Biologicals 39 (2011) 359–369

Contents lists available at SciVerse ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Review

Evaluation of the human host range of bovine and porcine viruses that may contaminate bovine serum and porcine trypsin used in the manufacture of biological products

Carol Marcus-Sekura [a,*], James C. Richardson [b], Rebecca K. Harston [b], Nandini Sane [b], Rebecca L. Sheets [c]

[a] Biotechnology Assessment Services, Inc. (BASI), 7413 Ottenbrook Terrace, Rockville, Maryland 20855, USA
[b] Advanced BioScience Laboratories, Inc., 9800 Medical Center Drive Suite D, Rockville, Maryland 20850, USA
[c] CAPT (USPHS), National Institute of Allergy and Infectious Diseases, 6700B Rockledge Drive Bethesda, Maryland 20892, USA

ARTICLE INFO

Article history:
Received 13 May 2011
Received in revised form
10 August 2011
Accepted 11 August 2011

Keywords:
Adventitious agent testing
Animal cell culture
Viral contamination
Bovine virus
Porcine virus
Human host range

ABSTRACT

Current U.S. requirements for testing cell substrates used in production of human biological products for contamination with bovine and porcine viruses are U.S. Department of Agriculture (USDA) 9CFR tests for bovine serum or porcine trypsin. 9CFR requires testing of bovine serum for seven specific viruses in six families (immunofluorescence) and at least 2 additional families non-specifically (cytopathicity and hemadsorption). 9CFR testing of porcine trypsin is for porcine parvovirus. Recent contaminations suggest these tests may not be sufficient. Assay sensitivity was not the issue for these contaminations that were caused by viruses/virus families not represented in the 9CFR screen. A detailed literature search was undertaken to determine which viruses that infect cattle or swine or bovine or porcine cells in culture also have human host range [ability to infect humans or human cells in culture] and to predict their detection by the currently used 9CFR procedures. There are more viruses of potential risk to biological products manufactured using bovine or porcine raw materials than are likely to be detected by 9CFR testing procedures; even within families, not all members would necessarily be detected. Testing gaps and alternative methodologies should be evaluated to continue to ensure safe, high quality human biologicals.

1. Introduction

Biological medicinal products for both human and veterinary use and the cell substrates used for their manufacture are currently tested for bovine and porcine adventitious viruses due to the widespread use of bovine serum and porcine or bovine trypsin in cell culture. Currently, testing for bovine and porcine adventitious agents is performed in compliance with the U.S. Department of Agriculture (USDA) regulations for veterinary products as specified in the 9CFR 113 regulations (9CFR). These regulations are intended to ensure that veterinary agents of concern are not introduced into new populations of animals (e.g., into new geographic regions or into previously unexposed herds or flocks), in order to prevent veterinary epidemics and protect the U.S. cattle and swine industries. Cell-culture derived vaccines for human use were developed in the 1950's. Since fetal calf serum and bovine or porcine trypsin were used in cell culture, the 9CFR tests developed for veterinary use to screen for viruses that can infect cattle and swine were implemented by the authorities regulating human vaccines. However, many viruses not of significant concern to the cattle and swine industry are not addressed by the 9CFR testing. Today, over half a century after cell culture-derived vaccines were initially developed, the human biologics industry is still using the methods specified in the 9CFR regulations for testing FBS and porcine trypsin. When these tests are applied to bovine and porcine-derived raw materials used to manufacture products intended for human use, they may or may not detect the most relevant agents of concern for biological products for human use. The USDA

Abbreviations: 9CFR, Code of Federal Regulations, Title 9; Ab, Antibody; BT, Bovine Turbinate cells; CBER, Center for Biologics Evaluation and Research; CDER, Center for Drug Evaluation and Research; FBS, Fetal Bovine Serum; HAd, Hemadsorption; HHR, Human Host Range; ICTV, International Committee on Taxonomy of Viruses; IFA, Immunofluorescence Assay; PCR, Polymerase Chain Reaction; PERT, Product-Enhanced Reverse Transcriptase assay; RBC, Red blood cells; RT, Reverse Transcriptase; ST, Swine Testicular cells; TEM, Transmission Electron Microscopy; USDA, Unites States Department of Agriculture; VERO, African green-monkey kidney cell line.

* Corresponding author. Tel.: +1 301 948 9412; fax: +1 301 948 0401.
E-mail addresses: crsek@aol.com (C. Marcus-Sekura), james.richardson@ablinc.com (J.C. Richardson), rebecca.harston@ablinc.com (R.K. Harston), nandini.sane@ablinc.com (N. Sane), RSheets@niaid.nih.gov (R.L. Sheets).

1045-1056/$36.00
doi:10.1016/j.biologicals.2011.08.003

regulations call for testing bovine serum for at least nine viruses; seven are tested specifically by immunofluorescence upon exposure and amplification in susceptible cells, and at least two more are tested by more general read-outs (hemagglutination/hemadsorption or cytopathic effect) upon exposure and amplification in susceptible cells. In addition, the USDA regulations require testing of porcine trypsin for porcine parvovirus by immunofluorescence upon exposure and amplification in susceptible cells.

In the context of the human biologicals industry, there are three main concerns with this approach. Firstly, it is not clear whether other known bovine or porcine viruses would likely be present in fetal bovine serum or porcine trypsin. Secondly, it has not been well considered which of these 9CFR-specified viruses have human host range. Thirdly, it has also not been systematically researched whether there are other bovine and porcine agents of equal or greater concern for human biologicals that are not specified or detected by the current testing.

To gather current relevant information to try and answer these questions, a research project was undertaken with the following goals:

1. To perform a literature search to define those viruses that could contaminate bovine and porcine raw materials used in production of human biologicals and which might pose a potential safety risk to recipients.
2. To evaluate current 9CFR testing for bovine and porcine viruses to predict whether those viruses defined as a potential risk to recipients of human biologicals can likely be detected by current 9CFR tests.

Serum and trypsin manufacturers use upstream sourcing controls and harvesting and pooling procedures to limit contamination of their products. However, these practices vary by manufacturer. In the absence of a consistent procedure for processing, the worst case in terms of potential contamination was assumed; i.e., that any virus worldwide that could contaminate bovine serum or porcine trypsin and has human host range is a potential contaminant. While this assumption is a stringent one, in fact, risk mitigation procedures can and should begin at the manufacturer of the animal-derived material and at the collection abattoirs. Regulatory agencies expect manufacturers of human biologicals to regularly audit their suppliers of serum, trypsin, and other animal-derived materials, to assure that proper risk mitigation procedures are followed.

Serum collection is a non-sterile procedure and the blood from 2500 to 3000 fetuses is used to produce a single 1500 L lot of serum [1]. Similarly, the production of porcine trypsin uses huge numbers of pancreases that are extracted to produce a lot (batch) of trypsin. One infected animal may contaminate an entire batch/lot and the sensitivity of current testing may not be adequate to detect diluted contaminants. The limitations of testing for viruses in pooled products have been amply demonstrated in the human blood industry, and introduction of the mini-pool testing approach has led to increased sensitivity of detection and thereby increased safety for human recipients of these products. An additional safety concern for bovine serum and porcine trypsin is whether equipment cleaning between processing of animal-derived material lots is adequate to prevent cross-contamination.

It should be noted that the lots of bovine serum and trypsin used to produce human biologicals are often subjected to virus removal and inactivation procedures, such as filtration, heat inactivation or gamma-irradiation, before use in manufacturing biological products for human use. What is unclear is whether the testing for adventitious viruses performed by the vendor or by the manufacturer using the materials is always conducted before the use of inactivation procedures, when the likelihood of detecting a contaminant would be higher, or whether it may occur after treatment, when detection would be significantly less likely due to the inactivation of the majority, but possibly not all, of the contaminant.

Many newer products are cell-based or utilize viral vectors and are more like traditional live-attenuated vaccines where manufacture provides less opportunity to remove/inactivate viral contaminants.

There have been several well-publicized contamination events caused by bovine/porcine agents that provide the opportunity to evaluate our current virus safety approach:

- Cache Valley Virus (CVV) –fermentors from multiple manufacturers have been infected. CVV, although not typically recognized as a bovine virus, is a multispecies virus with bovine host range [2].
- Calicivirus 2117 [vesivirus] -facility contamination forced a costly facility shutdown resulting in product shortage that deprived patients of product [3].
- Multiple contaminations of fermentors with reoviruses, another virus family with wide host range [4].
- Porcine circovirus type 1 and type 2 nucleic acid contamination of live rotavirus vaccines [5,6].

Contaminations of cell cultures during production of biological products often arise when huge scale-up procedures using large quantities of bovine serum or porcine trypsin are needed, and where the sensitivity of the tests currently being used is not adequate to detect a contaminant that starts at a low-level and amplifies over the long course of cell culture. Contamination has also been detected when new testing methods are applied, such as for porcine circovirus (PCV).

It is important to recognize that direct testing of bovine serum and porcine trypsin is not the only measure in place for assuring product safety and is used in conjunction with testing for viruses in cell banks and lot-to-lot in-process testing of unprocessed bulk harvest material. However, these other tests are not designed to specifically detect bovine or porcine viruses. Additionally, for some products, removal and inactivation of viruses during purification procedures must be demonstrated. This report will focus on bovine and porcine agents and their ability to be detected by the 9CFR procedures currently in use.

2. Methods

At the start of the literature search, the following goals were developed to guide and focus the search: (i) to determine whether the seven bovine viruses specified in 9CFR 113.47 [BVDV, REO, Rabies, BTV, BAd, bovine parvovirus and BRSV] are capable of infecting humans or display human host range (e.g., by infecting human cells in culture or producing antibodies in humans), (ii) to determine if, in addition to the 7 specific bovine viruses, there are other bovine viruses that would be detected by the hemadsorption/hemagglutination and cytopathic effect (CPE) procedures in 9CFR, (iii) to identify porcine viruses (in addition to porcine parvovirus) that have human host range and that could contaminate porcine trypsin, and (iv) if additional porcine viruses were identified, to determine whether the 9CFR procedure for testing of porcine cells [9CFR113.47] would detect them.

At the outset, the pathogen search sought to identify all known vertebrate viruses with bovine or porcine host ranges [natural infection, or detection of antibodies, or ability to grow in bovine or porcine cells in culture]. The second criterion for inclusion was that each virus identified also had to have human host range, which