

Use of Bovine Serum in the Manufacture of Human Vaccines – New Challenges and Risk Control

A virological perspective

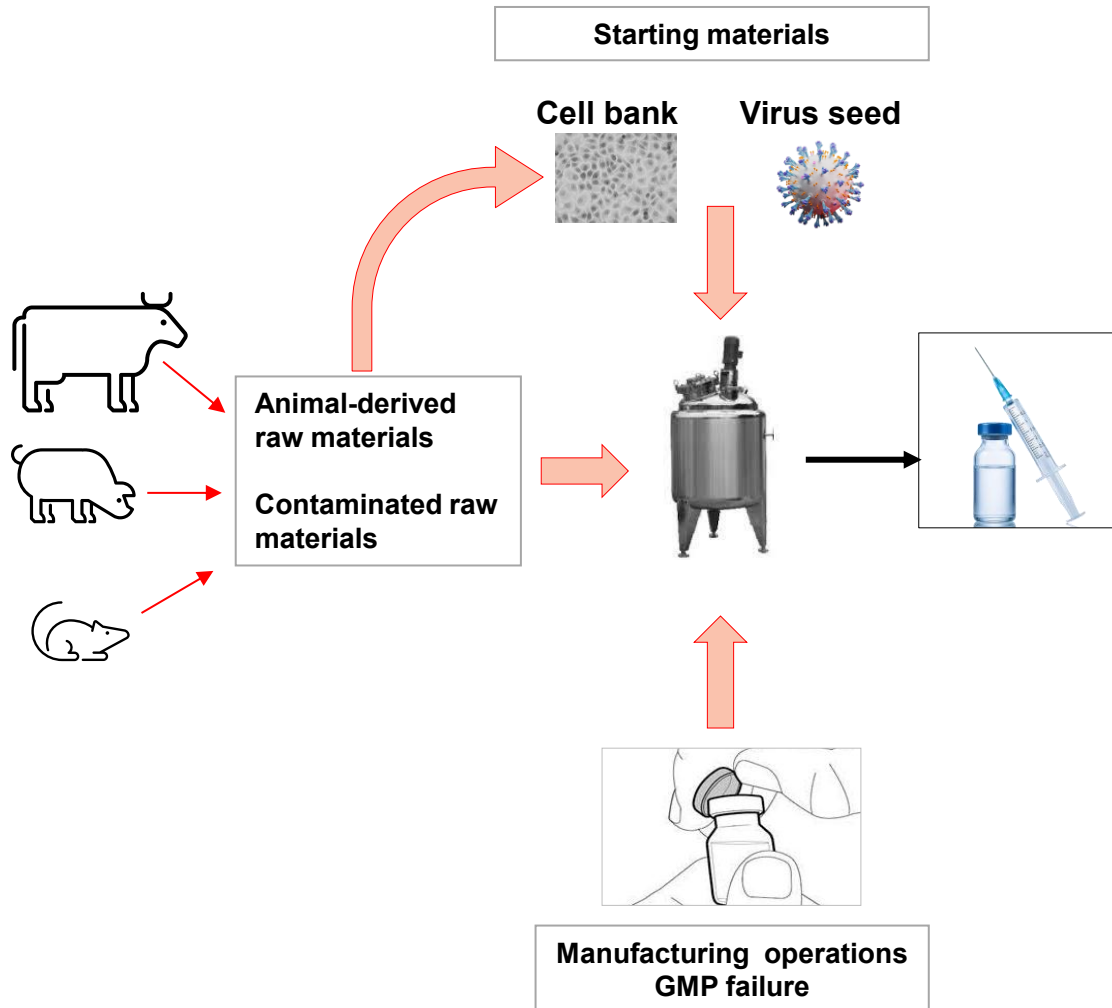
Dr. Florian Zielecki



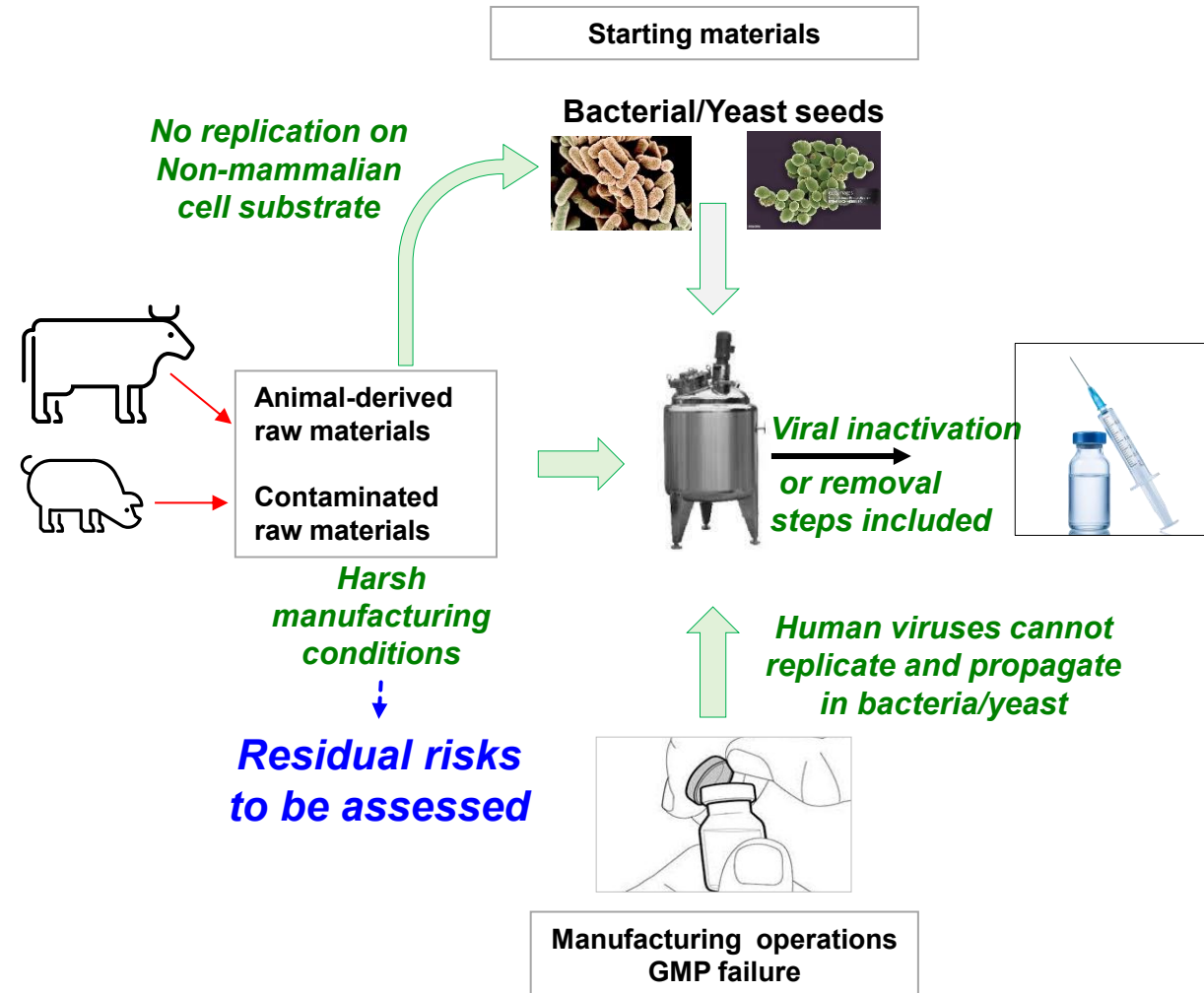
Dr. Florian Zielecki is an employee of the GSK group of companies.
This work was sponsored by GlaxoSmithKline Biologicals SA

Sources of Viral Contamination in Biomanufacturing

Cell culture-based processes



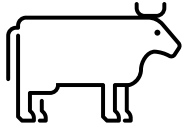
Bacteria / Yeast based processes



Bovine Serum – Risks and Challenges

EP 2262 / 9CFR

Bovine (fetal) serum



→ Fit for human consumption



Veterinary
examination



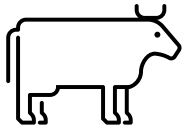
Detection of obvious disease symptoms e.g

- Foot and Mouth disease
- Bluetongue virus
- Cowpox

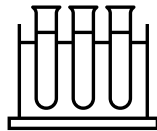
Bovine Serum – Risks and Challenges

EP 2262 / 9CFR

Bovine (fetal) serum



Veterinary
examination



EP2262
9-CFR
testing

***In vitro* testing on Vero and bovine cells**

The cell lines used are suitable for detecting hemadsorbing viruses such as bovine parainfluenza virus 3 and cytopathic agents such as bovine herpesvirus 1.

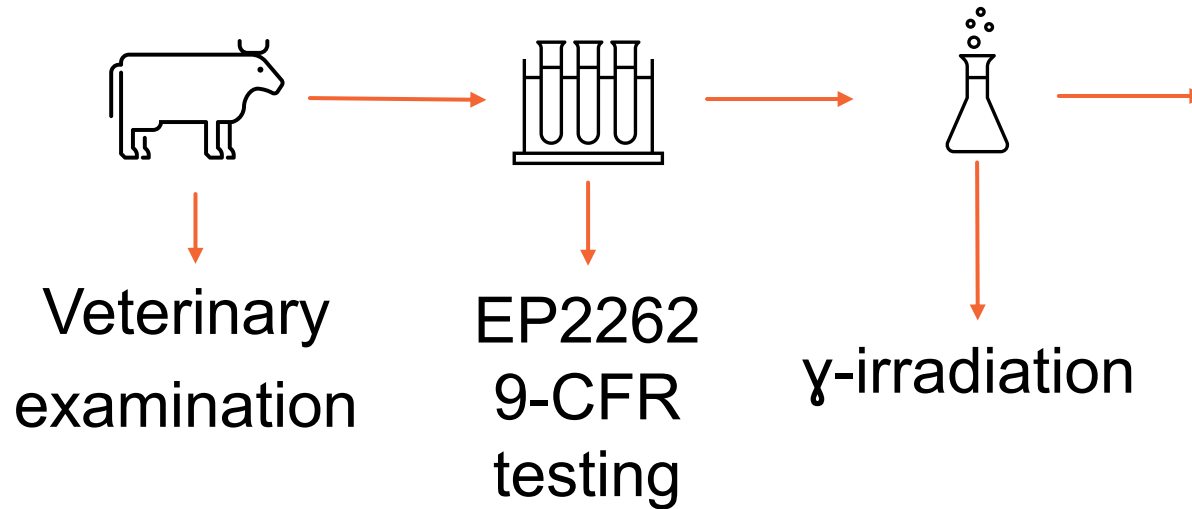
Antibody test

Specific tests (fluorescent antibody technique) for viral contaminants (if not detected by general tests), where relevant in view of the country of origin of the serum:

- Bluetongue virus
- Bovine adenovirus
- Bovine parvovirus
- Bovine respiratory syncytial virus
- Bovine viral diarrhoea virus
- Rabies virus
- Reovirus

Bovine Serum – Risks and Challenges

Bovine (fetal) serum

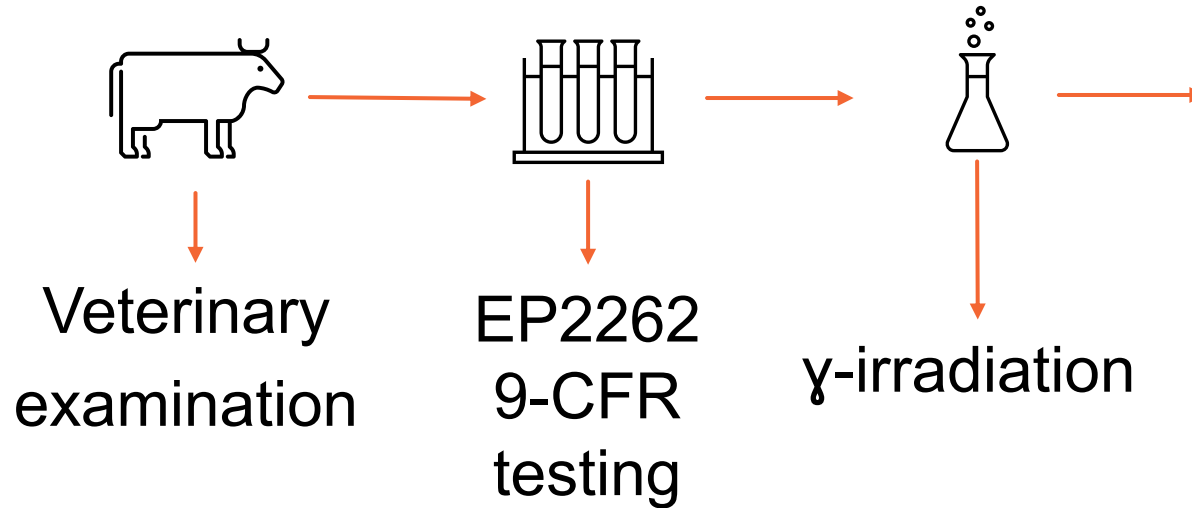


Virus families sensitive to gamma- irradiation ≥ 4 logs :

- Picornaviridae
- Astroviridae
- Retroviridae
- Caliciviridae
- Phenuiviridae (Bunya)
- Bornaviridae
- Flaviviridae
- Baculoviridae
- Rhabdoviridae
- Togaviridae
- Arenaviridae
- Peribunyaviridae
- Filoviridae
- Pneumoviridae
- Orthomyxoviridae
- Paramyxoviridae
- Nairoviridae
- Sedoreoviridae
- Coronaviridae
- Spinareoviridae
- Adenoviridae

Bovine Serum – Risks and Challenges

Bovine (fetal) serum



Potential challenges

- **Virus families (partial) resistant to γ -irradiation ≤ 4 logs :**
 - Circoviridae
 - Anelloviridae
 - Parvoviridae
 - Polyomaviridae

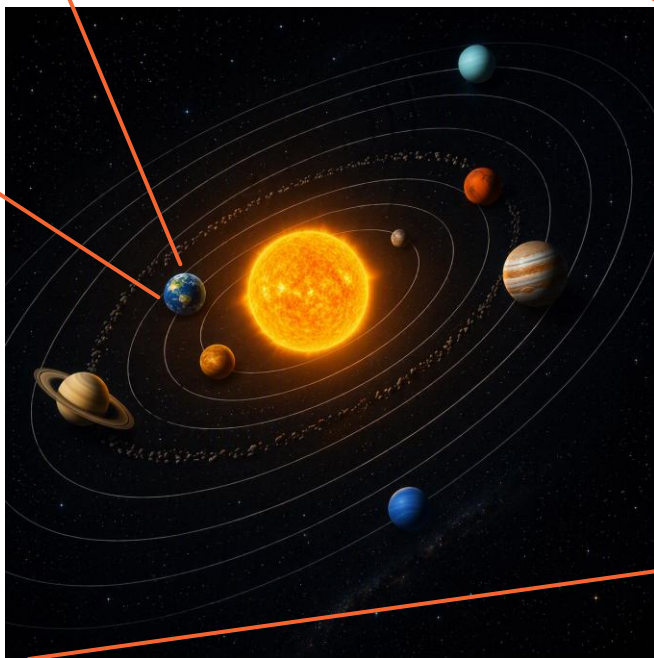
• **“Unknown”
viruses**

The unknown

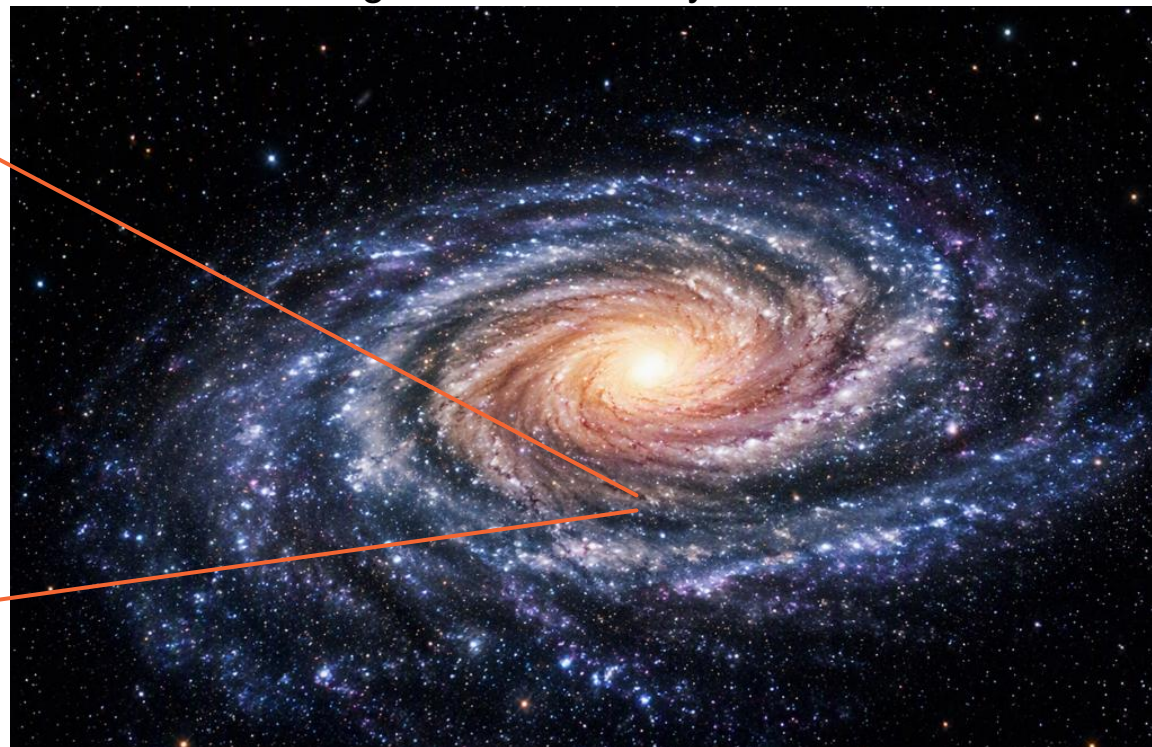
In vivo assay



In vitro assay



Broad molecular agnostic HTS analysis for Unknown viruses



All Images AI generated

Literature: Detection of Viral Sequences in Bovine Serum by NGS/HTS Analysis



Brief Report

Virome Characterization in Commercial Bovine Serum Batches—A Potentially Needed Testing Strategy for Biological Products

William P. Paim^{1,2}, Mayara F. Maggioli¹, Shollie M. Falkenberg³, Akhilesh Ramachandran⁴, Matheus N. Weber⁵, Cláudio W. Canal² and Fernando V. Bauermann^{1,*}

Zhang et al. Zool. Res. 2022, 43(5): 756–766
https://doi.org/10.2472/zj.issn.2095-8137.2022.093

ZR Zoological Research

Article

Open Access

Metagenomic analysis reveals presence of different animal viruses in commercial fetal bovine serum and trypsin

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Virome of US bovine calf serum



Mohammadreza Sadeghi^{a,b,c}, Beatrix Kapusinszky^{a,b}, Danielle M. Yugo^d, Tung Gia Phan^{a,b}, Xutao Deng^{a,b}, Isis Kanevsky^e, Tanja Opriessnig^f, Amelia R. Woolums^g, David J. Hurley^h, Xiang-Jin Meng^d, Eric Delwart^{a,b,*}

Biologicals 42 (2014) 145–152



Contents lists available at ScienceDirect

Biologicals

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



Unbiased analysis by high throughput sequencing of the viral diversity in fetal bovine serum and trypsin used in cell culture



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Metagenomic assessment of adventitious viruses in commercial bovine sera



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Bovine virus sequences detected in different types of Bovine Serum by NGS/ HTS described in scientific literature

- 19 virus sequences detected in Serum originating from AUS/NZ
- 45 virus sequences detected in Serum originating from US/North America
- Different Sequencing technologies and bioinformatic analysis used
- Not demonstrate to correspond to an infectious virus
- Large range of number of reads

Publication	United States	New Zealand / Australia
Gagnieur et al., 2014	Manufacture 1 vs 2 Pooled FBS from different Geographic origins	
Paim et al., 2021:	4 batches	2 batches
Sadeghi et al., 2017	4 bovine sera pools	/
Thoohey-Kurth et al., 2017	22 bovine Donor/ fetal serum batches	2 fetal bovine batches
Zhang et al., 2022	2x Pools (10 batches)	1x Pool (10 batches)
Total # of batches	34	7

Literature: Detection of Viral Sequences in FBS

FBS contains more viruses than expected

Family	Species/ Virus Name	Detected in # Publications	Detected in FBS from North America	Detected in FBS from AUS /NZ
Parvoviridae	Bosavirus = Ungulate copiparvovirus 5	4	Yes	Yes
Flaviviridae	Bovine viral diarrhea virus 1	4	Yes	Yes
Flaviviridae	Bovine viral diarrhea virus 2	3	Yes	/
Parvoviridae	Bovine parvovirus 2	3	Yes	Yes
Parvoviridae	Bovine parvovirus 3	3	Yes	Yes
Parvoviridae	Bovine hokovirus 2	2	Yes	/
Parvoviridae	Porcine parvovirus 4	2	Yes	Yes
Picornaviridae	Bovine kobuvirus	2	Yes	/
Polyomaviridae	Bovine polyomavirus 1	2	Yes	/
Anelloviridae	Torque teno virus	1	Yes	Yes
Caliciviridae	Norovirus genogroup 3	1	Yes	/
Caliciviridae	Norwalk virus	1	Yes	/
Flaviviridae	Bovine viral diarrhea virus 3	1	Yes	/
Herpesviridae	Bovine alphaherpesvirus 5	1	Yes	/
Parvoviridae	Dipteran brevidensovirus 2	1	Yes	/
Parvoviridae	Bovine copiparvovirus 3	1	Yes	/
Picobirnaviridae	Human picobirnavirus	1	Yes	/
unassigned	Chimpanzee faeces associated circular DNA molecule 1	1	Yes	/
unassigned	Gorilla smacovirus	1	Yes	/
unassigned	Lake Sarah-associated circular virus-27	1	Yes	/
unassigned	Nodamura virus	1	/	Yes

Bovine Serum Associated Virus (Bosavirus)

Sadeghi et al., 2017

Bosavirus is a bovine parvovirus belonging to the family **Parvoviridae**, genus Copiparvovirus. → Ungulate copiparvovirus 5

Is Bosavirus infectious or pathogenic?

- No confirmed zoonotic transmission has been reported to date.
- Limited clinical data exist in cattle;
- Bosavirus is mainly known from serum and virome studies, not disease outbreaks.
- Bosavirus is a well-documented, frequent agent in commercial FBS, detectable by metagenomics but not by standard regulatory tests. While its pathogenicity is unclear, its prevalence and resistance make it relevant for viral safety risk assessments.

Bosavirus

Weber et al., 2018

Evaluation of the serum virome in calves persistently infected with Pestivirus A (BVDV), presenting or not presenting mucosal disease

High-throughput sequencing (HTS) was applied for evaluation of the total virome in sera of

- (i) BVDV persistently infected calves displaying clinically apparent mucosal disease (n = 8);
- (ii) BVDV persistently infected calves with no signs of mucosal disease (n = 8)
- (iii) control, BVDV/Pestivirus A-free calves (n = 8).
- Genomes of **Parvoviridae**, such as **Ungulate erythroparvovirus 1** and **BosV**, were most frequently detected in all the three groups of calves.

Bosavirus

Across all published studies:

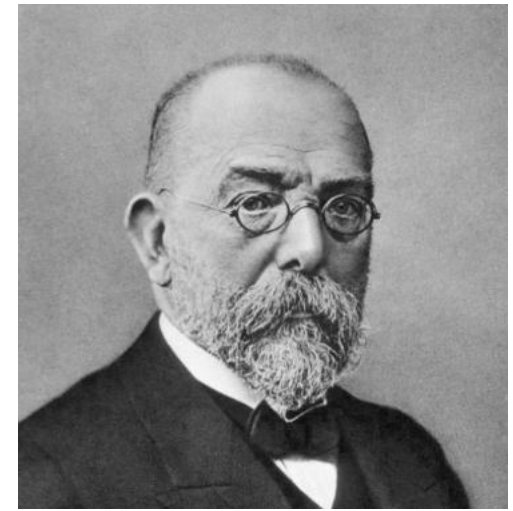
- No productive infection shown in any cell line
- No increase in viral genome copies over time
- No cytopathic effect (CPE)
- No serial passage
- No virus isolation

Notably: In the original virome study describing Bosavirus, authors explicitly framed it as a serum-borne parvovirus detected by sequencing, not as a cultured virus.

Later large-scale metagenomic surveys of commercial FBS likewise did not demonstrate replication, only frequent detection. To date, no paper reports successful *in vitro* propagation of Bosavirus in bovine or non-bovine cells.

The Four Classical Koch's Postulates

Does Bosavirus fulfil Koch's postulates?



1. The microorganism must be found in all cases of the disease
 - It should be present in diseased individuals
 - It should not be present in healthy individuals → **Bosavirus also present in healthy cattle**
2. The microorganism must be isolated and grown in pure culture
 - The pathogen must be taken from the host and grown in the lab → **Not shown**
3. The cultured microorganism should cause disease when introduced into a healthy host
 - A healthy organism exposed to the microbe should develop the same disease → **Not shown**
4. The microorganism must be re-isolated from the newly diseased host
 - It should match the original pathogen → **Not shown**

Central question:

How to address bovine viruses with the following characteristics?

- They are present in serum batches (detected by unbiased molecular technologies like HTS)
 - They are not known to be pathogenic either in cattle or human
 - They are partially resistant to gamma irradiation treatment
 - They cannot propagate on production cell culture
 - They could generate a positive PCR signal when tested
- ➔ Acceptance? Testing? Risk Assessment !
- ➔ Need to understand and categorize the real risk of viral nucleotide sequences present in bovine Serum ➔ **Not every virus might be a pathogen.**

Central question:

How to address bovine viruses with the following characteristics?

	BYDV	Borna disease virus
Present in Serum	Yes	Yes
Known to be pathogenic	Yes	No
Resistant to gamma irradiation	No	Yes*
Propagates in production cell culture	Bovine cells	No
Generate a positive PCR signal	Yes	Yes

*Resistance extrapolated from inactivation data using other related Parvovirus

- Acceptance? Testing? Risk Assessment !
- Need to understand and categorize the real risk of viral nucleotide sequences present in bovine Serum → **Not every virus might be a pathogen**

CSK