consumption of their meat could be a possible route of human exposure to MAP. However, few studies on MAP in ground beef are currently available. During the period November 2013–March 2014 we carried out a survey on the ground beef produced in an industrial meat processing plant. One-hundred and forty samples of ground meat were analysed by IS900-qPCR and culture (VersaTrek System1). The limit of detection (LOD) of qPCR was 630 MAP cells/g (107 CFU/ g) while the LOD for culture was 170–230 MAP cells/g (62–115 CFU/g). No samples were positive by direct IS900 qPCR, while two samples were positive by liquid culture. Our data suggest that the presence of live MAP in raw minced meat is possible. In order to avoid exposure for humans through the consumption of contaminated meat, proper cooking of meat is recommended.

Scaltriti°E, Sassera D, Comandatore F, Morganti°M , Mandalari°C, Gaiarsa S, Bandi C, Zehender G, Bolzoni°L, Casadei°G, Pongolini°S

**Differential single nucleotide polymorphism-based analysis of an outbreak caused by Salmonella enterica serovar Manhattan reveals epidemiological details missed by standard pulsed-field gel electrophoresis**

J Clin Microbiol. - Vol. 53 no 4 ( 2015). - p 1227-1238. - 48 bib ref [Nr. Estr. 6039]

We retrospectively analyzed a rare Salmonella enterica serovar Manhattan outbreak that occurred in Italy in 2009 to evaluate the potential of new genomic tools based on differential single nucleotide polymorphism (SNP) analysis in comparison with the gold standard genotyping method, pulsed-field gel electrophoresis. A total of 39 isolates were analyzed from patients (n = 15) and food, feed, animal, and environmental sources (n = 24), resulting in five different pulsed-field gel electrophoresis (PFGE) profiles. Isolates epidemiologically related to the outbreak clustered within the same pulsotype, SXB\_BS.0003, without any further differentiation. Thirty-three isolates were considered for genomic analysis based on different sets of SNPs, core, synonymous, nonsynonymous, as well as SNPs in different codon positions, by Bayesian and maximum likelihood algorithms. Trees generated from core and nonsynonymous SNPs, as well as SNPs at the second and first plus second codon positions detailed four distinct groups of isolates within the outbreak pulsotype, discriminating outbreak-related isolates of human and food origins. Conversely, the trees derived from synonymous and third-codon-position SNPs clustered food and human isolates together, indicating that all outbreak-related isolates constituted a single clone, which was in line with the epidemiological evidence. Further experiments are in place to extend this approach within our regional enteropathogen surveillance system.

Zanardi°G, Cosciani\_Cunico°E, Dalzini°E, Losio° N, Daminelli°P

## **Listeria monocytogenes in bulk tank milk and its behaviour during the cheese making**

The congress on controversies & consensus in bovine health, industry & economics : August 27-30, 2015 Berlin, Germany : congress program / [s.l. : s.n, 2015]. - p 66-67 [Nr. Estr. 7104]

Congress on controversies & consensus in bovine health, industry & economics : Berlin, Germany : August 27-30, 2015)

**Problem Statement:** *Listeria monocytogenes* is one of the main pathogen considered as a microbiological hazard associated with raw milk and its dairy products. Aim of this work is to describe a case of natural contamination by *L. monocytogenes* of raw milk and to assess the behaviour of *L. monocytogenes* during the cheese making and ripening (60 days) from two batches of contaminated milk. **Methods:** Bulk tank milk (BTM) from 258 on 1,500 (17.2%) dairy herds in province of Brescia — Lombardy Region, Italy — was examined for the presence of foodborne pathogens. In particular, *Listeria monocytogenes* was detected by Real-Time PCR (iQ-Check *Listeria monocytogenes* II (Biorad®) and confirmed by microbiological test (ISO 11290-1: 1999/Amd 1:2004). In order to assess the behaviour of *L. monocytogenes* during the cheese making and ripening, two batches of naturally contaminated milk (25 L milk/batch) were used. Milk, curd and cheese samples were evaluated for the presence of the pathogen, lactic acid bacteria (LAB) levels, pH and aw values. The temperature during the cheese making was registered by a data logger. All analyses were carried out in triplicate. **Results:** Real-Time PCR detected *L. monocytogenes* in 7 milk samples (2.7%), 3 of which confirmed by microbiology (1.2%). The follow up performed on three positive farms always showed *L. monocytogenes* from a quarter milk sample of a single cow as source of whole milk contamination. Neither general symptoms nor macroscopical abnormalities in milk were observed. One carrier asymptomatic cow, shedding 103 CFU/ml, was able to contaminate the BTM (e.g. 260 dairy cows for 65 q/die). Swabs from milking equipment and milk tank resulted always negative to the bacteriological tests. In two farms *Listeria gray* was detected in the maize feed as index of ideal microclimate conditions for the presence of *Listeria* spp. Bulk tank milk became negative after the removal or the antibiotic treatment of the affected cow. Regarding to the behaviour of *L. monocytogenes* during the manufacture of four cheeses, the LAB increased from 6.7 to 9 log CFU/g in the first four days. This increase in LAB levels generated a slight acidification of the cheese. An increase in the concentration of *L. monocytogenes* level from 3.5 to 5.7 log CFU/g was observed during the first days of ripening. Then, the growth of the pathogen stunted until the end of the ripening period. The results show that LAB are able to induce an early stationary state in *L. monocytogenes* and its growth is inhibited when LAB reached a critical density (Jameson effect). **Conclusion:** The source of *L. monocytogenes* was always identified in infected animals. Antibiotic therapy or the removal of the positive animal, hygienic improvement of milk production and the Good Manufacturing Practices applied to the feed production eliminated the bacteriological contamination of milk. Considering that the cheese making procedure in case of raw milk didn't guarantee the elimination of the pathogen but only its growth inhibition, the *L. monocytogenes* concentration in milk should not exceed 1CFU/ml to produce raw milk cheese ripened for 60 days or less. The risk analysis has also to consider the low contamination of raw milk by *L. monocytogenes*, because of an asymptomatic shedder dairy cow.