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REVIEW

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Fetal bovine serum: geographic origin and regulatory relevance of viral contamination

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Abstract

The article aims to clarify an existing misunderstanding by the users of fetal bovine serum (FBS), who assume that certain countries, like Australia and New Zealand, have fewer cattle disease viruses and pose less risk for the presence of viruses, than do the other FBS producing countries. The article reviews the 2013 information from the World Organization for Animal Health (OIE), regarding the presence and absence of the 14 viruses of concern for FBS in the cattle populations of the 30 major FBS producing countries of the world. United States Department of Agriculture (USDA) and European Union (EU) regulations have identified 8 adventitious viruses and 6 additional viruses of importation concern that need to be tested for or eliminated in FBS, viruses that can cross the placental barrier from the donor cow to the fetus. A comparison is made regarding the number of viruses of concern reported presently in each of the FBS producing countries. The results of the comparison reveal that four Scandinavian countries report the fewest number of viruses of concern for FBS (six in total), while Australia and the USA are among the countries reporting the highest numbers of viruses of concern for FBS (ten in total). FBS from Australia and the USA has thus no advantage over the other FBS producing countries, regarding the number of viruses needed to be tested for and eliminated.

Keywords: Adventitious viruses, Animal health status, European Medical Agency (EMEA), Fetal bovine serum (FBS), United States Department of Agriculture (USDA), Viruses of importation concern, World Organization for Animal Health (OIE)

Background

Within the fetal bovine serum (FBS) industry and among end users of FBS, there are misconceptions regarding the respective geographical origin when sourcing FBS, as suggested by Siegel and Foster in a recent Bioprocessing Journal article (Siegel and Foster 2013). One of the main preconceptions is that the 'best' FBS comes from New Zealand and Australia because of their favored animal health status, and that lesser quality FBS comes from other origins with less favored animal health statuses.

Bovine Spongiform Encephalopathy (BSE) used to be a disease factor which favored those countries that had never reported an outbreak, like New Zealand and Australia, as reported by Davis and Drake in another recent Bioprocessing Journal (Davis and Drake Hirschi 2014). This lead to the promotion of FBS from these two countries as "safer" than from other origins, regarding BSE and other cattle diseases, because Oceania is "isolated" from the rest of the world. However, as knowledge of BSE advanced, scientists and government regulators from the World Organization for Animal Health (OIE), the European Union (EU) and the United States Department of Agriculture (USDA) determined that BSE is not transmitted in bovine blood or blood products, when appropriate slaughter practices are adhered to.

The purpose of this paper is to compare and discuss the animal health statuses of the 30 major producing countries of FBS, relating to the viruses of concern for FBS. We will compare the latest (2013) animal health status of these countries using information from the OIE, as well as USDA and EU sources, and show which diseases of concern for FBS are present and which are absent.

FBS is the most commonly used supplement in animal cell culture media. Cell cultures have many clinical applications in medicine, such as the production of vaccines, diagnostic tests, and cell therapy. FBS used in cell cultures contains more growth factors than other animal

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serum media and has the advantage of very low levels of antibodies. On the other hand, it has the disadvantage of high costs and the need for testing and the elimination of adventitious viruses, as do other media of animal origin. Serum-free media have been developed to avoid the use of animals for cell culture media, and have met with great success, especially in the production of some proteins for medical uses (Cruz et al. 1998). However, pharmaceutical companies, diagnostic labs and researchers still depend heavily on FBS for most of their cell culture needs. Researchers have not been able yet to replicate all the growth factors present in FBS or to produce serum-free media on a large enough scale, allowing for a complete replacement of FBS. The pharmaceutical and biologics community will most likely continue depending on FBS for many more years.

Review

Origins, diseases and rule setting

Not all countries qualify for exporting FBS because of certain diseases in their cattle populations, and the related import restrictions imposed by importing countries. These restrictions are imposed because of the animal diseases present in the exporting country and the perceived or real risk of viruses being present in imported FBS. 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. Once having passed importation requirements, safety testing and/or sterilization treatment, the imported FBS is considered to be free of all viruses of importation concern, and comparable to FBS from any approved origin.

Given the fact that the two largest global markets for animal-derived products are the United States of America (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. Since the 1980's, the principal source countries for FBS are from North, Central and South America, and Oceania. Europe is being added to this list, now that Bovine Spongiform Encephalopathy (BSE) is no longer considered to be transmitted by blood and blood products. The 30 countries where FBS is collected all have in common their freedom from the major contagious viral diseases of cattle, such as Foot and Mouth Disease (FMD), Rinderpest, Peste des petits ruminants, and Rift Valley Fever.

The cattle diseases of concern for FBS are those which cross the placental barrier of the cow and infect the calf fetus, thus contaminating FBS and making it unsuitable for use in cell cultures. From a geographic perspective, some of these viruses (adventitious viruses) are considered to have a worldwide distribution, and others (viruses of importation concern) are limited to certain regions of the world. Table 1 compares the disease status of the 30 FBS exporting countries for eight adventitious viruses and for six viruses of importation concern. The sources for this information are the OIE, the USDA, and the EU.

Adventitious viruses

The USDA (USDA 9 CFR 113.46-53) and EU (EMEA-CPMP-BWP-1793-02) 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 the following eight adventitious viruses: bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR), parainfluenza 3 (PI3), rabies, reovirus 3 (REO3), bovine adenovirus (BAV), bovine parvovirus (BPV), and bovine respiratory syncytial virus (BRSV). Because these eight viruses affect cattle in all continents of the world, they may unintentionally be present in FBS from any origin. The cell culture testing procedures required by USDA and EU serve not only to detect these eight viruses, but also to detect hemagglutination/hemadsorption and cytopathic effects caused by other viruses, which can contaminate FBS.

Even though these eight adventitious viruses are considered to be present in all cattle producing areas, the OIE (http://www.oie.int/animal-health-in-the-world/official-disease-status/fmd/) reports the following exceptions:

Bovine Viral Diarrhea (BVD) is reported absent in three Scandinavian countries (Finland, Norway and Sweden). Several other European countries have also achieved significant success toward eradicating BVD (Switzerland, Austria, Scotland, Ireland, Sweden, Denmark and Germany).

Infectious Bovine Rhinotracheitis (IBR) is also reported absent in 4 Scandinavian countries, the same three countries mentioned above, plus Denmark.

Rabies has never been reported in New Zealand, and is reported absent in Australia, as well as the four Scandinavian countries mentioned above, plus Belgium, Germany and Ireland.

Viruses of importation concern

The viruses of concern for FBS which do not have a worldwide distribution are also of concern when importing FBS. Regulations from USDA (Veterinary Services Notice 1998) and EU (Regulation EC No 294-2013) identify six viruses of importation concern for the FBS producing areas of the world: Foot and Mouth Disease (FMD), Vesicular Stomatitis, Blue Tongue, Akabane, Aino (Veterinary Services Notice 1992), and Schmallenberg (USDA Schmallenberg Restrictions). FMD is the

Table 1 Regulatory diseases of concern for Fetal Bovine Serum—comparison of animal health status of countries of FBS origin

FBS exporting countries	Adventitious viruses of concern								
	Considered worldwide distribution by USDA and EU					Source: 2013 OIE data			Total adventitious viruses
	Parainfluenza 3	Reovirus 3	Bovines adenovirus	Bovine parvovirus	Bovine respiratory syncytial virus	Bovine viral diarrhea (BVD)	Infectious bovine rhinotracheitis.(IBR)	Rabies	
Finland	+	+	+	+	+	2010	1994	2007	5
Norway	+	+	+	+	+	2005	1992	2011	5
Sweden	+	+	+	+	+	2011	1995	1886	5
Denmark	+	+	+	+	+	+	2005	2002	6
New Zealand	+	+	+	+	+	+	+	–	7
Belgium	+	+	+	+	+	+	+	2008	7
Chile	+	+	+	+	+	+	+	+	8
Germany	+	+	+	+	+	+	+	2005	7
Ireland	+	+	+	+	+	+	+	1903	7
Uruguay	+	+	+	+	+	+	+	+	8
Argentina	+	+	+	+	+	+	+	+	8
Canada	+	+	+	+	+	+	+	+	8
Colombia	+	+	+	+	+	+	+	+	8
Dominican Republic	+	+	+	+	+	+	+	+	8
El Salvador	+	+	+	+	+	+	+	+	8
Guatemala	+	+	+	+	+	+	+	+	8
Honduras	+	+	+	+	+	+	+	+	8
Holland	+	+	+	+	+	+	+	+	8
Mexico	+	+	+	+	+	+	+	+	8
Nicaragua	+	+	+	+	+	+	+	+	8
Panama	+	+	+	+	+	2007	+	+	7
Paraguay	+	+	+	+	+	+	+	+	8
Peru	+	+	+	+	+	+	+	+	8
Poland	+	+	+	+	+	+	+	+	8
Australia	+	+	+	+	+	+	+	1867	7
Brazil	+	+	+	+	+	+	+	+	8
Costa Rica	+	+	+	+	+	+	+	+	8
France	+	+	+	+	+	+	+	+	8
Spain	+	+	+	+	+	+	+	+	8
United States	+	+	+	+	+	+	+	+	8

FBS exporting countries	Viruses of importation concern							Total viruses of FBS concern
	Source: 2013 data from OIE, USDA and EU						Total viruses of import concern	
	Foot and mouth disease (FMD)	Vesicular stomatitis (VS)	Bluetongue (BT)	Akabane	Aino virus	Schmallenberg virus		
Finland	1959	–	–	–	–	+	1	6
Norway	1952	–	2010	–	–	+	1	6
Sweden	1966	–	2009	–	–	+	1	6
Denmark	1983	–	2009	–	–	+	1	7
New Zealand	–	–	–	–	–	–	0	7
Belgium	1976	–	2008	–	–	+	1	8
Chile	1987	–	–	–	–	–	0	8

Table 1 continued

FBS exporting countries	Viruses of importation concern							Total viruses of FBS concern
	Source: 2013 data from OIE, USDA and EU						Total viruses of import concern	
	Foot and mouth disease (FMD)	Vesicular stomatitis (VS)	Bluetongue (BT)	Akabane	Aino virus	Schmallenberg virus		
Germany	1988	–	2009	–	–	+	1	8
Ireland	2001	–	–	–	–	+	1	8
Uruguay	2001	–	–	–	–	–	0	8
Argentina	2006	1986	+	–	–	–	1	9
Canada	1952	1949	+	–	–	–	1	9
Colombia	2009	+	2007	–	–	–	1	9
Dominican Republic	–	–	+	–	–	–	1	9
El Salvador	–	+	1997	–	–	–	1	9
Guatemala	–	+	1998	–	–	–	1	9
Honduras	–	+	2004	–	–	–	1	9
Holland	2001	–	2009	–	–	+	1	9
Mexico	1954	+	2010	–	–	–	1	9
Nicaragua	–	+	2009	–	–	–	1	9
Panama	–	+	No Info	–	–	–	2	9
Paraguay	2012	–	Unknown	–	–	–	1	9
Peru	2004	+	2004	–	–	–	1	9
Poland	1971	–	–	–	–	+	1	9
Australia	1871	–	+	+	+	–	3	10
Brazil	2006	+	+	–	–	–	2	10
Costa Rica	–	+	+	–	–	–	2	10
France	2001	–	+	–	–	+	2	10
Spain	1986	–	+	–	–	+	2	10
USA	1929	+	+	–	–	–	2	10

(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.

only one of these diseases which is not an insect vectored disease.

Non-insect vectored disease

Foot and Mouth Disease (FMD) All 30 countries are officially recognized by the OIE as free of FMD (http://www.oie.int/animal-health-in-the-world/official-disease-status/fmd/). The South American countries listed in Table 1 are free of FMD with vaccination (except Chile which is free without vaccination), and the remaining countries on the list are free of FMD without vaccination.

Currently, the major difference between US and EU importation rules for FBS is over the definition of FMD free status. The EU accepts FBS from countries free of FMD, both with and without vaccination, whereas the USA only accepts FBS from countries free of FMD without vaccination. As a result, today's commercialization of FBS from South American origins (with the exception of Chile) is limited to Europe and Asia.

Even with the conservative approach taken by USDA, it should be noted that there are no literature reports of the FMD virus or FMD antibodies ever being found in bovine fetuses or FBS.

Insect vectored diseases

Vesicular stomatitis virus (VSV) is of concern to the European countries because the virus only exists in the Americas and because its clinical presentation is identical to FMD, even though the two viruses are from different families (VSV from the Rhabdoviridae and FMD from Picornaviridae). VSV is spread by insects and is endemic in the tropical and semitropical areas of the Americas. Every few years, VS spreads into the adjacent temperate areas of North and South America.

Blue tongue virus (BTV) occurs in all tropical and semi-tropical climates of the world where biting midge vectors exist, and there are 24 distinct serotypes in different areas of the world (http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/BLUETONGUE.pdf). Importation requirements are in place for this virus, in order to prevent the introduction of new serotypes from other parts of the world. Every few years, the virus reappears in areas where biting midges exist. USDA requires that serum from all countries, except Canada and New Zealand, be tested for BTV. Canada has the same serotypes of BTV as the USA, and New Zealand has never reported BTV.

Akabane, aino and schmallenberg viruses (Simbu Serogroup viruses) are transmitted by insects and cause deformities and death in bovine fetuses. USDA requires Australian serum be tested for Akabane. The same test detects the Aino Virus, which is also present in Australia. USDA also has import restrictions for European animal byproducts relating to Schmallenberg.

Comparing origins of FBS

First and foremost, it should be noted that all 30 countries listed on Table 1 are officially recognized by the OIE as being free of FMD, a remarkable accomplishment requiring excellent disease detection and surveillance programs.

Regarding the other five viruses of importation concern: all 30 countries are free of Akabane and Aino viruses, except Australia; only the European countries are affected by the Schmallenberg virus; only the Americas are affected by VSV; and all three continents represented are occasionally affected by different strains of BTV.

Next, regarding the eight adventitious viruses: all 30 countries are considered to be infected with PI3, REO3, BAV, BPV and BRSV; six European countries, as well as Oceania are free of Rabies; and several Scandinavian countries report the absence of BVD and IBR.

The countries reporting the fewest (6–7) viruses of concern for FBS are Finland, Norway, Sweden, Denmark and New Zealand.

The countries reporting the most (10) viruses of concern for FBS are Australia, Brazil, Costa Rica, France, Spain, and the USA.

Conclusions

Regardless of geographical location, FBS from all 30 countries needs to be tested and treated for the presence of multiple viruses. No one continent or country which produces FBS seems to have a real advantage over the others, since all countries have viruses of regulatory concern needing to be tested for and eliminated in FBS.

The comparisons made in this article, concerning the animal health statuses of the major FBS producing countries, show why a correlation between geographical origin and the "quality" of FBS based on the country's animal health status is not reasonable.

Just because the prices of FBS from certain origins might be higher, does not mean that those origins are "safer", in terms of the number of viruses needing to be tested for and eliminated.

Once having passed importation requirements, safety testing and sterilization treatment, FBS is considered to be free of all viruses of importation concern, and comparable to FBS from any other approved origin.

Finally, safe FBS can come from any of the USDA and EU approved origins.

Abbreviations

BAV: bovine adenovirus; BPV: bovine parvovirus; BRSV: bovine respiratory syncytial virus; BSE: bovine spongiform encephalopathy; BTV: blue tongue virus; BVD: bovine viral diarrhea; EC: European Commission; EMEA: European Medicines Agency; EU: European Union; FBS: fetal bovine serum; FMD: foot and mouth disease; IBR: infectious bovine rhinotracheitis; OIE: World Organization for Animal Health; PI3: parainfluenza 3; REO3: reovirus 3; USA: United States of America; USDA: United States Department of Agriculture; VSV: vesicular stomatitis virus.

Competing interests

The author has been doing consulting work for Biowest (http://www.biowest.net/) for the last 10 years. This article has been written as part of the author's fee basis work for Biowest. The author declares that he has no competing interests.

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