Workshop on *Escherichia coli* (*E.coli*) / *Shiga* toxin-producing Escherichia coli (STEC) in the food sector, and, more especially, in the meat sector

jointly organised by
Instituto de Promoción de la Carne Vacuna Argentina (IPCVA) and
UECBV

Wednesday 29th October 2014

9:00-13:00

REPORT

Venue: COPA-COGECA
Rue de Trèves, 61
1040 Brussels, Belgium

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As announced, the UECBV/IPCVA Technical workshop on STEC took place on Wednesday, 29th October, am, in Brussels.

44 participants from different horizons met to exchange views on technical issues.

For the participation and the represented countries, please see the chart below:

Within the framework of the discussions on the EU food policy with regard to STEC, including the current draft aiming at a common approach to determine what is a high pathogenic *E.coli* STEC and how to manage it when positive STEC results are confirmed, UECBV and IPCVA felt there was a need for exchanging further views on the scientific and technical aspects.
The purpose of the workshop was to provide scientific information about STEC and the latest situation in the main beef producer and exporting countries, such as Argentina, and to have an open forum for discussion and debate from different realities: Europe, South America, Oceania, but also for the European Institutions to debate on a sensitive food hygiene issue that affects International food trade.

Gonzalo Alvarez Maldonado, President of the IPCVA, welcomed all the participants arriving from more than 13 countries, both from the EU and Third Countries.
He expressed the high expectations his organisation has put on this important event.
He underlined the objectives of the seminar, in particular:
- to look for a common opinion on what is a pathogenic *E.coli* STEC in relation to the Uremic Hemolytic Syndrome (HUS)
- to debate on the approach for the risk assessment and to improve the standardisation and harmonisation of microbiological sampling and analysis
- to look for a pragmatic way of managing risk and the latest knowledge on good hygienic practices and the use of other available new tools to control the risk. Read more

Hector Salamanco, Director of the IPCVA, as a co-chairman of the workshop, introduced the first speaker.

I. **Gerardo Leotta,** Veterinarian and Microbiologist, National Scientific and Technical Council in Argentina

For his presentation: Click HERE

In his first point on hazard identification, Gerardo Leotta started by explaining that, in the *E.coli* group producing shiga toxins, there is a great quantity of shiga toxins which are not causing diseases for humans, while others are.

Regarding the hazard characterisation, the main factors of virulence are the modified shiga toxin (*stx1* and, even more, *stx2*), while the principal adherence factor is intimin (*eae* gene) and other virulence factors are *aaiC* and *aagR*.
In 7 years, out of 1200 cases of toxi-infection in Argentina, 74.5% are due to O157:H7/NM (presence of *stx2* and *eae*).

In 2011, a major outbreak occurred within the EU due to STEC O104:H4 in sprouts. It was an unknown pathogenic strain. EFSA wanted to identify how this new pathogen emerged and, as a result, a new classification was proposed from which the main group is *eae*-positive or *aaiC*- and *aggR*-positive linked to the serogroups: O157, O26, O103, O145, O111, O104 (the 'big six'). This latest group (O104) is the one which caused severe diseases in 2011.

Regarding the exposure assessment, the main animal reservoirs are diversified: cattle, but also wild animals, rats, dogs, cats, goats, pigs, molluscs.
Products at risk are: minced meat, non-pasteurised milk, seeds, salads, frozen vegetables, non-pasteurised fruit juices and water.
The transmission between humans exists.

In relation to meat, the risk, when existing, remains at the surface. When the meat is further processed, the main risk categories are trimmings and minced meat (where the surface of meat is mixed) that can be eaten raw or with insufficient heat treatment.

Regarding the risk characterisation, it was referred to the US, EU and the ISO standard 13136/2012 approaches. The ISO standard is a good methodology, but it considers that all *shiga* toxin producing *E.coli* are pathogenic. Scientific research has shown that this is not always the case.

In Argentina, several actions have been taken to effectively reduce the risk posed by STEC in slaughterhouses, such as improving the chilling step, taking sanitary measures, disinfection of the environment and equipment, specific machine to remove the tails at an early stage, using lactic acid, reducing cross-contamination when moving carcasses etc.

A research project from March to September 2014 carried out in slaughterhouses has shown that very good results can be obtained after applying good hygienic practices. Out of 139 on 15000 samples (3000 analysis), VTEC was isolated in 10.6% of samples; however, none of them was genotype *eae*, *aaiC* or *aggR*.

One of the main conclusions from Gerardo was that, currently, there is not sufficient data to say that all STEC are pathogenic or not. The available methodologies today do not allow detecting and isolating all types of STEC. The zero-tolerance for all types of STEC in meat is not justified. For example, most of STEC outbreaks in Argentina have been associated with water and only two were associated with beef. The approach to control STEC must be at all steps of the food chain: primary production, industry, trade and consumer.

II. Presentation by **Gustavo Iabichella,** Dirección Nacional de Inocuidad y Calidad Agroalimentaria (DNICA) of Servicio Nacional de Sanidad y Calidad Agroalimentaria –SENASA–, Argentina

Gustavo Iabichella presented the risk management by the Argentinian authorities, i.e. SENASA. For his presentation: Click HERE

- STEC monitoring on carcasses has been put in place, in particular since 2012, with two aspects:
  - Detection on presence of STEC on bovine and ovine carcases
  - Obligation to monitor the risks within the HACCP systems

- Guidelines have been drafted to prevent cross-contaminations in slaughterhouses between carcasses and the digestive tract content/hides and skins

- A resolution allowing the use of lactic acid on carcasses has been adopted following the new possibility offered by the Regulation EU/101/2013

The basis of the legislation is to focus first on products at risk and, then, to identify the presence of one of the following relevant serotypes for meat: O145, O121, O26, O111, O113 and O157:H7.

A list of potential risk products has been specified:

- ✓ Ready to Eat Food
- ✓ Vegetables
- ✓ Minced meat
- ✓ Fresh charcuterie (mainly offal and trimmings)

A standing veterinary committee has been organised with other South American countries, such as Brazil, Uruguay, Chile. This committee monitors and evaluates the situation in the member countries.
The risk associated with STEC is complex, as it is difficult to differentiate between EHEC and VTEC; there is genomic plasticity and, therefore, the risk management must be based on the assessment on the pathogenicity of products.
Then, food safety must be first ensured by preventing the risk through the implementation of good hygiene practises, HACCP-based systems and micro-criteria checks (such as listeria, salmonella etc.).



In relation to risk communication, it is key that an interactive exchange of information and opinions is put in place between all interested parties, including consumers.

In conclusion, Argentinian authorities consider that, in case of identification of a positive STEC case, the food must be adequately treated before reaching consumers and that the risk communication to consumers must include recommendation on how to handle/cook food.

III. Presentation by **Maria Teresa Da Silva Felicio**, Scientific Officer, Biological Hazards and Contaminants (BIOCONTAM) Unit, European Food Safety Authority –EFSA–, Parma, Italy

For her presentation: Click HERE

Maria Teresa Da Silva Felicio presented the background of the EFSA mandate and how it has been managed around 5 terms of reference:

1. **The 'seropathotype' concept – the limitation to "relevant" serotypes O157, O26, O103, O111, O145, O121, O91, O104, O113[1], i.e. can pathogenicity be excluded for defined STEC serotypes?**
   EFSA used the seropathotype classification of Karmali et al. (2003), which classifies STEC based on their reported frequency in human disease, their known association with outbreaks and their severity of the outcome including HUS and HC. Nevertheless, it neither defines pathogenic STEC, nor does it provide an exhaustive list of pathogenic serotypes.

2. **Justification of the statement: 'seropathotypes D and E are not HUS-associated and are uncommon in man or only found in non-human sources'**
   There were no HUS cases reported for the serotypes included in seropathotype groups D and E, but there were 17 HUS cases reported that could not be assigned to a seropathotype group.
   Based on new data, when full serotyping has been undertaken, all serotypes associated with HUS have been categorised as seropathotype group 'HUS-associated serotype(s)' or HAS. The model may need to be updated as new information becomes available.

3. **An alternative concept based on detection of verocytotoxins, or genes encoding for verocytotoxins, in isolates**
   There is no single or combination of marker(s) that defines a 'pathogenic' STEC. Nevertheless, strains positive for verocytotoxin 2 gene (*vtx*2) and *eae* (intimin production) or [*aaiC* (secreted protein of EAEC) plus *aggR* (plasmid-encoded regulator)] genes are associated with a higher risk of more severe illness than other virulence factor combinations.

4. **The contribution by STEC to diarrhoeal cases and to more severe outcomes in the EU, based on hazard identification and characterisation, and under-reporting in EU**
   - Under estimation of STEC O157 is important and can greatly vary by MS
   - STEC infection resulted in HUS in ~10% of the cases
   - Difficulty of predicting the emergence of 'new' pathogenic STEC types by only looking at the presence of the *eae* gene or by focusing on a restricted panel of serogroups
   - The ISO/TS 13136:2012 standard improves the concept of detecting STEC in food



5. **The public health risk associated with the contamination of RTE foods with STEC, considering either the seropathotype concept or the detection of verocytotoxins or genes encoding the production of such toxins in isolates.**
   On the basis of the proposed molecular classification scheme, any RTE product contaminated with an isolate of one of the STEC serogroups of group I (O157, O26, O103, O145, O111, O104) in combination with *vtx* and [1] *eae* or [2] *aaiC* and *aggR* genes should be considered as presenting a potentially high risk for diarrhoea and HUS.
   For any other serogroups in combination with the same genes, the potential risk is regarded as high for diarrhoea, but currently unknown for HUS.

Some EFSA recommendations are:

- To perform **screening STEC for the presence of *aaiC* and *aggR* genes** on **isolates from human, food and animal** sources.
- To **screen,** for public health investigation of STEC infection, **clinical and/or food samples by PCR for the presence of *stx* genes.** If positive, to make all efforts to isolate and characterise the causative organism.
- To **verify and periodically revise the proposed molecular approach in light of new epidemiological information**.
- To **consider**, in accordance with the ISO specifications, **international harmonisation of nomenclature of VTEC and its virulence factors,** using **STEC instead of VTEC** and ***stx* instead of *vtx* or *vt*.**

ESFA has currently an on-going self-task mandate on the public health risks associated with enteroaggregative *Escherichia coli* (EAggEC) as a food-borne pathogen.

After the coffee break, **Doug Brydges**, President of the UECBV International Trade Section and co-chairman of the workshop, highlighted the commitment of the section in finding workable solutions. He introduced the following speaker, who presented the New Zealand experience:

IV. Presentation by **Andrew Hudson**, Head of Microbiology, Animal and Plant Health Agency – APHA– (merger between FERA and AHVLA), UK

For his presentation: Click HERE

Andrew Hudson explained that *E. coli* O 157:H7 has been routinely looked for since 1999. In 2012, six additional serotypes are added to the routine checks: O26, O103, O45, O111, O121, O145.

In New Zealand, STEC caused 1.4% of foodborne outbreak cases in 2013, from which serotype O157:H7 dominates clinical cases (85-90%).
None of the cases was associated with commercially produced food. Origin was person-to-person, water, raw milk, but mostly unknown.

New Zealand regulator (NZFSA, MAF, MPI) has commissioned "Risk profiles", a number of which are available on STEC: raw milk, red meat and meat products, salami, leafy vegetables.

A testing programme is put in place at a number of laboratories in New Zealand by GDS (genetic detection) PCR method reports presence/absence. Positive samples are sent to ESR reference laboratory for confirmation. The testing is using Real Time PCR in combination with immunomagnetic separation.
For the immunomagnetic isolation, 2 PCR are used at the beginning of the genetic testing, in order to exclude some points. Then, you grow them into colonies; however, some false positives can be detected. Then, you pick the colonies and make the agglutination. The modified rainbow agar used is the same than in US.
Andrew Hudson presented a table showing the probability of missing contamination in lots.
When you are sampling very low numbers, the probability of missing them is quite high, as well.
Some of the risk assessments produced were made on hamburgers/ground beef in the US, as well as on tenderised vs. un-tenderised steaks.
The results were similar for tenderised or un-tenderised steaks (1 illness per 15.9 million for intact steaks and 1 illness per 14.2 million for non-intact (tenderised) steaks).
For ground beef: 1 illness per 1 million servings.

Eating preferences give different risk of exposure. Burgers are eaten well cooked in UK, NZ, IE, while 18% Americans are eating burgers cooked rare.

Regarding food sources, two different studies (Batz and Painter) are showing quite different results.
Control measures are available, such as: treating food animals (e.g. vaccination); decontaminating hides (e.g. chemical, bacteriophages); decontaminating carcasses; proper cooking/heat treatment; preventing cross-contamination.

Andrew Hudson believes that defects on the controls can be modelled with a risk assessment.

V. Presentation by **Philippe Cartier**, Meat Service Research Engineer – Institut de l'Elevage, France

For his presentation: Click HERE

Philippe Cartier gave some background on the monitoring system to prevent any contamination in France and in Europe, including through good practices and HACCP-based systems, which are essential. His presentation was focused on the analytic strategy. In France, the monitoring system focuses, in particular, on the top 5.
Since 1996, there is a specific monitoring of HUS for children under 15 years old. Around 120 to 150 cases per year are raised. There were 152 cases in 2013. Among them, 138 cases were sporadic cases, 7 were family outbreaks/foyer and 1 was an outbreak. The isolation was not possible in all of them. O157 was the most common pathogenic strain found.
In 75% cases, it was possible to isolate a strain. Top 5 strains cover 55% of cases and 75% of isolated strains.
The scientific background follows the ANSES and EFSA opinions. Group 1 is the most dangerous. Cattle do not carry strain O104.

The French approach takes into account the methods available. Detection of O157:H7 is done on minced meat. The frequency of analysis can be up to each frozen minced meat batch and, on average, once a week in chilled minced meat.

More recently, industrial rapid methods have been developed to isolate strains of the top 5: genetic phase and an Antigene/Antibodies reaction.
Since 2012, top 5 can be looked for once a week in frozen minced meat.

In addition to the companies monitoring, Competent Authorities included the research of top 5 (top 7 since 2012) in their annual monitoring plan.

Philippe Cartier highlighted that a screening based only on *stx/stx-eae* is not selective enough.
In practice, the consequence would be higher cases of presumptive cases, which means more products blocked waiting for the confirmation. It would be difficult to manage for stocking the products/logistics, for laboratories, regarding the best-by-date and the delay to get the results and from the economical point of view.
The step of confirmation would be fastidious (50 colonies and need to characterise the strain for each suspect colony).
Because of the lack of specificity of the method, the risk is to miss highly pathogenic strains. It is already hard work to isolate strains and it would become even more difficult.

Companies could be tempted to monitor less to avoid the difficult consequences.

Since French Competent Authorities are looking for the top 7, the two additional serotypes have not been found.

Philippe Cartier warned regarding the draft Commission guidance that looking for all STEC strains is not feasible today and it is disproportionate if we look at the cost/benefice ratio.
In addition, the guidance does not lay down the harmonised condition for testing. The high risk/low risk classification makes the analysis for the high risk profile complicated and more difficult to use.

VI. Presentation by **Martial Plantady**, Legislative officer, Food, Alert System and Training Unit, Veterinary and International Affairs Directorate, Health & Consumers Directorate-General (DG Sanco), EU Commission, Brussels

For his presentation: Click HERE

Martial Plantady specified that the guidance has one single target: recommendation to Competent Authorities when they receive the result from the laboratory and STEC is detected.
He reminded the background of the draft guidance.
Until 2010, the seropathotype approach within the EU was focusing on the top 5.
But in 2011, an outbreak in sprouts revealed a new pathogenic serogroup.
Following this, EFSA published an opinion in 2013 revisiting the “seropathotype” concept of Karmali and colleagues (2003).

Due to the plasticity of the genome, it looks difficult to design individual serotypes as pathogens. The former approach is challenged.

According to the EFSA opinion, it is not possible to predict the potential to cause human disease.

The reasoning followed in the guidance is in 3 steps:

1) Hazard characterisation

Due to the new uncertainty brought by the sprout outbreaks, a new approach is desirable.

2) Exposure assessment

a. at retail

A differentiation is done between a food profile 1 (with RTE and non-RTE that can be eaten not enough cooked) and a food profile 2 (non-RTE food with an appropriate treatment).

b. not yet at retail

The same differentiation is made with an extra category for products with an insufficient traceability in food profile 1.

3) Recommendations for a harmonised application of Article 14 of Regulation (EC) No 178/2002

It is recommended, based on the molecular approach, to detect the presence of an *E.coli* strain positive for

[1] *stx* and *eae* genes or

[2] *stx*, *aaiC* and *aggR* genes

In case a positive is detected, a differentiation is made between food profile 1 [with RTE (like carpaccio) and non-RTE that can be eaten not enough cooked (like burgers or minced meat)] and food profile 2 [non-RTE food with an appropriate treatment (like steaks)]. The serotyping is recommended only in the case of food profile 2 before knowing if an action is necessary. For food profile 1, an action is necessary in case of positive finding of the genes (it is considered that there is no need to look for the serogroups).

There is not yet an agreement among the Member States on the content of the guidance. Some are considering it is not strict enough.

In order to clarify the positions of the Member States, the Commission services sent them a questionnaire. A clearer view is expected in January/February 2015.

DISCUSSIONS

Moderator: **Javier Dominguez**, Food Standards Agency –FSA–, UK

Javier Dominguez introduced the discussions making a parallel with the issue of campylobacter on which the UK is working a lot due to the number of cases and the severe consequences for public health. In recent years, consumers have become more aware of food safety issues and they are more demanding about those, but, at the same time, there is a lower level of understanding, mainly in younger generations, of good hygienic practices at home while storing, handling and cooling food.

He highlighted the key areas to explore, such as:

- ❑ Common understanding of the main pathogenic STEC strains (integration of animal food and human epidemiological data and better sharing of these data)
- ❑ More research to standardise sampling methods and progress on the availability of a rapid test
- ❑ Reinforcing a common approach on assessing the risk and making a risk-based analysis
- ❑ Continue to improve hygiene practices, plus additional/novel tools
- ❑ Proportionate Official Controls based on sound science/evidence
- ❑ Common approach for international trade

❖ A first question was asked to EFSA regarding the positive cases where the serotyping is not made. They may be from the top 5.

The EFSA representative acknowledged the data gap. It is an inherent limitation and a challenge for risk managers. In many sporadic cases, no link with a specific food can be made. There is a need to improve data collection.

❖ Regarding a plan to improve the monitoring, EFSA mentioned that now there is a report every year. For the time being, for any new combination, Member States must ask EFSA. Nevertheless, there is a big project in the pipeline, called FoodEx, which should facilitate the work once put in place in 2015.

❖ The guidance of the Commission was welcomed to look for harmonisation. Nevertheless, it was mentioned that, for the time being, due to gaps in science, there is a need to find proportionate solution to avoid destructing meat and protecting consumers.
The COM representative indicated that, if the foodstuff is not yet made available to the consumer, it can undergo a cooking process. The guide is clear and it is covered by article 19 of the general food law. The responsibility is for Food Business Operators (FBOs) and the Member States to check the reliability of the operators to make sure the law is enforced.

❖ It was asked what to do to determine if a STEC is pathogenic or not. The ISO standard 1336 is not clear. It is a story of the half-empty or half-full glass. All STEC are not pathogenic and some conditions must be filled in to be pathogenic. We need to find a clear way to communicate to the public.
The EFSA representative indicated that we know that some STEC are pathogenic as we have data on some diseases, but we have to keep in mind the uncertainties. The molecular model from the COM explains this.

❖ It was mentioned that the precautionary principle is understandable, but it is also a problem to overestimate the risk. One answer could be to balance measures destined to further mitigate the risk and measures to collect data to increase our knowledge on STEC and be able to carry out a better risk assessment.
A discrepancy appeared here between the willingness of Competent Authorities to ensure the lowest possible risk for consumers and the need for the industry to be more targeted on the main risks to have proportionate and pragmatic testing regimes and interventions to manage the situation. Here, there is possibly a need for a dual approach: one for collecting more data for better monitoring the risks and avoiding what happened with the O104 in seeds and another approach for the industry to monitor according to the present knowledge and available tools.

- Answering to the question where the best place to test in terms of cost/efficiency is, a meat company remarked that it may depend on the measures to be taken afterwards. It needs to remain proportionate to be efficient.

- Another meat industry representative commented that analyses are done to check if the hygiene procedures are correct.

- A scientist remarked that it is difficult to get sterilised food. In addition, it would contradict some aspects we expect from food. The monitoring system could be completed by campaigns explaining to consumers how to handle/cook food, in particular for the most sensitive population.

- There was the general view to keep *E.coli*/STEC as a process hygiene criterion.

- A question was raised on statistics. It is not because nothing is found that it gives a guarantee. It is up to the risk manager to assess this.

- There was a fear, as well, that there would be a demand from retailers to test all products. A high amount of products could be blocked if there is no serotyping.

- There was a warning to avoid over-reactions. In Belgium, for example, there is a high quantity of meat eaten raw or rare and there is no disease linked to this consumption.

- Regarding the feasibility, the necessity to keep in mind the delays for products with very short best-by-date was underlined. If the serotyping is not done from the beginning, then too many products would be blocked and wasted. It is a critical point for the decision-making people.

CONCLUSIONS

The lively discussions allowed reaching the following conclusions presented by Javier Dominguez:

1. The meat sector is already taking steps to control STEC:

- Good manufacturing practices from slaughterhouses to the final consumer are in place and improving;
- Meat is as safe as other food, as described by the EFSA report on zoonosis;
- Prevention and monitoring by FBOs and verification by Competent authorities is in place;
- In case of positive findings, treatments are available (which is not always possible for other food).

2. What more can be done:

- It must be risk-based. Science is showing that some data is missing to bring certainties on what is pathogenic or what is not. Nevertheless, authorities and companies need to work with what is available. Eventual new pathogenic strains cannot be predictable. A dual approach may be necessary to continue improving the knowledge, but allowing pragmatic approach for companies with acceptable delays. The pragmatic approach today in the meat sector is to focus the monitoring on the top 5 pathogenic strains, which are recognised as the more pathogenic ones.

- FBOs are responsible for food safety. Analyses are done in order to check whether the hygienic process is under control. They must remain feasible, otherwise it would become non-manageable and counterproductive (risk of testing less).

- Efforts must continue to improve hygienic production – Key! Prevention is better than cure. The presentations showed a large range of possibilities available to mitigate the risks. In addition, additional/novel/a combination of tools are available (for example, the possibility to use lactic acid as an extra tool for hygiene is new within the EU). Nevertheless, efforts continue to be made to improve further.

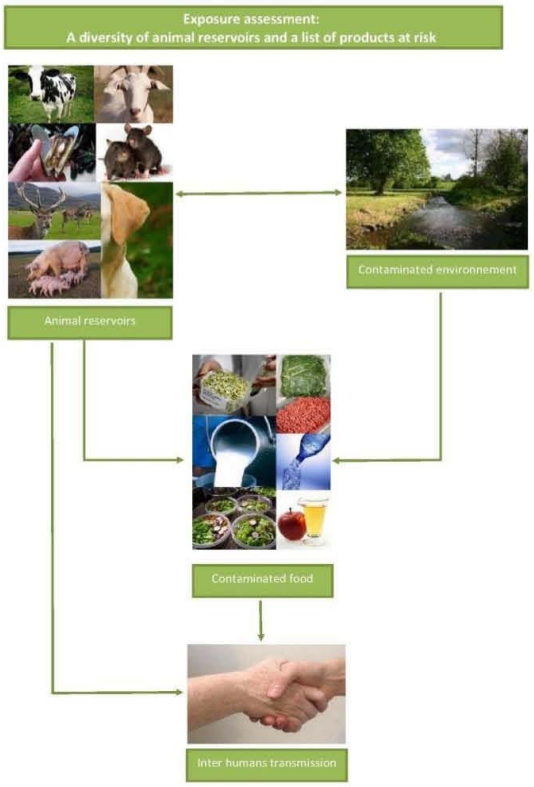
- Working on communication, in particular towards consumers, was also highlighted as an important point of action. Ideas, such as campaigns in all media, more information on the packaging on how to handle the products, were also given as examples.

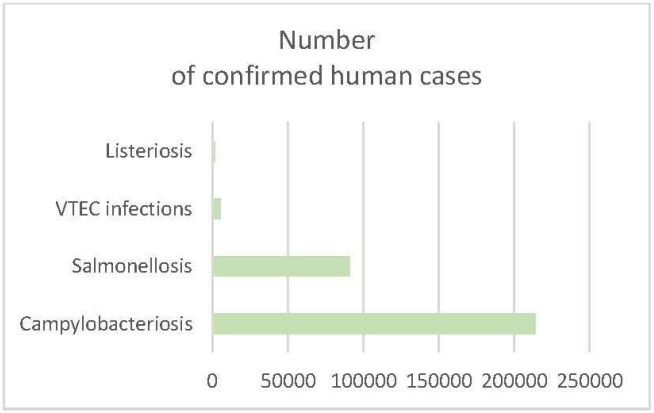
- Regarding the EU Commission draft guidance, it was highlighted that the difference must be done between monitoring the risks and managing the risks (when a positive case is found). The focus on risky products is relevant at the monitoring stage, while, when a positive case is found, all food must be treated the same way, with strict channelling treatment, to ensure that the risk is mitigated before the product reaches the consumers.

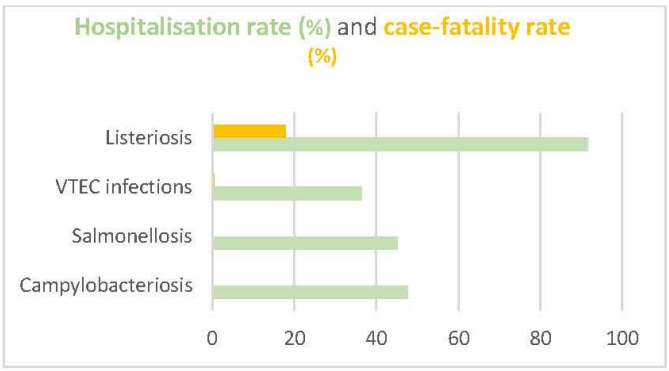
- It must be the same level playing field for the domestic production and imports.

Jean-Luc Mériaux, UECBV Secretary General, underlined some points of the conclusion, such as the need to put this issue in perspective: the meat sector is working on this issue, but meat is largely as safe as other food as it is reported each year in the EFSA zoonosis report. We must also fully use the new tools available. He warmly thanked the Argentinian colleagues for this initiative and closed the meeting.

Summary of the VTEC issue in images for a risk-based management decision



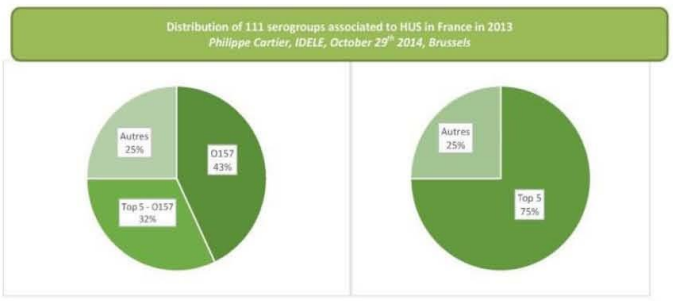




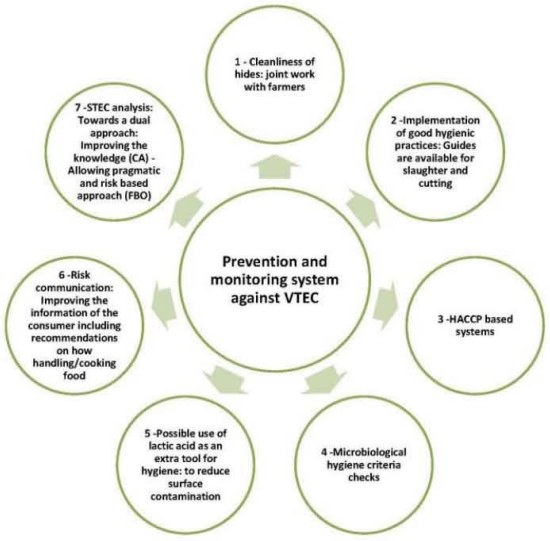
Source: EFSA

The STEC reservoirs are diverse as well as the products at risk. Meat is only one among others

STEC/VTEC is far from being the first risk in food



The Top 5 serotypes cover 75% of the serogroups associated to HUS



There is a large range of tools available to improve the prevention of diseases by STEC.
STEC analysis is only one monitoring tool coming after others