

EVALUATION OF THE HUMAN HOST RANGE OF BOVINE AND PORCINE VIRUSES WHICH MAY CONTAMINATE BOVINE SERUM AND PORCINE TRYPSIN USED IN THE MANUFACTURE OF BIOLOGICAL PRODUCTS

Carol Marcus-Sekura, PH.D.
BASI Consulting

PROJECT RATIONALE

- ✘ A literature search and evaluation of the zoonotic nature of bovine/porcine viruses which could contaminate materials used in the production of Biologicals
- ✘ Biological products have a great safety record in terms of virus contamination—BUT
- ✘ Have we been doing our bovine and porcine virus testing right or have we just been lucky?
- ✘ Can we relax testing standards and do fewer tests since there are reports that there have been no positive results for some tests?
- ✘ Can we make better use of new testing methods?

TESTING OF HUMAN BIOLOGICAL PRODUCTS WHEN ANIMAL-DERIVED MATERIALS ARE USED IN PRODUCTION

RISKS

- ✘ no direct testing of unprocessed bulk product for bovine or porcine agents
- ✘ no evaluation of cell banks to determine which raw-material-derived bovine/porcine viruses are capable of replication
- ✘ not clear whether viruses of concern to the human biologics industry are detected by the currently required tests for porcine and bovine viruses
- ✘ new products: cell-based or viral vectors more like live-attenuated vaccines with less opportunity to remove/inactivate virus contaminants

GOALS OF RESEARCH PROJECT

- ✘ Search for viruses which can infect cattle or swine
- ✘ Determine which viruses present a risk to humans
- ✘ Assess whether these viruses could be detected by the 9CFR tests for FCS and porcine trypsin
- ✘ Determine which of those viruses could contaminate FCS or porcine trypsin
- ✘ Identify viruses which have contaminated products [or process]
- ✘ Provide recommendations

CONCERNS ABOUT FCS

- Manufacture has some analogy to human blood collection – but without donor screening
- Serum collection NOT a sterile procedure; multiple animals and huge volumes are involved
- Manufacture involves pooling material from many animals which results in cross-contamination; testing sensitivity may not be adequate to detect low-level contamination
- Common equipment may be used at serum producer [or biologicals manufacturer] without adequate cleaning/inactivation

CONCERNS ABOUT 9CFR FOR SERUM

- Beginning with cell-culture derived vaccines developed in the 1950's, 9CFR was used to screen for viruses which can infect cattle and swine; many viruses not of concern to cattle and swine industry are not addressed by 9CFR
- Human biologics industry using FCS and trypsin testing for vet vaccines; in 2011- we're still relying on cattle and swine industry procedures to protect human products

OTHER CONCERNS ABOUT FCS

- ✘ Focus of cattle industry is on keeping the animal healthy until slaughter [not until old age] and preventing disease transmission- and is a cost/benefit approach
- ✘ Animals live outdoors; when farmed they are in large numbers and in close proximity
- ✘ Animal may have received vaccine; and vaccine may contain a live-attenuated virus

REVIEW OF 9CFR BOVINE 9CFR113.53

- ✘ monolayers of VERO and of a cell line of same species as the product [e.g, BT cells]
- ✘ 3.75 ml or 15% of ingredient used in test
- ✘ 21d test with at least 2 subcultures
- ✘ examine throughout test for cpe; reject cpe+ product
- ✘ at 21d: examine for cpe and/or hemadsorbing agents
- ✘ use guinea pig and chicken rbc [9CFR113.46]
- ✘ 2 temps: - 4C, and if no positive, try 20-25C
- ✘ reject positive product
- ✘ examine for bluetongue, bovine adenovirus, bovine parvovirus, bovine respiratory syncytial virus, bovine diarrhea virus, rabies virus, reovirus using fluorescent Ab [9CFR113.47]
- ✘ # animal sera pooled to produce a "lot" not defined

9CFR PORCINE; PORCINE TRYPSIN 9CFR113.53 [D]

- ✘ Inoculate primary porcine cells or a cell line of proven equal sensitivity to PPV
 - ✘ At least 5g of trypsin dissolved, centrifuged, reconstituted
 - ✘ Inoculate onto monolayer of PPV-sensitive porcine cells
 - ✘ 14d maintenance period with subculture
 - ✘ Cells then tested with a fluorescent Ab to PPV
- [Test is known not to be sufficiently sensitive and 21d is often recommended]

9CFR PORCINE CELLS [9CFR113.47]

- ✘ Vero, ST or PK cells
- ✘ CPE, hemadsorption
- ✘ IFA for 6 viruses
 - + bovine viral diarrhea virus (BVDV)
 - + rabies virus
 - + reovirus
 - + porcine adenovirus
 - + transmissible gastroenteritis virus (TGEV)
 - + porcine hemagglutinating encephalomyelitis virus (PHE-CoV)

DATABASE APPROACH FOR VIRUSES

- ✘ Perform literature search to cast a wide net
- ✘ Identify all viruses that could infect cattle and swine [bovine or porcine host range]
- ✘ Identify the subset of viruses with human host range and label these "agents of concern"
- ✘ Evaluate this subset further for ability to be detected by 9CFR methods
- ✘ Store notes and references in a database format so easily searchable

DEFINITION OF HOST RANGE

- *Ability to infect animal or human
 - symptomatic infection
 - production of antibodies by host
 - infection of host cells in culture

HUMAN HOST RANGE

- **3 categories**
 - Frankly zoonotic - cause disease [rabies virus]
 - Human Ab+ : exposure, no known disease [PCV]
 - Infect Hu cells in vitro [e.g. SWV, BPIV3, FMDV]
- [many biological products are injected and bypass normal host defenses]

PLAN FOR SUBSET DEFINED AS AGENTS OF CONCERN

- AOC = human infection [disease or sero+ or infection of human cells in vitro]
- × Collect information on:
 - × geographic areas from which we source raw material
 - × fetal transmission or viremia or pancreatitis [trypsin]
 - × history of iatrogenic transmission or cell culture contamination

Predict detection by 9CFR

- cell lines infected in vitro; isolation procedures;
- ability to produce cpe and hemagglutination

FINDINGS

- × Huge number of viruses that can infect cattle and swine and huge literature; many not of concern to cattle and swine industry so ignored by 9CFR
- × 2,011 species vertebrate viruses; host range for many individual viruses in a family unknown; so organize data by virus family or groups of related viruses
- × Database currently has ~325 separate entries for viruses [or virus groups] with bovine, porcine and/or human host range

DATABASE ENTRIES

- × A Database entry may include
 - + Individual viruses
 - + Multiple names used for a given virus
 - + Closely related viruses in large virus families
 - × e.g. each flavivirus does not have a separate entry
 - + Strains of the same virus

VIRUS CONTAMINATION EXAMPLES

- × Production or Products
 - + MVM, CVV, BPyV, REO, BHV-4, PCV-1, PCV-2, PERV, Vesivirus 2117 [SV40, polio in vaccine, Hu Ad]
 - + Endogenous retroviruses in CHO, murine cell lines
- × FCS – Akabane, Bovine enteroviruses, BPIV3, BPyV, BVDV, CVV, IBR, EHDV, FMDV, Pseudorabies, others
- × Cells in vitro
 - + BVDV, PPV, Bovine Kobuvirus

RESULTS: BOVINE VIRUSES

- × Entries with bovine host range >99
- × Bovine viruses labelled agents of concern >87
- × Viruses detected by 9CFR IFA = 7
- × When include + controls for cpe and HA_g = 9
- × Viruses with bovine and human host range >79
- × Viruses with bovine and human host range and bovine viremia >58

VIRUS FAMILIES: BOVINE/HUMAN HOST RANGE

- ✘ 21 virus families were identified which contain viruses with bovine and human host range;
- ✘ Adenoviridae, Anellovirus, Bornaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Flaviviridae, Hepeviridae, Herpesviridae, Orthomyxoviridae, Papillomaviridae, Paramyxoviridae, Parvoviridae, Picornaviridae, Polyomaviridae, Poxviridae, Reoviridae, Retroviridae, Rhabdoviridae, Togaviridae

ISOLATION VERSUS INFECTION

- ✘ 9CFR- VERO and Bovine turbinate – not all viruses that grow in these cell lines can be ISOLATED in these cells
- ✘ Although laboratory strains can grow in some cell lines, wild isolates [field strains] may not readily replicate. For many agents, multiple rounds of passage of original isolate was necessary to derive a strain that grew well in laboratory cell lines
- ✘ Swarm/mutation/selection
- ✘ Laboratory tests used to screen for virus contamination of cell lines might miss agents not previously adapted to cell culture

**POSSIBLE BOVINE AGENT OF CONCERN
DETECTION BY 9CFR CPE OR HA**

✘ Infect Vero	35
+ Isolation in Vero	12
✘ Cpe and/or HAd/HAg in 9CFR-VERO	28
✘ Infect BT	5
+ Isolation in BT	3
✘ Cpe and/or HAd/HAg in 9CFR-BT	1

**EXAMPLES OF VIRUSES WITH HU + BOVINE
HOST RANGE LIKELY NOT DETECTED BY 9CFR**

- ✘ Borna disease virus, bovine circovirus, bovine enterovirus, bovine foamy virus, bovine leukemia virus, bovine polyomavirus, Cache Valley virus, hepatitis E virus, norovirus, Ross River virus, rotavirus, SLE virus, others

IMPORTATION AND REALITY – A SURPRISE

- ✘ High prices for FCS have encouraged an illegal market
- ✘ Publication indicated illicit trade of serum from other nations coming into US through Canada and Mexico
- ✘ Viruses which might contaminate FCS may be derived from additional countries rather than being limited to the US, Australia and New Zealand
- ✘ If preparations of FCS are contaminated with newborn calf serum or bovine serum, many additional viral agents may be contaminants

LESSONS LEARNED - BOVINE VIRUSES

1. 9CFR designed for bovine biological products; i.e. bovine vaccines to protect cattle from diseases of bovines that impact industry – NOT human products using FCS. i.e. some viruses of concern to products for human use may not be covered by 9CFR
2. Many new agents have been identified since 9CFR was put in place; some bovine agents once thought to be zoonotic have human counterparts and are different agents; many viruses have had name changes
3. Some bovine agents replicate in human cells in vitro
 - two non-pathogenic viruses being developed as vectors for live-attenuated human vaccines
 - another being evaluated as anti-cancer therapy

LESSONS LEARNED - BOVINE VIRUSES

4. Laboratory virus that infects a cell line may not predict virus isolation from infected animal [field strain]
5. Viruses in nature are often swarms or able to mutate, so with enough passages it is often possible to adapt strains to grow in a cell line
6. Illicit serum
7. Lessons from contaminations: MMV, CVV, BPyV, Reo.....others

PARTIAL SOLUTION

- ✘ Transplacental Ab transmission does not occur in cattle and therefore if Ab is found in FCS it suggests
 - + either virus transmission in utero
 - + or contamination with bovine serum
- ✘ Testing for the presence of antibodies in FCS may be a useful technique to screen for illicit spiking or sloppy collection, both of which can impact the chance of virus contamination of the FCS

RESULTS: PORCINE VIRUSES

- ✘ Agent targeted by 9CFR for trypsin: PPV
- ✘ Many viruses inactivated by trypsin
- ✘ 3 additional agents detected by 9CFR IFA for porcine cells
 - + porcine adenovirus
 - + porcine hemagglutinating encephalitis virus
 - + transmissible gastroenteritis virus - TGEV
- ✘ Viruses with porcine host range - >85
- ✘ Viruses with porcine and human host range- >67

VIRUS FAMILIES:PORCINE/HUMAN HOST RANGE

- ✘ 17 virus families were identified which contain viruses with porcine viruses and human host range:
- ✘ Anellovirus [no family name], Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Filoviridae, Flaviviridae, Hepeviridae[tentative family name], Herpesviridae, Orthomyxoviridae, Paramyxoviridae, Parvoviridae, Picornaviridae, Reoviridae, Retroviridae, Rhabdoviridae, and Togaviridae.

EXAMPLES OF VIRUSES WITH HU+PORCINE HOST RANGE LIKELY NOT DETECTED BY 9CFR

- ✘ Hepatitis E virus, norovirus, porcine circovirus [PCV-1 and PCV-2], porcine CMV, porcine endogenous retrovirus [PERV], porcine enterovirus, Powassan virus, rotavirus, Seneca Valley virus, TTV, others

LESSONS LEARNED PORCINE VIRUSES

1. Pancreas arrives from abroad for processing in huge frozen blocks with hundreds of organs; no opportunity to test individual organs or make minipools
 - assume any virus with porcine host range [not inactivated by porcine pancreatic enzymes] is a risk
2. Requirement for functional enzyme impacts ability to inactivate contaminating viruses
3. Presence of pancreatic nucleases, proteases, and other enzymes
 - may inactivate virus contaminants
 - may interfere with implementation of PCR-based techniques for virus detection

FAMILIES CONTAINING ≥1 VIRUS WITH BOV/HU HR LIKELY MISSED BY 9CFR "B"

FAMILIES CONTAINING ≥1 VIRUS WITH PORC/HU HR LIKELY MISSED BY 9CFR "P"

✘ Anelloviridae	B?	P	Papillomaviridae	B	
✘ Bonaviridae	B		Paramyxoviridae		P
✘ Bunyaviridae	B	P	Parvoviridae	B	P
✘ Caliciviridae	B	P	Picornaviridae	B	P
✘ Circoviridae	B	P	Polyomaviridae	B	
✘ Coronaviridae	B		Poxviridae	B	
✘ Flaviviridae	B	P	Reoviridae	B	P
✘ Hepeviridae	B	P	Retroviridae	B	P
✘ Herpesviridae	P		Togaviridae	B	P

CONCLUSIONS

- ✘ Many more viruses with bovine and porcine host range than we currently test for using 9CFR procedures
- ✘ Illicit serum further increases the virus risk
- ✘ 9CFR bovine testing using Vero cells and HAd/HAg likely detects many additional viruses, but has never been validated for field strains
- ✘ Viral clearance steps used in production of some biological products give added safety
- ✘ PCR technology may be useful [contaminating proteases and nucleases and continuous sequence variation in field strains, and detection of non-infectious nucleic acids may be an issue].

RECOMMENDATIONS

- ✘ Possible requirement for gamma-irradiation of FCS
- ✘ Possible implementation of testing of FCS for Ab to common viruses to detect contamination with newborn calf or bovine serum
- ✘ Possible implementation of PCR-based assays to detect a wider spectrum of agents
- ✘ Possible augmentation of porcine 9CFR for viruses other than PPV
- ✘ Your input - CRSEK@AOL.COM

ONE HEALTH INITIATIVE

The convergence of people, animals, and our environment has created a new dynamic in which the health of each group is inextricably interconnected.....

.....of the 1461 diseases now recognized in humans, approximately 60 percent are caused by multi-host pathogens characterized by their movement across species lines. And, over the past 3 decades, approximately 75 percent of new emerging human infectious diseases have been zoonotic. Our increasing interdependence with animals and their products may well be the single most critical risk factor to our health and well-being with regard to infectious diseases.

- ✘ **"One Health - One Medicine": linking human, animal and environmental health-** AVMA, AMA, ASTMH, ASM, CDC

PROJECT TEAM

- ✘ BASI
 - ✘ Carol Marcus-Sekura
- ✘ NIAID-DAIDS
 - + Becky Sheets
- ✘ ABL
 - + James Richardson
 - + Nandini Sane
 - + Rebecca Harston

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