FETAL BOVINE SERUM

Handout
TOPICS FOR DISCUSSION AND ANALYSIS

Presented by Biowest
The production of FBS is not just another industry. Our activity is in constant evolution and is inextricably linked with some of the most remarkable scientific and medical discoveries in the history of mankind.

At first chaotic and disorganized, the FBS market gradually developed and matured along with the countless scientific and technological discoveries that have punctuated the last fifty years. Experience and research have perfected collection and treatment methods of FBS in order to supply a safe and high-quality product.

The vast majority of serum manufacturers have worked professionally, respecting good practice rules and taking traceability and supply chain control in earnest. Thanks to the implementation of strict traceability rules by the European Union (EU) and other countries, as well as ownership changes in poorly-managed companies involved in questionable practices, illegal schemes are now unlikely to occur.

Currently, all reputable serum producers readily adhere to strict codes of ethics. However, the risk of misrepresentation of country-of-origin still can exist in cases where serum passes through a network of intermediaries. To fully deter questionable, or even illegal, activities in our industry, economic incentives to engage in such behavior must be tackled at their roots. This consists of international standardization of rules governing imports, along with educating serum users and regulators, based on the latest scientific knowledge.

To meet the hopes and expectations of biopharmaceutical companies, researchers and stakeholders in general, our industry has no choice but to strive for excellence and to provide, without compromise, the best possible products to our customers.
SUPPLY SHORTAGE

Today, because of pregnancy testing improvements and meat market-related factors, producers face a steady decrease of the availability of FBS, while demand continues to surge. For a time, FBS collection in additional "new" countries compensated for the decrease in supply. However, even with this, the overall world supply of FBS has been declining for several years.

The ongoing short supply of FBS increases the urgency of harmonizing import rules, and ensuring transparency and science-based education to FBS users.

HARMONIZATION OF IMPORT RULES – INPUT FOR ANALYSIS AND DISCUSSION

As a thirty-year player in the industry and one of ISIA's founding companies, Biowest is aware of its responsibilities and strives to contribute to a constructive dialogue among manufacturers and serum users. We believe that several important FBS issues must be addressed and discussed. We have outlined some of the more important topics in this folder, and hope that it generates constructive discussion and analysis towards developing a common industry position that can be promoted to regulators and serum users.

Enjoy the reading.

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And

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Fetal Bovine Serum (FBS) History, Another lesson learned

**HISTORY**

FBS is critically important for research, diagnostics, vaccines, and biopharmaceuticals. Its use has resulted in significant contributions to the improvement of human and animal health. The use of FBS in cell culture started in the USA in the 1960s, based on serum collections in North America. In Europe, FBS was mainly imported from New Zealand and Australia until the 1980s. Supply was tight and prices were high. European Union (EU) import rules differed from country-to-country which made smuggling and misrepresentation of origin fairly simple and profitable for those willing to ignore the rules. “Serum brokers” were specialists in imports into the EU, as described in an article published by *Der Spiegel* in 1993.

Collection in Latin America started in 1979 in Mexico. FBS from Mexico and Central America was allowed into the USA. FBS from South America was only allowed into Europe where the serum industry, instead of purchasing directly from producers as in Mexico and North America, collaborated with serum brokers as described in the *Spiegel* article. This network of brokers became the main route for FBS from South America into European FBS companies.
Biowest was one of the few companies buying FBS directly from serum producers in South America. While Biowest had originally been formed in cooperation with German brokers to produce FBS in France, that cooperation ended and Biowest continued its serum collection activity directly with French abattoirs.

**ANOTHER LESSON LEARNED**

In 1994, the Serascandia group, a supplier of South American FBS, acquired an ownership interest in Biowest, becoming its sole owner a decade later. During a review of operations, Biowest learned that one of its managers had been involved in a scheme to misrepresent the origin of FBS. Biowest took immediate action by terminating the responsible manager, reporting the facts to the French police and USDA, and notifying affected serum customers. This unfortunate learning experience resulted in Biowest proposing that the leading serum companies create an industry organization to prevent such activity, which was the genesis of ISIA.

In an ideal world, the story would have ended there. However, the investigation by the French authorities initiated by Biowest’s report continued and led to additional findings. The French authorities found that a company operating in eastern France engaged in a scheme over the course of nearly a decade involving the mislabeling and adulteration of approximately 110,000 liters of Brazilian-origin FBS. Of this, approximately 50,000 liters were misrepresented as being of French origin, being sold as CE-marked French FBS (for IVD applications), and shipped to Germany with false veterinary certificates. The destination and declared origin of the remaining FBS is unknown to us at this time. However, as reported by the French authorities, in addition to misrepresenting the country-of-origin, the FBS in question was adulterated by an unknown "mix."

Additional information concerning this matter has been provided to ISIA’s Board of Directors for review because, in our opinion, it affects the integrity of the serum industry. ■
Future, Moving Forward

As long as import rules are different between countries, and resulting serum prices vary by up to a factor of 10, traceability-related crimes can occur. Illegal activities must be stopped because they not only negatively affect serum users and the image of the serum industry, but the long term viability of FBS as a product. ISIA must intensify efforts to educate and to promote the harmonization of international import rules.

Since FBS is initially a non-utilized byproduct available from slaughterhouses worldwide, the global supply of FBS has permanently exceeded global demand. Today, conditions are favorable for uniform and stable purchasing and sale prices at more affordable levels. The problem has been barriers to trade and the lack of scientific information to FBS users. This has generated a fragmentation of the serum world, with different supply/demand constellations in different segments, and price differences between origins sometimes exceeding 1,000%.

If FBS is dealt with in a transparent, customer-friendly, scientifically-correct way and trade barriers and misinformation are eliminated, FBS will be more abundant and less expensive, and there will be no economic incentives for criminal activities. This is how it should be. Instead, historically, the serum industry has occasionally interrupted collection, creating shortages; objected to opening imports of FBS into the USA from South America as proposed twice by the USDA; and misinformed serum users and regulators about the safety of
different sources. The concept that Oceania is safer is still part of some marketing campaigns, even after BSE has been found to be a spontaneous event in all cattle populations (Nobel Prize winner Stanley Pruziner), and it has been determined by OIE not to be transmitted by blood products.

The latest case of FBS fraud, as uncovered by the French authorities, was elaborate and had a low risk of being detected. It was discovered by pure chance and may have continued indefinitely under slightly different circumstances, like the PAA case. This type of activity can only be eradicated by eliminating economic incentives, via science, education, and harmonization.

The damage done to researchers and other serum users includes inexact or invalidated results in research and diagnostics, virus risks, product recalls, etc. From a financial viewpoint, losses include costs of lost research, plus the difference between FBS prices actually paid and the much lower prices that should have been paid for the same product if it had been justly declared. The damage to competitors consists of lost FBS business and the cross-effects on sales of media, reagents, plastics, and equipment.

The vast majority of serum companies have always worked legally, taking traceability compliance seriously and controlling their supply chain. With the traceability laws that have been implemented by the EU and other countries, as well as the new ownership of companies historically associated with questionable practices, illegal schemes are no longer likely to happen. Today, all serum companies adhere to strict codes of ethics. However, the risk of misrepresentation still exists in cases where the product goes through a chain of intermediaries. To eliminate potential illegal activities, economic incentives must be attacked at the root, by the harmonization of import rules and the education of serum users and regulators, based on science.
WE PROPOSE TO REVISE THE ISIA TRACEABILITY APPROACH, TAKING ADVANTAGE OF THE INTRODUCTION OF THE ISIA ETHICS CODE.

The serum world has changed dramatically over the last ten years. What made sense ten years ago when we created ISIA is not needed any longer. All suspected illegal activities have stopped. New miscreants may show up, but the ISIA traceability certification makes no difference in this context. Such activities take place downstream, outside the reach of the ISIA.

If a simpler and less expensive way can be found to achieve the same goals, modifications of the approach should be considered. The ISIA approach can be: "Sign on to the ethics code, agree to an audit at any facility at any time, by customers or by ISIA-appointed auditors, for as far back as desired up to ten years." There is no need for companies to spend resources to comply with complicated rules, which were justifiable when created, but are no longer needed. We propose to make the Code of Ethics the central point, with the Traceability Certification program being adopted by those who feel they need it, but not by those who don't.

ISIA Board members should be asked to provide a written confirmation that they are not aware of cases of infractions in
their companies, which have not been investigated and disclosed to affected victims as required by the ISIA Code of Ethics. The serum industry needs no more revelations of traceability crimes, especially not from Board members’ companies. ISIA members, of course, are compliant. Further, certification does not guarantee that a company is compliant. If the agreed-upon traceability guarantee is to be maintained, the seal name should be changed from "Quality Seal", which is misleading, to "Traceability Seal". Since traceability is taken for granted by the serum users, it should not be used as a sales argument.

As expected by the serum users, responsible companies have always assumed accountability for their supply chain, performing audits as needed and basing the frequency and depth of the audits on specific factors, such as trust and experience with individual suppliers and the value of the product. There is a difference in the relevance of the audits between old supplier relations and start-ups, and between Australian FBS at > 1,000 US$/Liter and South American FBS at < 100 US$/Liter. All known illegal activity involved Oceania, US or France. None involved Brazil or Venezuela.

For example, based on the presentation by Life Technologies in Bruges (informing that it cost $200K for the certification process), as well as conversations with fellow members and non-members, we believe the traceability program should be redefined as proposed above. The only effective way to eliminate smuggling and misrepresentation is to eliminate the economic incentives. Harmonizing rules and educating serum users and regulators based on science will reduce the price differences that are the origin of the criminal activities. This is where ISIA’s efforts should be focused.

The vast majority of serum companies have always worked legally, taking traceability compliance seriously and controlling their supply chain. With the traceability laws that have been implemented by the EU and other countries, as well as the new ownership of companies historically associated with illegal practices, illegal schemes are no longer likely to happen. Today, all serum companies adhere to strict codes of ethics. However, the risk of misrepresentation still exists in cases where the product goes through a chain of intermediaries. To eliminate potential criminal activities, economic incentives must be attacked at the root, by the harmonization of import rules and the education of serum users and regulators, based on science.

NOTE

This position paper was written by Biowest and reflects the current views of the company.

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FBS Quality vs. Countries of Origin

Since the early days of fetal bovine serum (FBS) use for cell cultivation, geographical isolation and purported favored animal health statuses of a few FBS countries of origin have been used as common sales arguments. These arguments have been used so much that, within the FBS industry and among users, there is a persistent belief that the highest quality FBS necessarily comes from New Zealand and Australia.

With its many years of experience in serum manufacturing and after conducting numerous research studies, Biowest has demonstrated beyond any doubt that the quality (adaptability to cell lines) of FBS is in no way related to its country of origin, but rather to how the FBS is collected, processed, cleared of contaminants, and safety and quality tested.

On behalf of Biowest, serum specialist Leonel Del Cid in 2014 studied 244 batches of FBS coming from six countries representing three continents, analyzing the correlation between the physical and biochemical parameters and cell growth. The results showed no observable difference in cell growth performances among the six FBS source countries for Sp2/0 – Ag14, HeLa, L929 and MRC-5 cell lines. See Table 1.
In order to dispel another widespread misbelief, Biowest compared the latest animal health status of 30 FBS-producing countries, using OIE\footnote{Organisation internationale pour l’Élevage (International Organisation for Animal Health)}\textsuperscript{1}, USDA\textsuperscript{2}, and EU\textsuperscript{3} data sources. The purpose was to identify which diseases of concern for FBS were actually present and which were absent, country by country. The results shown in Table 2 are surprising and demonstrate the lack of rationality and scientific basis of the assumptions about good-quality FBS origins.

This lack of information has serious consequences for FBS prices. Currently, Australian serum is sold at a price sometimes ten times higher than FBS from other origins, even though their growth performances are equal. Paradoxically, serum from Australia and the US actually contains the greatest number of viruses of concern for importation.

**ORIGINS, DISEASES, AND RULE-SETTING**

Not all countries qualify to export FBS because of certain diseases in their cattle populations and of unavailability of material. At this time, there are only 30 countries in the world where FBS is collected. Restrictions are imposed, not because of how the FBS from that country will perform in cell cultures, but mainly because of the animal health status of the exporting country. The purpose of import requirements is to guarantee the absence of viruses of concern, by either prohibiting importation or by other measures, such as safety testing, and gamma irradiation.

Since the two largest global markets for animal-derived products are the USA and Europe, the import requirements from the USDA and the European Commission (EC), to a great extent, have become the veterinary control standards for the FBS industry.

<table>
<thead>
<tr>
<th>Country origin of the FBS</th>
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<td>Brasil</td>
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Table 1 - Comparison of Cell Growth Performance for Different Origins of FBS.
Since the 1980s, the principal source countries for FBS are from North, Central and South America, Oceania, and Europe. As BSE is no longer considered to be transmitted by blood and blood products, FBS is no longer subject to import restrictions related to the BSE status of the exporting country, provided that a proper slaughter method is used.

The cattle diseases of concern for FBS are those which cross the placental barrier of the cow and infect the calf fetus. From a geographic perspective, some of these viruses (adventitious viruses) have a worldwide distribution, and others (viruses of import concern) are limited to certain regions of the world. Table 2 compares the disease status of 16 of the 30 FBS exporting countries for eight adventitious viruses and for six viruses of importation concern. The sources for this information are the World Animal Health Organization (OIE), the USDA, and the EU. See Table 2.

### Table 2 - Regulatory Diseases of Concern for FBS - Comparison of Animal Health Status of Countries of FBS Origin

<table>
<thead>
<tr>
<th>FBS exporting countries</th>
<th>Adventitious Viruses of concern</th>
<th>Viruses of Importation Concern</th>
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<tbody>
<tr>
<td></td>
<td>Considered worldwide distribution by USDA and EU</td>
<td>Source: 2013 OIE data</td>
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<td></td>
<td>Considered worldwide distribution by USDA and EU</td>
<td>Source: 2013 OIE data</td>
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<tr>
<td>FINLAND</td>
<td>+ + + + + + 2010 1994 2007</td>
<td>5</td>
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<tr>
<td>SWEDEN</td>
<td>+ + + + + 2011 1995 1866</td>
<td>5</td>
</tr>
<tr>
<td>DENMARK</td>
<td>+ + + + + + + + 2005 2002</td>
<td>6</td>
</tr>
<tr>
<td>NEW ZEALAND</td>
<td>+ + + + + + + - 7</td>
<td>7</td>
</tr>
<tr>
<td>CHILE</td>
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<tr>
<td>URUGUAY</td>
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<tr>
<td>CANADA</td>
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<tr>
<td>COLOMBIA</td>
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<tr>
<td>HOLLAND</td>
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<tr>
<td>MEXICO</td>
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<td>PARAGUAY</td>
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<tr>
<td>AUSTRALIA</td>
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<td>BRAZIL</td>
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<tr>
<td>FRANCE</td>
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<tr>
<td>UNITED STATES</td>
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<td>8</td>
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</tbody>
</table>

(year) indicates year disease last reported
(+ disease is present
( - disease has never been reported

Sources: OIE Animal Health Status / USDA 9 CFR 113.46-53 / EMEA-CPMP-BWP-1793-02
**ADVENTITIOUS VIRUSES**
The US and EU regulations require that all FBS, regardless of country of origin, be tested and/or treated (by heat or gamma irradiation) to assure its freedom of eight adventitious viruses. These eight viruses are referred to as adventitious viruses because they are considered to have a worldwide distribution and therefore may unintentionally be present in FBS from any origin.

**VIRUSES OF IMPORT CONCERN**
The viruses that do not have a worldwide distribution are of special concern when importing FBS. Regulations from the USDA and EU identify six viruses of importation concern for the FBS-producing areas of the world.

**COMPARING ORIGINS OF FBS**
Considering all viruses of import concern, Chile, New Zealand, and Uruguay are the only countries found to be clean. All of the other listed FBS-producing countries report between six and ten of the viruses of concern for the USDA and EU. Among the countries reporting the most (ten) viruses of concern are Australia and the USA.

In conclusion, here are the key points to consider about FBS origin:

- The comparison of six different countries shows that origin does not have an influence on growth promotion (Table 1).
- A comparison of animal health statuses of the major FBS-producing countries shows that there is no correlation between “geography” and “FBS quality” based on the country’s animal health status (Table 2).
- Higher prices for certain origins of FBS do not mean that those origins are “better” or “safer”.
- Quality depends on sourcing, processing, quality control, treatments, and batch-specific testing.
- Once viruses are tested for and removed, the quality of a batch of FBS depends on physical and biochemical quality parameters, which are part of the Certificate of Analysis.

**CONCLUSION**
High-quality, safe FBS can come from any of the USDA - and EU - approved origins.

**REFERENCES**
2. USDA 9 CFR 113.46-53
3. EMEA-CPMP-BWP-1793-02
4. OIE Terrestrial Animal Health Code Chapter 11.4. Article 11.4.27
5. OIE Terrestrial Animal Health Code Chapter 11.4. Article 11.4.1
6. USDA 9 CFR 113.46-53
7. EMEA-CPMP-BWP-1793-02

**AUTHOR:** PERCY W. HAWKES, DVM, Regulatory Consultant, Retired Foreign Service Diplomat for USDA (APHIS)
BSE and FBS
30 years later

WITH THE OCCURRENCE OF BSE IN THE 1980S, MANY REGULATIONS HAVE BEEN ESTABLISHED TO REDUCE THE RISK OF BOVINE- DERIVED PRODUCTS IN HUMAN CONSUMPTION AND OTHER APPLICATIONS. THESE REGULATIONS HAVE STRONGLY INFLUENCED THE PREFERENCES AND SELECTION OF FBS IN CELL CULTURES. SIGNIFICANT SCIENTIFIC WORK HAS BEEN DONE IN THIS FIELD, AND MUCH MORE IS UNDERSTOOD NOW ABOUT THE OCCURRENCE AND TRANSMISSION OF THIS DISEASE.

BACKGROUND INFORMATION

When BSE was first discovered in the UK in 1986, little was known about the cause of the disease or how it was transmitted. As a result, countries throughout the world responded by banning the importation of all bovine products, including blood products, from affected countries. After the discovery of the prion by Stanley Prusiner as the causative agent of BSE and his report detailing how it is spread by feeding Meat and Bone Meal (MBM) to cattle, the World Animal Health Organization (OIE) and regulatory authorities throughout the world established standards that banned the feeding of mammalian MBM to cattle and adopted slaughter practices that minimize the risk of BSE transmission.

Thanks to these efforts, the number of BSE cases has fallen dramatically, from 37,280 cases in 1992 in the U.K. alone, to only seven cases worldwide in 2013, two of which were atypical or spontaneous BSE cases.

Despite the dramatic reduction (almost elimination) of BSE cases in the world and proper risk-reduction procedures being implemented in cattle-exporting countries, many importing countries maintain disproportionate BSE-related bans on bovine blood and blood byproducts.

BSE REGULATIONS

In 2011, the European Union recommended the use of the OIE classification to replace the former GBR risk-class system. Presently, there are 25 countries in the "negligible-risk level", including Australia, Brazil,
Chile, Colombia, Denmark, New Zealand, Panama, Paraguay, the USA, and Uruguay (See Table 1).

The European Union states that blood is safe when coming from "negligible BSE risk" and "controlled BSE risk" origins.

Since 1998, the EU has had a mandatory BSE-monitoring program in place – ID passports for all animals and BSE-testing for cattle, covering 100% of animals in predefined risk groups. The traceability system is also useful for the tracking of other animal diseases and making Eu origin the preferred choice in some applications.

For Fetal Bovine Serum (FBS), the BSE risk has been known for many years to be ZERO, regardless of origin. The incubation time for BSE is recognized to be several years, which has been taken into consideration in the EU BSE control program, initially covering animals older than 24 months. Additionally, studies have shown that via embryo transfer, BSE from infected mother cows is not transmitted to the offspring.

**REGULATIONS FOR SERUM USAGE FOLLOW THESE DEVELOPMENTS**

Other authorities follow the OIE recommendation and have adapted their regulations as well. The latest version of the 9 CFR 95.12 states that blood and blood products derived from bovines must come from animals that were "not subjected to a pithing process or to a stunning process with a device injecting compressed air or gas into the cranial cavity." This is the only USDA restriction relating to BSE for blood and blood products derived from bovines.

**CONCLUSION**

According to the OIE and other regulatory entities in the USA and EU, it can be concluded that BSE is irrelevant when selecting origins of bovine serum.

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**REFERENCES**


**AUTHOR:** PERCY W. HAWKES, DVM, Regulatory Consultant, Retired Foreign Service Diplomat for USDA (APHIS)
Bluetongue Virus and FBS

DESCRIPTION OF THE VIRUS: BLUETONGUE IS AN INSECT-TRANSMITTED, NON-CONTAGIOUS VIRAL DISEASE THAT AFFECTS DOMESTIC AND WILD RUMINANTS. THE WORST-AFFECTED DOMESTIC SPECIES IS SHEEP. GOATS AND CATTLE USUALLY HAVE MILD, SELF-LIMITING CASES. WHITE-TAIL DEER AND PRONGHORN ARE AMONG THE WILD SPECIES THAT CAN BE AFFECTED BY BLUETONGUE VIRUS. BLUETONGUE DOES NOT AFFECT HUMANS.

Bluetongue virus (BTV) is an Orbivirus from the Reoviridae family, a non-enveloped, double-stranded RNA virus, measuring 60-80 nm. It is primarily spread by biting insects but can also rarely be transmitted venereally by infected semen and transplacentally from mothers to offspring.

The primary insect vector is the biting gnat or midge (Culicoides spp.), which exists from tropical to temperate regions worldwide. Large outbreaks of the disease are seen when these gnat populations reach peak numbers. The disease is considered seasonal in areas where insect activity is limited by inclement weather (freezing) and is more prevalent in temperate to tropical areas.

DISTRIBUTION

BTV was first described in South Africa in 1902, and since then there have been a total of 26 serotypes identified throughout the world. Historically (before 1998), BTV serotypes had been identified in Africa, Asia, South America, North America, the Middle East, India, and Australia (Fig. 1).

However, since 1998, seven of the existing serotypes and one new serotype of BTV (25) were identified in Europe, coinciding with large outbreaks of Bluetongue in sheep and other ruminants. (The European continent serotypes, which do not appear on the map below in Fig. 1, are: 1, 2, 4, 6, 8, 9, 11, 16, and 25).

CLINICAL SIGNS

The clinical signs in sheep include high fever; depression; labored breathing; sores or vesicles on the tongue, mouth, or nostrils; lameness associated with laminitis and coronitis of the hooves; facial and tongue edema (swelling), where the disease...
gets its name; loss of wool, weight loss; abortion; and even death.

As mentioned earlier, sheep are the hardest hit domestic species. The degree of susceptibility in sheep varies depending on age, breed, and disease serotype. In flocks infected for the first time, the morbidity (percentage of animals affected) can reach 50-75% and mortality can reach 20-50%.

Again, cattle are not usually affected with clinical signs as badly as sheep, but the Culicoides spp. are much more attracted to cattle, and cattle serve as the primary reservoir and amplifying host for the virus as they develop a high level of viremia (high levels of virus in the blood stream)\(^5\).

Cows that become infected during pregnancy usually abort the embryo, and less frequently, the cow may give birth to a malformed fetus or calf. Early embryonic loss and a decreased reproductive efficiency are more frequently seen than malformed or aborted fetuses\(^6\).

**REGULATORY CONCERNS REGARDING IMPORTED FETAL BOVINE SERUM (FBS)**

Even though Bluetongue is widely distributed throughout the world, it requires the country of origin to certify that either: a) there have been no cases of Bluetongue, seropositive animals, or vaccination of animals for Bluetongue in the last 12 months; or b) if seropositive animals are present in the country of origin, the FBS product must be transported directly from the Border Inspection Post (BIP) to the consignee. (Regulation EC No. 294/2013)\(^7\)

The USDA requires safety testing by sheep inoculation of all imported FBS for Bluetongue virus, except for FBS from Canada and New Zealand\(^8\). It is very rare for the Bluetongue virus to be found in FBS. As an example, during the past 35 years (since 1980), Biowest has exported a total of approximately 2,800 batches (1.4 million liters) of FBS of Mexican and Central American origin to the United States. None of these 2,800 batches of FBS have tested positive for Bluetongue by the USDA.

**CONCLUSION**

It is very unlikely for BTV to be present in imported FBS because:

- Cows infected with BTV during the first part of pregnancy will normally abort the embryo/fetus.
- Technicians are not allowed to collect blood from dead or malformed fetuses.
- The processing of FBS normally includes gamma irradiation between 25-40 kGy for adventitious contaminants as required by the 9CFR 113.53.\(^9\)

**REFERENCES**

2. http://edis.ifas.ufl.edu/in768

**AUTHOR:** **PERCY W. HAWKES, DVM, Regulatory Consultant, Retired Foreign Service Diplomat for USDA (APHIS)**
Foot and Mouth Disease (FMD) and FBS

BACKGROUND INFORMATION

With the globalization of the animal products market and a growing degree of market integration worldwide, Foot and Mouth Disease (FMD) has increased in significance as a major constraint to international trade in live animals and animal products, and it is one of the viruses of concern when importing FBS.

Contrarily to BSE which only loomed in the 1980s, Foot and Mouth Disease has existed since the beginning of bovine breeding. As early as 350 B.C., the philosopher Aristotle mentions a cattle plague causing fever and vesicular lesions on the mouth and hooves of cloven-hooved animals. Since the 16th century, FMD has been recognized as one of the most significant epidemic diseases threatening livestock. Its first clinical description was made in 1546 by Italian physician Hieronymus Fracastorius, and for the following two centuries, the number of FMD outbreaks increased in Europe.

In 1898, German physicians Loeffler and Frosch showed that the disease was caused by a microscopic, filterable, transmissible agent smaller than any known bacteria, thus discovering the first vertebrate virus. It was not until 1920 that a convenient animal model for the study of the FMD virus was established, and with the later in-vitro cell culture systems, the chemical and physical properties of the FMD virus were elucidated, culminating in 1989 with a complete description of the three-dimensional structure of the virion. FMD
virus is classified as a species in the Aphthovirus genus of the family Picornaviridae and measures 27-28 nm in diameter. Seven main serotypes exist throughout the world, as well as numerous subtypes.

The FMD virus can be found in all secretions and excretions from acutely infected animals, including expired air, saliva, milk, urine, feces, and semen.

However, evidence of the FMD virus crossing the placental barrier in bovines has not been demonstrated. In October of 2000, the European Commission's Scientific Committee on Animal Health and Animal Welfare reported that the FMD virus does not cause viremia in bovine fetuses. In 2007, the Institute for International Cooperation in Animal Biologics (IICAB), an OIE Collaborating Center, also reported that the FMD virus does not cross the placental barrier. However, more recently, the IICAB edited their FMD information, stating that an experimental infection of FMD virus in sheep crossed the placental barrier and caused death to the fetus, as reported by Ryan, et al. in 2007.

During the last 100 years, the trade of livestock products has been greatly influenced by the presence or absence of the disease in different regions of the world. Most countries and regions of the world have had FMD at one time or another, and have gone to great efforts to eliminate the disease and keep it, at great financial cost, from recurring.

During the last few decades, much has been accomplished to eradicate FMD, to establish standards for FMD-free status recognition, and to ensure that animal products being imported and exported are not contaminated with the virus. The World Animal Health Organization (OIE) has led this effort, with the participation of 180 member countries of the OIE. The OIE Terrestrial Code, Chapter 8.7, sets the standards for recognizing countries that have achieved the FMD-free status, either with vaccination or without vaccination. Countries with either FMD-free status (without vaccination and with vaccination) must show scientific evidence and certify that the FMD virus has not been found circulating in their cattle herds.

VACCINATION

Like many other viruses, the FMD virus ceaselessly evolves and mutates. One of the difficulties in vaccinating against it is the huge variation between, and even within, serotypes. Therefore, FMD vaccines must be highly specific to the strain involved in the epizootic.
DISTRIBUTION OF FMD IN THE WORLD

The OIE official FMD Status Map, as well as the lists of FMD-free countries without vaccination and with vaccination\(^7\), show that North and Central America, Oceania, Europe, Japan, and parts of Southeast Asia are recognized free of FMD without vaccination. Most of South America is recognized as free of FMD with and without vaccination, with the exception of Venezuela, Ecuador, Surinam, and some of the northern Amazon basin of Brazil. Africa and Asia remain as continents where FMD has not been eradicated, except for part of South Africa.

STANDARDS AND REQUIREMENTS FOR IMPORTING FBS

The International OIE standard (see OIE Terrestrial Code Chapter 8.7 on FMD) for importing FBS from FMD-free countries (with or without vaccination) states that the Veterinary Authorities of the importing country only need to require an international veterinary certificate attesting that the product comes from animals from an FMD-free country, zone, or compartment.\(^8\)

The European Union importation requirement for FBS from FMD-free countries (see Regulation EC No. 294/2013) follows the OIE standard, requiring
that the exporting country certify that no cases of FMD have occurred in their country in the last 12 months. Gamma irradiation at 25 kGy is another option for importing FBS into the EU.

The United States importation requirement relating to FMD does not follow the OIE standards. The USDA takes a more conservative approach, only allowing the importation of FBS from countries free of FMD without vaccination. Gamma irradiation is not currently allowed by USDA as a treatment option for importing FBS into the United States.

**SUMMARY**

Despite all efforts, FMD outbreaks still occur throughout much of the world, and while some countries have been free of FMD for some time, its wide host range and rapid spread represent a permanent cause for international concern. The 180 member countries of the OIE have worked together to develop international standards for disease reporting, surveillance and mapping, laboratory diagnosis, vaccine production, disease eradication standards, and trade requirements, which greatly reduce the risk of FMD being spread through the trade of animals and animal products. Only when importing blood products from FMD-infected countries is there good reason to believe that the FMD virus can be present in the blood of live cattle presented for slaughter. There are no literature reports of the FMD virus ever being found in bovine fetuses, nor are there any reports of FBS ever testing positive for FMD.

**ADDITIONAL WEB SOURCES OF INFORMATION ABOUT FMD**


**REFERENCES**

8. CFR 94.1
Gamma irradiation is routinely used by FBS companies to sterilize or clear FBS of potential contaminants, as requested by "end user" clients and as required by USDA\(^1\) and EU\(^2\) regulations. Currently, each serum company has its own irradiation procedures and standards, depending on client requests, yet this information is not published or shared throughout the industry. The standard irradiation dose for most US and EU customers is 25 kGy, but biopharmaceutical companies generally require a minimum of 30-35 kGy or higher. Some companies require that FBS bottles be packed on wet ice during irradiation, while others require the serum to be frozen on dry ice. There are also differences in the geometry of how boxes to be irradiated are positioned, as well as the number and placement of dosimeter verification devices. Some companies irradiate raw FBS before it is tested for BVD to avoid testing positive to BVD, while other companies prescreen individual jugs of FBS for BVD, making sure the entire lot is negative to BVD before irradiation for the other potential adventitious viruses. The lack of standardization of these procedures for the irradiation of FBS, often leads to doubt, mistrust, and in some cases, deceptive practices within the industry.

We believe that standardized irradiation guidelines need to be developed, validated, and published for inactivating potential virus contaminants of animal sera, just as validated guidelines have been developed and published for eliminating pathogens in human tissues\(^3\) and implants\(^4\), and for microbial contaminants in meat products\(^5\) and for plant pests for fruits and vegetables\(^6\).
The 9 CFR 113.46-53 requires that potential contaminants in the serum be inactivated by heat sterilization or other sterilization methods acceptable to the USDA (APHIS), or that the serum tests negative for each specific pathogen of concern. Since heat sterilization destroys many growth factors of FBS, irradiation is widely used to inactivate pathogens of concern, especially the BVD virus, which is always present in non-irradiated and non-prescreened lots of raw pooled FBS. Most biopharmaceutical companies want raw FBS to test negative for BVD before it is irradiated, as required by the EU regulation (EMEA-CPMP-BWP-1793-02). However, to avoid problems, some FBS companies irradiate raw FBS prior to testing for BVD, without disclosing this information to the biopharmaceutical company, and choose tests for BVD that do not detect the presence of inactivated virus or that are less sensitive. Again, the lack of standardized procedures for the irradiation of FBS and for testing for BVD sometimes leads to deceptive practices within the industry.

During the last 25 years, a tremendous amount of published and unpublished work has been done on virus inactivation in human and animal tissues by gamma irradiation. Today, producers of FBS and biopharmaceuticals are not only concerned about proper doses of irradiation needed to inactivate the viruses listed in 9 CFR 113.46-53, and 9 CFR 94 regulations, but are also concerned about doses high enough to inactivate other smallest-sized viruses7 (not mentioned in the 9 CFR 113), without adversely affecting the quality of the serum.

We believe that transparent and verifiable standards should be developed jointly by a "FBS Irradiation Working Group", made up of interested FBS companies, biopharmaceutical companies, and experts from academia and regulatory agencies like the USDA and FDA. The purpose of the Working Group would be to share irradiation procedures and research relating to:

- Irradiation doses and protocols.
- Standards for irradiation facilities.
- Preservation of serum quality.
- Testing and inactivation protocols for BVD and other viruses of concern, including the small-sized viruses and other viruses not mentioned in 9 CFR 113.

We believe that the harmonization of irradiation standards and guidelines must start within the industry itself and include the input and participation of government regulators and academia. Having such standards in place for raw and for finished FBS will result in a much-needed positive transparency and perception of the FBS industry as a whole, and at the same time, an increased assurance in the safety of FBS products in general.

REFERENCES
1. USDA 9 CFR 113.46-53
2. EMEA-CPMP-BWP-1793-02
7. Circoviridae, Parvoviridae, Picornaviridae, and Polyomaviridae

NOTE
This position paper was written with input from: GE Life Sciences (HyClone Laboratories), SAFC (Sigma Alrich), and Biowest.

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