TAB. 20.3 - RICERCHE EFFETTUATE

IGIENE DEGLI ALIMENTI AD USO UMANO

**Ricerca di Escherichia coli O157:H in alimenti tramite multiplex PCR**

Bertasi° B, Agnelli° E, Pavoni° E, Daminelli° P, Boni° P


Escherichia coli 0157:H7, incluso nella lista dei patogeni emergenti dall'Organizzazione Mondiale della Sanità (OMS), è ritenuto essere, con sempre maggiore frequenza, responsabile di gravi forme cliniche, quali coliti emorragiche e sindromi emolitico-uremiche. Poiché si ritiene che il rischio di infezione sia quasi esclusivamente associato al consumo di alimenti infetti, risulta evidente la necessità di disporre di metodiche che, con elevata sensibilità e specificità, consentano un adeguato controllo delle matrici alimentari. Obiettivo del presente lavoro è stato la messa a punto di una metodica PCR dotata di elevata sensibilità 1101 ufc/g e specifico per i geni della virulenza e dell'adesività, con l'applicazione della stessa per lo screening delle matrici alimentari al fine di dare una stima reale del rischio associato.

**Ribotipizzazione automatica al servizio dell'indagine epidemiologica: l'esempio di Listeria monocytogenes**


Listeria monocytogenes è un patogeno ubiquitario presente in differenti tipologie di alimento; alcuni ceppi del microrganismo possono causare l'insorgenza di listeriosi nell'uomo, che può diventare particolarmente grave in soggetti immunocompromessi. Genotypic characterisation methods are useful to study pathogenicity/strains associations, because traditional techniques are influenced by environmental factors instead. Automatic ribotyping can be used to identify and characterise strains isolated from food, environmental samples and biological samples, to identify eventual correlations and to perform a more specific risk analysis.

**Presenza di Coxiella burnetii e Mycobacterium paratuberculosis nel latte crudo:**

Bertasi° B, Maccabiani° G,Tilola° M., Daminelli° P, Boni° P

*X Congresso Nazionale Societa’ Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) : 22-24 Ottobre 2008 : volume degli atti / [s.l. : Societa’ Italiana Diagnostica di Laboratorio Veterinaria (
Related to increase of raw milk consuming, it is necessary to control products about pathogens contaminations. Mycobacterium paratuberculosis and Coxiella burnetiì are zoonotic agents that can contaminate raw milk. M. paratuberculosis is the causative agent of a degenerative disease primarily in ruminants, but there is a possible link with development of Crohn's Disease in humans. Coxiella burnetii is a causative agent of Q-fever, a widespread zoonosis. It is not well know how are the links between microorganisms presence and disease in humans, so it is necessary to perform monitoring and epidemiological studies. Isolation of these microorganisms it is very difficult and it takes very long time when it is performed by traditional culture techniques; so in this field molecular biology methods seem to be the most useful techniques.

Osservazioni preliminari sulla presenza di PCDD/F e PCD-dl nel latte bovino = Preliminary observations on presence of PCDD/F and dl in cow's milk

Il lavoro riporta alcune osservazioni in merito al livello e al tipo di contaminazione da alcuni composti organoclorurati PCDD (polychlorinated dibenzodioxin) PCDF (polychlorinated dibenzofuran) e PCB-dl (polychlorinated dioxin like) riscontrata nel latte bovino prelevato da autocisterne che lo raccolgono in allevamenti siti nel comune di Brescia e nei comuni della cintura urbana. Quest'area è stata la sede di una industria produttrice di PCB dal 1958 al 1983 (1) e ancora oggi vede la presenza di numerose ferriere ed acciaierie. Per questo il problema dell'inquinamento da PCB è conosciuto e monitorato da tempo ma, l'applicazione delle nuove normative (Regolamento CE n°91881/2006 e Regolamento CE n°91883/2006) (2), (3) che rivedono i limiti e la composizione dei contaminanti considerati, impone nuovi studi epidemiologici in grado di fornire dati ed indicazioni per una corretta analisi del rischio sanitario. I risultati delle analisi del latte di autocisterna hanno evidenziato valori di PCDD/F e PCB-dl WHO-TEQ fra 2,589 e 3,03 pg/gr/grasso mostrando un livello di contaminazione inferiore ai limiti di legge pari a 6,00 pg/gr/grasso, ma superiore ai limiti di azione (2,00 pg/gr/grasso) per il contenuto dei singoli gruppi di sostanze PCDD/F o PCB-dl (raccomandazione CE 144/06) (4). I risultati mettono in evidenza come i polichlorobifenili (PCB) siano i principali responsabili di tale situazione rappresentando oltre il 65 % delle sostanze inquinanti mentre le diossine (PCDD + PCDF), risultano decisamente inferiori (tab 1.).


Purpose of this work is to highlight the level and type of contamination by organochlorine compounds; PCDD (polychlorinated dibenzo-p-dioxin), PCDF (polychlorinated dibenzofurans) and dl-PCB (polychlorinated dioxin like) of milk tanker composed by diaries bulk milk of Brescia and hinterland. In this area, in the past industries had produced PCBs (1) and still today it's site of many ironworks and steelworks; pollution problem is known and it was monitored, but, the application of new regulations (Reg. CE n°1881/2006 and Reg. CE n°1883/2006) on the limits and the composition of contaminants had imposed new epidemiological studies to provide data and tools for risk evaluation and analysis. Milk tanker analysis showed WHO-PCDD/F PCB-TEQ values between 2,589 and 3,03 pg/gr of fat, lower than legal limit (6,00 pg/gr. of fat) but higher than action limit 2,00 pg/gr. of fat (raccomandazione CE 144/06) The results indicate PCB as primarily contamination compounds, representing more than 65% of pollutants, lower than dioxins (PCDD/F). Food for human consumption environmental contamination is certainly widespread, given the huge dispersion of these substances into environment, but there are a few epidemiological studies that describe the magnitude and type of risk.

In this study, samples of "Ciabuscolo", a raw fermented meat product listed among the traditional products of the Marche Region, were collected from four meat processing plants and analysed to evaluate microbiological process and product's standards.

Bolzoni° G

In difesa del latte intero


Bolzoni° G, Marcolini° A, Consolini° M, Varisco° G

Pagamento a qualità meno euro ma più mercato


La microbiologia predittiva degli alimenti quale strumento per l'analisi del rischio : dinamica di comportamento dei patogeni durante il processo produttivo


The aim of this paper is the presentation of the experimental draft used by Food Department of Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna to demonstrated the safety of food processing by use of challenge test combined with mathematic model of predictive microbiology.

Caprai° E, Accurso° D, Galletti° G, Licata E, Carone° V

Contaminazione da aflatoossina M1 nel latte bovino : risultati di cinque anni di sorveglianza in Emilia-Romagna = Aflatoxin M1 contamination in bovine milk : results during five years of surveillance in Emilia Romagna region


A seguito della contaminazione da aflatoossina B1 verificatasi nel 2003 negli alimenti di uso zootecnico e del conseguente innalzamento dei valori di aflatoossina M1 nel latte, il Servizio Veterinario Igiene degli Alimenti della regione Emilia Romagna ha predisposto un apposito piano di sorveglianza per monitorare l'andamento di tali residui nel latte bovino, a tutela della salute dei consumatori. Il piano di sorveglianza attuato dal 2004 prevedeva un campionamento mensile di circa 300 aziende bovine. Sulla base dei risultati ottenuti, il numero dei campionamenti è stato progressivamente ridotto nel corso degli anni (100 campionamenti mensili nel 2007), mantenendo comunque un monitoraggio continuo di tutte le aziende bovine della regione. Nel corso del 2004 il livello medio delle concentrazioni di aflatoossina M1 rilevato dalle analisi effettuate si è ridotto da
Owing to proliferation of aflatoxin B1 in feed during the 2003 and the consequent increase of aflatoxin M1 concentration in bovine milk, the Veterinary Service of Emilia Romagna region started a particular plane to monitor the depletion of these residues in bovine milk, to preserve public health. The monitoring plane carried out from 2004 consisted on a monthly sampling in 300 bovine farms. According to the results obtained, the number of samples analysed has been reduced during the years (100 samples in 2007) keeping a bovine farm monitoring anyway. During the 2004 the average concentration of aflatoxin M1 in the samples analysed has been reduced from 0.027 pg/kg to 0.022 pg/kg, thanks to preventive measures actuated owing to the emergency. The change of main aflatoxin M1 concentration from 2005 to the beginning of 2008 was the same of the 2004, but with an average value fixed around 0.010 pg/kg. These concentrations movements showed however an annual increase between August and November; it would be necessary to respect the number and the frequency of monthly sampling in the farms and dairy, as the regional plane required and eventually to increase the controls attendant in the critical period (the beginning of autumn).

Cosciani_Cunico° E, Bonometti° E, Finazzi° G

Validation of Listeria predictive growth model with italian meat product


International ICFMH symposium (21st : Arbeen, Scotland : 1-4 September, 2008)

Zampone is a typical Italian food product made with minced pork meat and fat. It is not an RTE food; the pre-cooked slices eaten without or after insufficient heat treatment, is potentially a risk for the consumers. In this paper, the ability of L.monocytogenes to grow in Zampone at refrigeration and abuse temperatures was studied. Three strains of L.monocytogenes were spread onto slices of pre-cooked Zampone in the concentrations of ca 10^3 cfu/ml. The contaminated meat slices were then vacuum packed and stored a 4-10-15-20°C. The growth of the pathogen was observed by planing it on selective medium at regular intervals. The samplings were carried out in duplicate: The growth rates were calculated using the model of Baranyi and Roberts (1994). In pre-cooked Zampone, L.monocytogenes grew at each used temperature values; the accuracy of the predictive model was of 1.36 (Baranyi et al.1999). The discrepancy between the predictive model and the experimental data was in the range of what was previously described in literature (Pin et al. 1999). Since Zampone represents a good substrate for L.monocytogenes, to consume this meat without following the label instruction could be a risk.

Cosciani_Cunico° E, Bonometti° E, Finazzi° G, Daminelli° P

Survival of Salmonella typhimurium in some italian meat products


International ICFMH symposium (21st : Arbeen, Scotland : 1-4 September, 2008)

Many of the most well-known Italian meat products are made from raw meat, in which Salmonella Typhimurium could be present as contaminant (European Rapid Alert System for Food and Feed, www.ec.europa.eu). In this study, spiced Salame, Salame alla cacciatora, Pancetta arrotolata,
Coppa piacentina, were inoculated with high concentration of pathogen at the beginning of food processing. Behaviour of S. Typhimurium, temperature, pH, aw, controlling parameters profiles, during food processing and storage, was studied and the D values were calculated. Three different S. Typhimurium strains were propagated, the inoculum was collected at the final concentration of \(10^8\) cfu/ml and was spread into the minced raw meat or over the meat surface (Hinkens et al. 1996). For each challenge tests, sampling was carried out in duplicate and enumerations were performed at regular intervals by plating food suspension onto selective medium. S. Typhimurium had decreased with a linear function depending on the rate of aw decreasing (Cosciani et al. 2005). In spiced Salame and Salame alla cacciatora, that are commercialized after 21 days of storage, the D value was, respectively, 4 days and 15 hours with a standard error of 0.51, and 18 days and 7 hours with a SE of 0.32. Storage of Pancetta arroto is 50 days and S. Typhimurium D value in this product was 33 days and 10 hours with a SE of 0.28. Coppa piacentina is usually commercialized after 90 days of storage, and D value of Salmonella on the surface was 17 days and 8 hours with a SE of 0.61. The process parameters were of the commercial product. Since the considered safe decrease of cell concentration was not always observed (USDA 2001, 66 FR 12590), we suggest that better control of the raw materials, the process parameters and the possible longer product storage are necessary in order to guarantee the safety of these products.


La microbiologia predittiva quale strumento per l'analisi del rischio: dinamica di comportamento di Listeria monocytogenes durante la shelf life degli alimenti RTE


The aim of this paper is the presentation of the experimental draft used by Food Department of Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna to demonstrated the safety of food processing by use of challenge test combined with mathematic model of predictive microbiology.


Sicurezza alimentare : compiti, ruolo e strumenti del veterinario ispettore negli stabilimenti di trasformazione = Food safety : veterinary inspection role in food plants


Food safety is based on good knowledge of products and of productive process as well as explained in European Community Legislation (Reg. CE 2073/2005 integrated by Reg. CE 1441/2007), Food Department of Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna edited
guidelines to support of food business operators compliance with microbiological criteria and, at the same time, to explain veterinary inspection role in food plants.

Daminelli° P, Monastero° P, Finazzi° G

Techniques for rapid pathogens detection raw milk

International ICFMH symposium (21st : Arbeen, Scotland : 1-4 September, 2008)

Recently in Italy the idea regarding "natural food" is very popular among consumers; consequently, according to Regulation EC No 853/2004, farm increase raw milk commercialisation through automatic dispenser (Bolzoni et al. 2007). Pathogens like Listeria monocytogenes, Salmonella spp., Campylobacter coli/jejuni, can be found in raw milk, so it is necessary to organize controls during production and distribution to ensure an adequate level of food security for consumers. Lombardia Region is the most important land of Italy for number and quality of milk farmers, and so the Sanitary Authority organised a monitoring plan to verify sanitary condition of milk directly sold. Molecular biology methods were applied to obtain results quickly in order to allow raw milk trading; in particular was used specific real time PCR for detection of L. monocytoges and Salmonella spp., whereas Campylobacter coli/jejuni were detected by traditional PCR technique. Microbiology methods were used only to confirm positive results obtained by PCR techniques. The sampling was made in two period: at first from September 2005 to May 2006, and then from January to August 2007. 1984 raw milk samples collected from 218 milk farms were analysed by PCR (for all pathogens). The prevalence of pathogens in raw milk detected by PCR in the first period of sampling was 2,62% for L. monocytogenes, 1,60% for Salmonella spp., 0,29% for Campylobacter coli/jejuni. the second period the pathogens prevalence reduced for L. monocytogenes (0,28%) and Salmonella spp (0,70%) while increased for Campylobacter coli/jejuni (0,88%). Salmonella spp and L. monocytogenes were isolated with microbiology methods respectively only 1 and 5 times while in any time was isolated Campylobacter coli/jejuni. This results show that the use of PCR methods is very useful because it permits to detect microbiological hazard at low level, before it becomes critical for health consumers..

Dusi° G, Bozzoni° E, Assini° W, Gasparini° M, Ferretti° E

Development and validation of a confirmatory method for the determination of resorcylic acid lactones in urine sample by liquid chromatography-tandem mass spectrometry

Euroresidue : conference on residues of veterinary drugs in food (6th : Egmond aan Zee (Netherlands) : 19-21 May, 2008)

The determination of zeranol, its metabolites taleranol and zearalanone is complicated by the occurrence of other resorcylic acid lactones (zearalenone, (x- and P-zearalenol). The aim of this study is a very quick analytical procedure for the determination of all six resorcylic acid lactones. The urine sample was subjected to an enzymatic deconjugation and then simply cleaned up on an immunoaffinity column. The analytes were detected by liquid chromatography- negative ion electrospray tandem mass spectrometry using deuteriumlabelled internal standards. The method was validated as a quantitative confirmatory method according to EU Decision 2002/657/EC. The results obtained show good linearity, accuracy and ruggedness. The decision limits obtained were around 0.6 pg L-1 for all the analytes.
Comportamento di microrganismi patogeni in formaggi o caprino fresco e stagionato = Dynamic of several pathogens on artificially contaminated fresh and seasoned Caprino cheeses

In questo lavoro sono riportati i risultati di un challenge test eseguito per valutare il livello di sicurezza del caprino, formaggio prodotto a partire da latte crudo di capra. A tale scopo differenti aliquote di latte sono state contaminate ognuna con una sospensione di 10^8 ufc/mL di una miscela di 3 ceppi per ciascuno dei patogeni Salmonella typhimurium, Listeria monocytogenes, Escherichia coli O157:H7, Staphylococcus aureus tossigeno (Genigeorgis et al.,1991). In parallelo è stata eseguita anche una caseificazione con latte non contaminato. Lo fase di acidificazione e coagulazione del latte a temperatura di 20°-22°C per 24 ore non si è dimostrata in grado di modulare la presenza dei microrganismi contaminanti. Tuttavia la successiva conservazione delle forme in cella frigorifera a 6°-8°C, grazie all’azione combinata di pH acido e di bicompetizione parte della flora lattica, ha determinato in 5-6 giorni un rapido decremento tra 4 e 5 logaritmi della concentrazione di Salmonella, e di 1 logaritmo di E. coli. Tale effetto risulta ancor più evidente prolungando la fase di stoccaggio/stagionatura del formaggio. Per quanto riguarda L. monocytogenes invece, per assistere alla riduzione decimale di tale microrganismo è stato necessario conservare il caprino per oltre 2 settimane. Staph. aureus, da ultimo, per quanto abbia mostrato un decremento nel numero di ufc/g, è risultato capace di produrre la tossina già al termine della fase di coagulazione del latte.

The aim of this trial was to value the behaviour of some pathogens during the processing and ripening of Caprino, an Italian raw goat milk cheese. Different groups of Caprino cheese were made: one as negative control and other four were made contaminating the goat mille with suspensions of different strains of Salmonella typhimurium, Listeria monocytogenes, E. coli O157:H7 and toxigenic Staph. aureus., with a concentration of nearly 10^8 cfu/mL each. The coagulation phase of milk, obtained to 20°-22°C far 24 hours, wasn’t able to determine any effect on pathogens concentration, but ripening the cheese at 6°-8°C for 5-6 days, the combined action of low cheese pH and great amount of lactic bio-competitive flora was able to determine the rapid decrease of Salmonella (4-5 log) and of E. coli O157:H7 (1 logo), too. To obtain a decrease of L. monocytogenes population was necessary to extend ripening of the cheese far at least 2 weeks. Staph. aureus capabilily to produce the toxin was demonstrated till the earlier phases of cheese processing.

Valutazione della dinamica di sopravvivenza di L. monocytogenes nel formaggio Grana Padano DOP grattugiato = Survival dynamic of Listeria monocytogenes in Grana Padano DOP grated cheese

E stato predisposto un challenge test con lo scopo di valutare la dinamica di sopravvivenza di L. monocytogenes nel formaggio Grana Padano DOP grattugiato. A tal fine sono state preparate aliquote di prodotto contaminate con una sospensione di 3 diversi ceppi del microrganismo in modo da ottenere una concentrazione finale compresa tra 105-1011 ufc/g. Diverse aliquote sono state poste ad incubare a 4°, 10°, 15° e 20°C e sottoposte a periodo campionamento per valutare l’andamento del patogeno. I risultati speri-mentali sono stati confrontati con modelli di microbiologia predittivi consultabili sul sito www.combase.cc (1). Ad una temperatura di refrigerazione ottimale del campione (4°C) la popolazione di L. monocytogenes, tende lentamente a calare mentre il modello matematico, basato sui dati di crescita in brodo, stimava un aumento evidente a partire do circa il quarantesimo giorno di conservazione. A 10°C Listeria tende a colera leggermente, nelle prime tre settima-ne per poi mantenersi su livelli costanti fino alla fine del secondo mese, mentre il modello matematico stimava invece una crescita del microrganismo a partire dal quindicesimo giorno per arrivare ad un incremento di circa 2 logaritmi al sessantesimo. A temperature di abuso termico, 15° e 30°C, dopo una iniziale fase di latenza, rispettivamente 15 e 9 giorni, si osserva un incremento
del patogeno fino ad arrivare ad un valore superiore di circa 2 logaritmi rispetto a quello di partenza in circa un mese, mentre le stime del modello ma-te matico prevedevano una fase di latenza decisamente più breve. Le differenze tra valori osservati e valori attesi sono riconducibili al fatto che i modelli matematici utilizzati si basano su simulazioni di crescita che tengono conto del pH e dell'altimo dell'alimentazione e della temperatura di conservazione, ma non della presenza di floghe competitive, date do batteri lattici che risultano essere invece presenti in quantità rilevanti nel Grana Padano DOP grattugiato ottenuto da forme con 10 mesi di stagionatura. Grazie a questa situazione il prodotto è risultato non favorire la crescita di L. monocytogenes a temperature di conservazione ottimali o per lo meno usualmente rilevabili nella realtà, e in grado di contrastarne lo sviluppo anche in situazioni di abuso termico.

In this challenge test Grana Padano DOP grated cheese was contaminated with a suspension of three different L. monocytogenes strains in order to value the pathogen population dynamic of different storage temperature (4°, 10°, 15° and 20° C). Data observed were compared with theoretical growth curves calculated applying predictive microbiology models. At 4°C, ideal storage condition, Listeria slowly decreases while in the predictive model was expected it should increase beginning from 40th day. At 10°C the pathogen population doesn't change while was predicted an increase beginning from 151 day. At 15° and 20°C Listeria increases but slower comparing to what expected. Grano Padano DOP grated cheese, a food with a high amount of lactic flora able to bio-compete with pathogens, doesn't support the growth of Listeria monocytogenes to ideal and at usual storage temperature, and is also able to oppose the microbial replication in situation of thermal abuse.

Finazzi° G, Daminelli° P, Cosciani° E, Bonometti° E

Behaviour of Listeria monocytogenes in sliced mortadella stored at different temperatures in presence of sodium lactate


International ICFMH symposium (21st : Arbeen, Scotland : 1-4 September, 2008)

Listeria monocytogenes is one of the most important microbiological risks in ready to eat food such as Mortadella (Beumer et al. 1996), an Italian salami, known also as bologna, made with pork meat finely minced, combined with pork fat cubes and spices and than sacked and cooked at 70°C or more for several hours. In this work two kind of Mortadella, regular and added with Sodium Lactate 3.5%, were surface contaminated with a suspension of three different strains of Listeria monocytogenes in order to obtain a final concentration of around 103-104 cfu/cm² (Glass, 2002). Different kinds of Mortadella were then sliced, packaged in ma condition and stored for 90 days at different temperatures 14, 10, 15 and 20°C) to value the behaviour of Listeria. In regular Mortadella, stored at 4°C, L. monocytogenes doesn't grow (SE 0.28), while in Mortadella added with sodium Lactate the pathogen died with a biphasic function with a D value in the first step of 22 days and 17 hours and a second phase occurring after 38 days in which Listeria population stabilizes (SE 0.22). At 10°C Listeria increases with a duplication time of 1 day and 17 hours (SE 0.23) reaching plateau phase after nearly 11 days, while in presence of sodium Lactate the pathogen decreases with a biphasic function with a D value in the first step of 5 days and 7 hours and after 7 days and 15 hours D becomes more than 97 days (SE 0.12). At 15°C Listeria increases with a duplication time of 18 hours ± 4 hours reaching plateau phase after nearly 9 days (SE 0.56), while in presence of sodium Lactate the pathogen population keeps the sure level during the storage period (SE 0.591). Finally at 20°C the pathogen increases in both situation but in regular Mortadella the duplication time of Listeria is 8 hours ± 2 reaching plateau phase after nearly 3 days (SE 0.43), while in presence of sodium Lactate the duplication time is 1 day and 17 hours and the plateau phase occurs after nearly 8 days (SE 0.46). After this trial is evident how the addiction of sodium Lactate during production of Mortadella is useful to inhibit Listeria monocytogenes at usual storage temperature, and to slow down pathogen replication in situations of thermal abuse.
In collaborazione con un produttore è stato condotto un challenge test che ha previsto la contaminazione di bresaole di bovino, taglio punta d'anca, al termine della fase di salamoia con sospensioni di Salmonella typhimurium, L. monocytogenes e E. coli O157:H7 e successivo insacco e legatura. Le bresaole nel corso del normale processo di asciugatura/stagionatura sono state periodicamente campionate in superficie per valutare l'andamento delle popolazioni dei patogeni. Salmonella typhimurium diminuisce con andamento bifosico e il tempo di riduzione decimale (Di) tempo necessario per avere la diminuzione di 1 logaritmo del microrganismo, nella prima fase è pari a 8 ore e 16 minuti, mentre nella seconda a 20 giorni e 11 ore. Anche E. coli O157:H7 diminuisce, ma con andamento lineare caratterizzato da D pari a 8 giorni e 20 ore. L. monocytogenes diminuisce anch'essa in maniera lineare ma con tempiotiche sensibilmente più lente mostrando una D pari a 30 giorni e 17 ore.

The aim of this trial was to evaluate the dynamic of the pathogen population, such as Salmonella typhimurium, L. monocytogenes and E. coli O157:H7, during the seasoning of traditional Valtellina Bresaola PGI. Different Bresaola were artificially contaminated on their surface after brine-salting with a 10^7 cfu/cm² concentration of pathogens, and then soaked and seasoned. Samples were taken from the surface of each bresaola during the seasoning time of 5 weeks, and analysed to value the populations of pathogens. Salmonella decreased with a biphasic trend characterized by a first phase in which the decimal decreasing time (Di) was 8 hours and 16 minutes, and a second phase in which D became 20 days and 11 hours. However, during the usual seasoning period, the decrease of Salmonella was equal to 5 logarithms. E. coli O157:H7 decreased with a line or trend in which D was 8 days and 20 hours, and during the usual seasoning period, the decrease of the pathogen was equal to 4 logarithms. The population of L. monocytogenes decreased with a linear trend in which D was 30 days and 17 hours, and so during the usual seasoning period the decrease of the pathogen was less than 2 logarithms.
La valutazione dell'efficacia degli alimenti funzionali mediante i test della tossicologia in vitro

= In vitro toxicology methods for the evaluation of the efficacy of functional food


La commercializzazione e la vendita dei prodotti alimentari definiti "funzionali" è attualmente caratterizzata da un forte incremento, sostanzialmente sostenuto dalla nuova concezione di alimento come promotore di benessere. La legislazione in materia di alimenti funzionali è ancora carente anche se sempre più pressante è la richiesta di strumenti atti a dare evidenza oggettiva delle proprietà dichiarate. Le colture cellulari ed i metodi alternativi della tossicologia in vitro risultano tra i più promettenti strumenti in grado di evidenziare specifiche attività dei principi attivi sulle funzioni cellulari, garantendo quindi sostenibilità scientifica alle ulteriori azioni finalizzate alla garanzia di sicurezza alimentare. Su questa base, vengono di seguito descritte alcune possibili applicazioni delle colture cellulari e dei test della tossicologia in vitro nella evidenziazione di alcune delle più comuni proprietà dichiarate dagli alimenti funzionali rappresentate dall'attività probiotica, intiossidante ed immunomodulatoria.

"Food as benefic product" is a recent concept shared by science and consumere. Recently, it has been a relevant increase in the trading of food meant as well-being promoter. At present, European low has a shortage about the regulation of food proposition as wellness proposer, but many parts of food trading and production are asking for new rules able to protect the characteristics that define each ype of product. Cell culture and in vitro toxicology-alternative methods are important means to detect the activity of some specific molecules on the cellular behaviour, so they are the scientific tools to rely on for the proposition of bills directed to warrant the food safety. On this bases, they will be described some possible cell culture applications and in vitro toxicology tests, to screen the most common properties of functional foods such as probiotic activity, anti-oxidant and immunomodulating activity.

Allergie e intolleranze alimentari = Food allergies and intolerances


Consumo di latte crudo : valutazione del livello di esposizione ai principali patogeni batterici attraverso metodiche culturali e biomolecolari = Raw milk consumption : evaluation of exposition level to main pathogens using culturall and biomolecular methods


Norovirus (NoV) is recognized as the most important cause of nontlacterial acute gastroenteritis in adults, responsible of outbreaks especially in closed institutions such as restaurants, nursing homes and hospitals. Diagnostic and detection of Norovirus presence in food are limited because of the difficulties of replication in cell cultures and the gold standard for detection is represented by reverse
transcription PCR, although it is very difficult to find an appropriate primer pair that is both sensitive and specific, due to the high genetic diversity among noroviruses. Presently, various real time PCR assays have been reported for NoV detection but data that support a method of choice definition are not available. Therefore it is necessary to compare and standardize methods to apply in surveillance systems. The aim of the present study was to compare in terms of efficiency, sensibility and specificity three different methods for NoV detection in fecal and food samples.

Menotta° S, Caprai° E, Masselli° M, Nocera L, Taus L, Massirio° I, Fedrizzi° G

Emergenza aflatossine 2003: controllo dei formaggi a lunga stagionatura prodotti in Emilia Romagna = Emergency during year 2003: control of aflatoxin M1 levels in cheese samples produced in Emilia Romagna region


Nel 2003 il particolare andamento della stagione agronomica ha causato una forte contaminazione da aflatossine nelle granaglie destinate all’uso umano e all'alimentazione zootechnica: ciò ha inevitabilmente portato ad un innalzamento dei residui di aflatossina M1 nel latte causando una situazione di emergenza per la tutela della salute dei consumatori. Per affrontare tale emergenza la regione Emilia Romagna ha impostato un piano di controllo di tutte le aziende produttrici di latte e suoi derivati al fine di individuare situazioni a rischio. Nella regione oltre il 70% del latte è destinato alla caseificazione e l'autorità sanitaria pur permettendo la trasformazione del latte prodotto nei giorni successivi al riscontro di una positività, aveva posto in vincolo cautelativo i corrispondenti lotti di formaggio. In tal modo, in attesa di disposizioni, erano state sequestrate negli stabilimenti circa 8000 forme di formaggio a pasta dura in stagionatura, prodotte nel periodo compreso fra novembre 2003 e dicembre 2004. Nell'agosto 2004 il Ministero della Salute emanava una nota che stabiliva un limite provvisorio di 0,450 pg/kg per i formaggi a pasta dura a lunga stagionatura e che elencava i punti salienti del metodo analitico consigliato e delle modalità di campionamento. In tal modo si è potuto procedere alle fasi successive: per ogni unità produttiva posta in vincolo erano stati analizzati 3 campioni e all'analisi seguendo criteri stabiliti dai servizi veterinari della Regione. Nel periodo compreso tra dicembre 2004 e febbraio 2005 sono stati analizzati 378 campioni di formaggio appartenenti a 144 lotti. Il 4,8% dei campioni risultava superiore ai limiti provvisori previsti; il 9,3% presentava una concentrazione compresa tra 0,300 e 0,450 pg/kg, il 49,2% tra 0,100 e 0,300 pg/kg e il 36,8% inferiore a 0,100 pg/kg.

During the summer 2003 the particular atmospheric conditions caused the proliferation of aflatoxins (B and G) in cereals and feed. The resulting massive contamination from aflatoxin M1 in cow milk and derivatives cried for a rapid and exhaustive analysis of all Emilia Romagna products. The Regional Authorities started a particular plan to improve the controls of regional cheeses produced between November 2003 and December 2004. In Emilia Romagna region the production of matured cheese is a very important reality, so it was necessary to analyse these products before the merchandising. An LC-MS/MS analytical method was developed and 378 cheese samples were analysed. These samples Game from 144 batches of matured cheese that the veterinary authorities abducted because produced with suspected irregular milk. Totally the 95,2% of samples was regular with concentration of aflatoxin M1 lower than 0,45 pg/kg. Thirteen batches of cheese were irregulars: they had one or more cheese with residues of aflatoxin M1 more than 0,450 pg/kg.

Menotta° S, Carone° V, Bolognesi° E, Caprai° G, Fedrizzi° G

Determination of chloramphenicol residues in royal jelly with liquid chromatography-tandem mass spectrometry: validation of the method based on 2002/657/EC

Fourth International Conference on Antimicrobial Agents in Veterinary Medicine (AAVM) : Prague, Czech Republic, August, 24-28, 2008 : program and abstracts / [s.l. : s.n., 2008]. - p 60 [Nr. Estr. 3843]

International Conference on Antimicrobial Agents in Veterinary Medicine (AAVM) (4 : Prague, Czech Republic : August, 24-28, 2008)
During the summer of 2005 some consumer associations accused a massive contamination of chloramphenicol (CAP) in Royal Jelly imported from China. CAP is a broad-spectrum antibiotic but it's been included in Annex IV Regulation 2377/90 because its heavy toxically effects demonstrated in human. It's illegally used against bee's diseases or to preserve honey and its derivatives from microbiological contaminations. Royal jelly, because of its singular properties (restorative characteristics) is consumed by particular class of people, such as old people and children. To preserve public health, Italian labs began to analyse this compound, very different from other bees products. The Royal Jelly chemical characteristics impeded to use screening techniques, so a rapid method for determination and confirmation of CAP was developed. Following addition of d5-Chloramphenicol as internal standard, Royal Jelly was finely mixed with silica-powder and extracted with ethyl acetate. After centrifugation, a fraction of supernatant was evaporated. The residue was reconstituted with methanol-water and analysed in LC-MS/MS. By using an MRM acquisition method in negative ionization mode, the transitions 321>152, 321>194 and 326>157 were respectively used for quantification, confirmation and internal standard. The method validation was based on EU-decision 2002/657. The CAP linear range was from 0,1 to 0,6 gg/kg. Intra-laboratory reproducibility was 7,7%; repeatability was 10%. The mean recovery was 80,6%. CC(alpha) and CCP were 0,14 µg/kg and 0,16 µg/kg respectively. During the emergency phase (from August 2005 to March 2006) more than 200 samples coming from China were analysed and 17% presented levels over the MPRL fixed by EC for CAP.

Menotta° S, Fedrizzi° G, Macrì S, Scandurra S, Saggiora M

Results of avilamycin residues monitoring plans for the experimental use in Italy


World rabbit congress (9 : Verona (Italy) : June 10-13, 2008)

The digestive disease is the main cause of mortality in industrial fattening rabbit farms. Recently, avilamycin has been experimentally used by rabbit producers in Italy as a new option to control digestive syndrome. This experimental use was exceptionally authorized by the Italian Health Ministry in order to reduce the losses due to Epizootic Rabbit Enteropathy (ERE) in the rabbit breeding. Although the ERE pathogenesis is not yet completely known in all its aspects, the presence of Clostridium perfringens has been reported as associated agent in most of cases. The objective avilamycin experimental use was to evaluate the efficacy of the drug in feed for the control of digestive signs associated with Clostridium spp. in rabbits at a dose of 5 mg avilamycin/kg body weight/day, equivalent to 60=120 g11000 kg of feed on the basis of age, body weight and feed consumption for all animals in the weaning phase. During the experimental use some different residues monitoring plans were performed by Italian health authorities and Elanco Animal Health with the aim to ensure public health on treating food-producing animals with an experimental therapy. According to the guidelines of the Italian Health Ministry an official avilamycin residues monitoring plan was conducted by Elanco during the last two years and other experimental plans were performed by regional authorities and foodstuff producers. An HPLCMS/MS method was developed by Elanco, performed and validated by the "Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna" according to European legislation (EEC/657/2002) and applied to monitor avilamycin residues in the different plans actuated in Italy. The Avilamycin was analyzed by hydrolysis to dichloroisoverninic acid (DIA), the residual marker analyzed. The avilamycin linear range was from 50 to 250 gg/kg (approximately concentration of DIA from 10 to 50 pg/kg). Within laboratory data (reproducibility intra-laboratory) were from 11% to 17% for muscle and liver. Repeatability was included between 10% and 19% for both tissues. The mean recovery was 85% for muscle and 81% for liver. According to different monitoring plans, more than 250 samples of rabbit treated with avilamycin were collected and their tissues (muscles, livers or both tissues) were analyzed. The results obtained from these analyses demonstrate the very low risk due to residues and the high level of safety for avilamycin used in rabbit as food-producing animal.
Menotta° S, Fedrizzi° G, Macrì S, Scandurra S, Saggioro to M

Results of avilamycin residues monitoring plans for the experimental use in Italy


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Caratterizzazione delle tossine PSP (Paralytic Shellfish Poisoning) in mitili raccolti in differenti aree marine italiane = PSP toxic profile of mussels collected in various marine areas of Italy


During the period November 2006 - August 2007, PSP toxins have been detected in mussels from three different Italian marine areas. Here we report about the toxic profiles of these molluscs, as revealed by HPLC-FL. Differences among the three areas are presumably related to the presence in the presence in the Alexandrium (Dinophyceae) species: A. catenella in Sardinia and A. minutum in Sicily and Emilia Romagna.
Rugna\textsuperscript{a} G, Bardasi\textsuperscript{b} L, Vecchi\textsuperscript{c} G, Mazzini C_Z, Bacchi M, Illetti\textsuperscript{d} G, Merialdi\textsuperscript{e} G, Fontana\textsuperscript{f} MC

**Effetto di un trattamento di pasteurizzazione post-confezionamento su porzioni di mortadella nei confronti della contaminazione da Listeria spp.**


The presence of Listeria monocytogenes in ready-to-eat (RTE) meat products is cause of public-health concern. To reduce the risk of food borne infection and ensure food safety, RTE meat cooked-products can be pasteurized after packaging. In this study the efficacy in reducing L. innocua superficial contamination of three in-package pasteurization protocols (A: 89 °C x 6 min; B: 89 °C x 8 min; C: 89 °C x 10 min) was evaluated. Treatment C ensured superficial contamination reduction also in particularly irregular surfaces like those at the edges of product and can be an effective intervention to reduce L. monocytogenes contamination risk in such products.

Sangiorgi\textsuperscript{a} E, Saccani G

**Circuito interlaboratorio per la determinazione del contenuti di nitriti e di nitrati in matrice carne**


Nel corso del 2005 si è svolto un circuito interlaboratorio per la determinazione dei nitriti e nitrati in matrice carne tra laboratori pubblici (Istituti Zooprofilattici, Stazione Sperimentale Conserve Alimentari ed il Laboratorio Alimenti dell'ISS) e laboratori di alcune industrie alimentari, per un totale di 20 partecipanti. Il lavoro presenta i risultati ottenuti, la loro elaborazione statistica ed un confronto tra i metodi spettrofotometrico e quelli che utilizzano la cromatografia ionica. I risultati mostrano una dispersione dei dati elevata, dovuta in parte al differente trattamento dei campioni nei vari laboratori ed ai diversi metodi di rilevazione mentre non ci sono differenze statisticamente significative tra la spettrofotometria e la cromatografia ionica.

In the year 2005 there has been a proficiency test for nitrite and nitrate determination in meat with government laboratories (Istituti Zooprofilattici Sperimentali, Stazione Sperimentale Conserve Alimentari, Alimenti's Laboratory of Istituto Superiore di Sanità) and food industries laboratories (20 partecipants at all). This paper shows the results, their statistical elaboration and the comparison between spectrophotometric and ion chromatography (IC) methods results. The high dispersion of the data is due to the different sample treatment and detection methods probably; spectrophotometric methods Have no statistically significant difference with (IC) methods.

Sangiorgi\textsuperscript{a} E, Simoni\textsuperscript{b} M, Berneri\textsuperscript{c} R, Ferretti D, Pi ro\textsuperscript{d} R

**Utilizzo della spettroscopia FTIR per la caratterizzazione e le analisi di legge di mieli italiani**


Simposio Italiano di Spettroscopia NIR (3 : Lazise (VR) : 22-23 Maggio 2008)

Il miele riveste particolare importanza nell'alimentazione odierna in relazione alle sue caratteristiche nutrizionali, di salubrità e di genuinità. L'analisi del miele è complessa, nonostante l'apparente semplicità della matrice, comprende tecniche chimico fisiche come rifrattometria e conducibilità per la determinazione di umidità e ceneri, tecniche di cromatografia liquida per la determinazione di
zuccheri e idrossimetilfurfurale, titola Aone per l’acidità, reazioni enzimatiche per la determinazione dell’indice diastasico. L’utilizzo della spettroscopia NIR permette di ottenere un elevato numero di parametri con una semplice diluizione del campione. È stato utilizzato uno spettrometro NIR a trasformata di Fourier per liquidi (Milkoscan FT2, Foss Electric) con tre gruppi di lunghezze d’onda nell’intervallo spettrale tra 240 e 1299 nm (250-405 nm, 445-460 nm e 735-770 nm) dotato di software WINISI II 1.50 per l’elaborazione dei dati. I mieli, circa 1400 mieli italiani delle annate 2005, 2006 e 2007, provenivano da un concorso selezione mieli a livello nazionale. Per la calibrazione dei vari parametri sono stati utilizzati i dati delle analisi chimiche di un set di campioni variabile da 390 (zuccheri principali) a 40 (zuccheri minori) i campioni di miele, dopo una diluizione con acqua, sono stati analizzati per i parametri umidità, glucosio, fruttosio, saccarosio, zuccheri minori, acidità, HMF, conducibilità, indice diastasico, colore Pfund, polarimetria. Le curve dei principali parametri di legge si sono rivelate affidabili e altre degli zuccheri minori sono promettenti. Con la tecnologia NIR è quindi possibile effettuare i principali controlli di legge ed una valutazione quantitativa della composizione del miele in modo veloce ed economico. I dati ricavati da alcuni mieli unifloreali sono stati confrontati con i profili fisico-chimici dei mieli europei (L.Persano Oddo Apidologie 35, 2004) ottenendo una buona corrispondenza dimostrando così il possibile utilizzo di questa tecnica per una valutazione qualitativa e di caratterizzazione dei mieli unifloreali.

Scordella G, Bresolin R, Rubini° S, Fedrizzi° G

Food safety and quality requirements for importing aquaculture products in European Community : present and future


The European Union is collectively the largest importer of seafood in the world, with individual member states making up five of the world’s seven largest edible seafood importers. In 2002, the EU member states imported edible seafood valued at €24.4 billion. Over the past decade total EU seafood imports have almost doubled in value. The import rules for finfish and shellfish are harmonised, meaning that the same rules apply in all EU countries. The European Commission’s Directorate General for Health and Consumer Protection (DG SANCO) receives and distributes notifications to the food and feed inspection services of member state, as well as to other countries. Its rules for fish and shellfish products seek to guarantee that all imports fulfill the same standards as products from the EU Member States. The rather strict food safety rules and regulations in EU is a direct consequence of recent food scandals (BSE, Dioxin problem, Salmonella, Listeria and Cholera scares) that entered the minds of EU consumers as potential threats. Furthermore, in the past years, the number of alerts and notifications from the EU Rapid Alert and Safety for Food and Feed (RASFF) has risen significantly (from 698, in 1999, to 3024, in 2002). Imports of fishery products into the EU are subject to official certification, based on the recognition of the competent authority of the non-EU country by the European Commission. Public authorities must ensure credible inspections and controls throughout the production chain, which cover all relevant aspects of hygiene, public health and also animal health. The eligibility criteria are:

1. Exporting countries must have a competent authority which is responsible for official controls throughout the production chain.
2. Live fish, their eggs and gametes intended for breeding and live bivalve molluscs must fulfill the relevant animal health standards.
3. The national authorities must also guarantee that the relevant hygiene and public health requirements are met.
4. A control plan on heavy metals, contaminants, residues of pesticides and veterinary drugs must be in place to verify compliance with EU requirements.
5. Imports are only authorised from approved establishments, inspected by the competent authority of the exporting country and meet EU requirements.
6. Inspections by the Commission’s Food and Veterinary Office are necessary to confirm compliance with the above requirements.

Because of high expectations regarding the safety and quality of their food, the food law of the EU
implements the principle of quality management and process-oriented controls throughout the food chain - from the aquaculture farm to the consumer's table.

Scordella G, Rubini° S, Fedrizzi ° G, Bresolin R

Seaf ood safety laws in E.U. markets

To meet citizens high expectations, European Union seafood regulations implement quality management and process-oriented controls throughout the food chain. E.U. food businesses must work to reduce risks for consumer health. Imports must meet E.U. requirements and come from approved establishments inspected by the competent authority of the exporting country. When problems arise, the Rapid Alert System for Food and Feed notifies member states and the European Commission.

Terzano C, Gamba° V, Moretti S, Galarini R, Dusi° G

Development and validation of a confirmatory method for the determination of sulphonamides in milk by HPLC with diode array detection

Euroresidue : conference on residues of veterinary drugs in food (6th : Egmond aan Zee (Netherlands) : 19-21 May, 2008)

A simple multiresidue method for the determination of 7 sulphonamides residues (sulfadiazine, sulfapyridine, sulfamerazine, sulfamethazine, sulfamonomethoxine, sulfadimethoxine and sulfaquinoxaline) in milk samples was developed and validated. The drugs were extracted with a mixture chloroform/acetone and simply cleaned up on a action exchange solid phase extraction column. The analytes' determination was carried out using HPLC with UV-DAD detection at 270 nm. The procedure was validated as a quantitative confirmatory method according to the EU Decision 2002/657/EC. The developed method shows good linearity, specificity, precision, ruggedness and is able to confirm each sulphonamide residue above 20 pg kg-1. Decision limits around 110 µg kg-1 and recovery above 60% were obtained for all the analytes. The results of the validation process demonstrate that the method is suitable for application, as confirmatory method, in European Union statutory veterinary drug residue surveillance programmes.

Zarengi L, Fedrizzi° G, Masselli° M, Caprai° E, Ungari D, Gorreri M, Nocera L, Menotta° S

Valutazione del fattore di trasferimento di aflatossina M1 nei formaggi a pasta dura a lunga stagionatura e studio della sua distribuzione nella forma = Aflatoxin M1 concentration levels inside cheese samples and evaluation of its distribution between whey and cheese during the production

La contaminazione da aflatossina M1 del latte nel 2003 ha coinvolto anche la produzione dei
formaggi, realtà molto importante per la regione Emilia Romagna. Tre forme di formaggio, di cui era nota la concentrazione del latte con cui erano state prodotte, sono state destinate ad un lavoro sperimentale. Ciascuna forma è stata idealmente suddivisa in quattro anelli concentrici a partire dall'interno: nucleo centrale, mezzo raggio, sottocrosta e crosta. Il materiale è stato omogeneizzato separatamente ed in ciascuna porzione è stata effettuata la determinazione dell'aflatossina M1 e dell'umidità. Parte del formaggio è stato omogeneizzato in toto escludendo la crosta e sono state effettuate le stesse determinazioni. A seguito delle opportune valutazioni statistiche è stata verificata l'omogeneità di distribuzione dell'aflatossina M1 all'interno del formaggio. Nella seconda fase del lavoro sono stati analizzati 29 campioni di formaggio prodotti con latte non conforme a concentrazione nota e provenienti da 13 caseifici diversi allo scopo di valutare sperimentalmente quale fosse la concentrazione dell'aflatossina M1 durante la trasformazione del latte in formaggio (coefficiente di trasferimento). Le concentrazioni del latte di partenza erano comprese fra 0,055 e 0,280 pg/kg, quelle delle corrispondenti forme erano comprese fra 0,080 e 0,640 pg/kg. I coefficienti di trasferimento erano compresi fra 0,7 e 7,7. Dai risultati non è stato possibile individuare alcuna correlazione fra le concentrazioni del latte e quelle delle corrispondenti forme. Dal lavoro sperimentale si evince però che il 90,7% dei formaggi analizzati presentava un fattore di concentrazione minore o uguale a 5.

The contamination from aflatoxins (B and G) in cereals during 2003 caused a massive contamination from aflatoxin M1 in cow milk and consequently in milk derivatives. In Emilia Romagna region the production of matured cheese is a very important reality, so it was necessary to analyse these products before the merchandising. These kind of cheese has a long ripening time, its weight is about 30 kg and no informations were about the aflatoxin M1 distribution inside the cheese. As first step of the work the concentrations of aflatoxin M1 in different areas of cheese were analysed. Three different cheeses were subdivided in 4 parts each one from external to internal area (rind, sub-rind, medium area and centre). Each part was analysed separately and aflatoxin M1 concentration was evaluated. In the same time aflatoxin M1 was analysed from a portion of total cheese. By means of statistical evaluations it was possible to set the homogeneous distribution of aflatoxin M1 inside the cheese. The second aim of this work was to know how aflatoxin M1 was transferred from milk to cheese during the production phases and if it would be possible to evaluate which factors influence this aflatoxin M1 repartition. The cheeses product from milk with aflatoxin M1 concentration between 0,055 and 0,280 pg/kg had concentrations between 0,080 and 0,640 pg/kg. The calculated transfer factors were fra 0,7 to 7,7 but 90,7% were lower than 5. No one correlation was detected to explain these differences from cheese to cheese.

Zavanella M, Muliari R, Mioni R, D'Incau° M

Microbi e alimenti