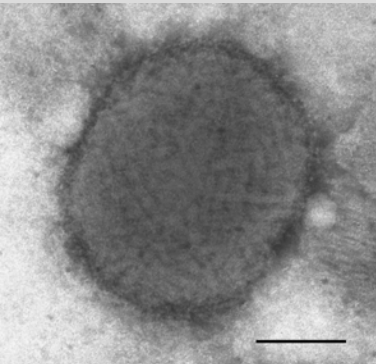


Investigations of endemic outbreaks of cutaneous fowlpox in chickens and turkeys in Austria

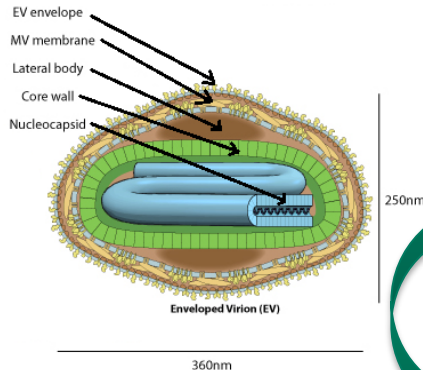
Miguel Matos

Clinic for Poultry and Fish Medicine, Department for Farm Animals and Veterinary Public Health,
University of Veterinary Medicine Vienna, Austria

Fowlpox virus (FWPV)



100 nm



Family *Poxviridae*

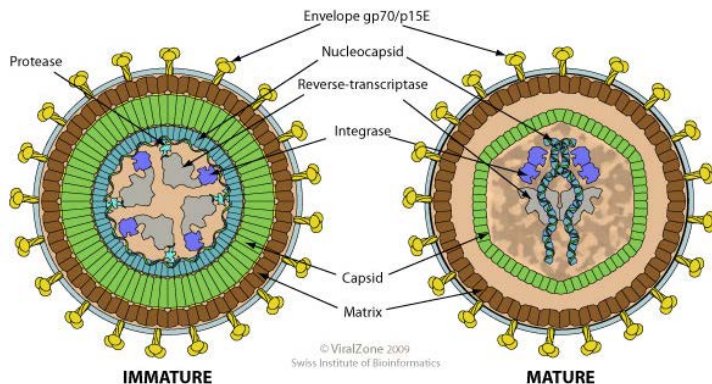
Subfamily *Chordopoxvirinae*

- ☐ Genus *Avipoxvirus*
- ☐ brick-shaped virions
- ☐ linear dsDNA ~ 300kbp
- ☐ field strains frequently carry an integrated, active copy of the **Reticuloendotheliosis Virus (REV)**

Family *Retroviridae*

Subfamily *Orthoretrovirinae*

- ☐ Genus *Gammaretrovirus*
- ☐ responsible for:
 - ☐ chronic lymphoid neoplasia
 - ☐ runting disease syndrome
 - ☐ acute reticulum cell neoplasia



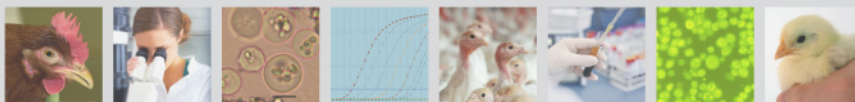
integrate into the genome of cells and of large DNA viruses, including Marek's disease (MD) and FWPVs



Fowlpox: clinical presentation

■ Fowlpox:

- cutaneous form: development of proliferative lesions and scabs on unfeathered areas
- diphtheric form: development of diphtheric lesions on the upper parts of the digestive and respiratory tracts (ddx: ILT)
- control by vaccination:
 - wing-web method or thigh (turkeys)
 - live attenuated vaccines – CEO and TCO
 - 8-12 weeks of age
 - live nonattenuated vaccines – Pigeonpox vaccine
 - less pathogenic for chickens and turkeys
 - recombinant FWPV vectored vaccines
 - TROVAC®: AIV, H5, NDV
 - Vectormune®: FP MG, FP LT/AE, FP N



Outbreak description & flock data

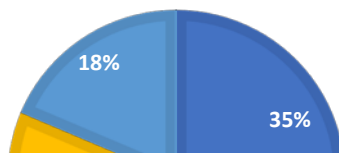
- 64 outbreaks of cutaneous fowlpox
- from fall 2018 to early 2020 (most cases between October 2018 and June 2019)
- flocks were not vaccinated against FWPV



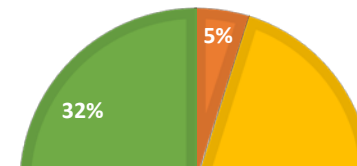
▲ layers
○ broilers
■ turkeys

■ < 5000 ■ 5000-10000 ■ 10000-15000 ■ 15000-20000 ■ >20000

■ cage ■ BA ■ CFR



flock size



husbandry system

Epidemiological picture is rather a reflection of the Austrian poultry industry



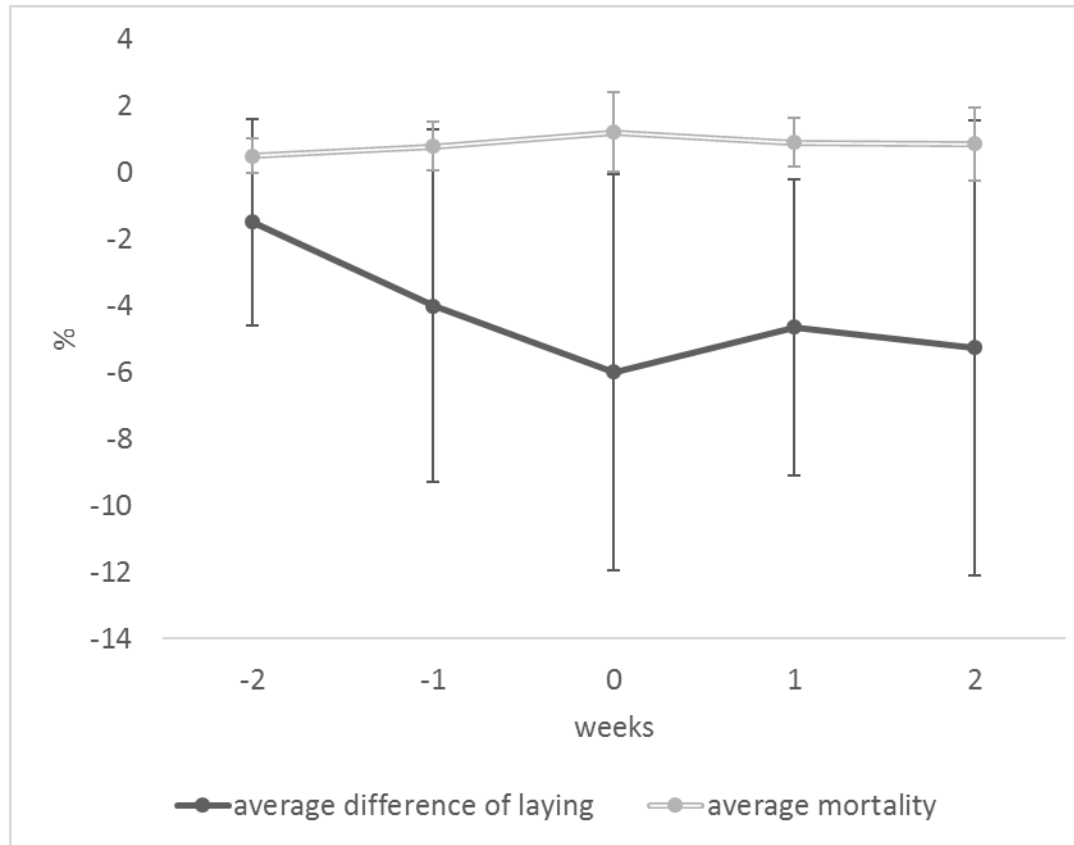
22.10.2020

Endemic outbreaks of cutaneous fowlpox in Austria

Clinical signs

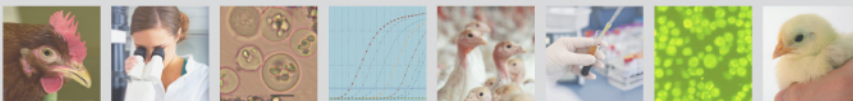
■ field data from selected flocks (n=15)

■ week 0 = veterinary visit and sampling



■ a mean drop of 6% in egg production was recorded

■ weekly mortality had a slight increase up to 1.2%



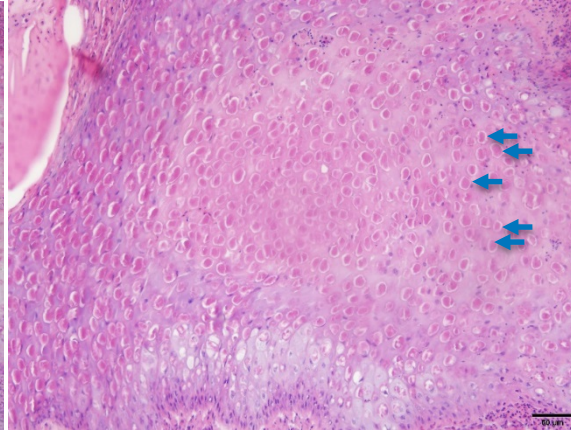
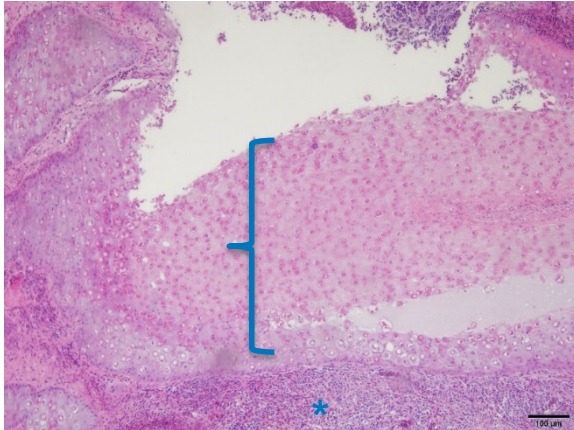
Post mortem investigations



Gross lesions

comb, wattle and eyelids

- ☐ nodular lesions
- ☐ crusty scabs



Histopathology

epidermal cells

- ☐ marked hyperplasia ({)
- ☐ degeneration, lymphoid infiltration (*)
- ☐ intracytoplasmic eosinophilic inclusion bodies (Bollinger bodies) (←)



Molecular investigations

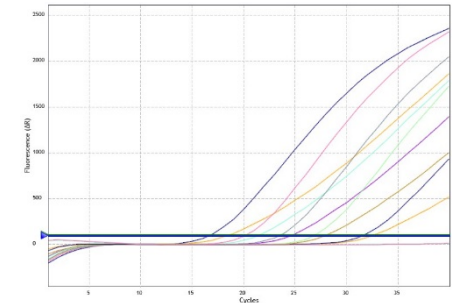
■ Establishment of a multiplex qPCR – FWPV and REV



→ DNA extraction →



→



amplified gene	primer/probe	sequence 5' 3'	position within the gene sequence
FPV 4b core protein	qP 1 (primer forward)	tcagcagtttggtacaagaca	1825-1845
	qP 2 (primer reverse)	ccatttccgtgaatagaatagtat	1933-1910
	qP S (probe)	FAM-atctccgccgtcgcaactcca-BHQ1	1890-1869
	qR 5 (primer forward)	gttttctatacacaccagcctacct	1716-1740
REV gag	qR 6 (primer reverse)	tcctgacctcccgctact	1827-1809
	qR S (probe)	HEX-ctgtcctcaccctctccctctcctcca-BHQ1	1806-1781

(Hauck et al., 2009)



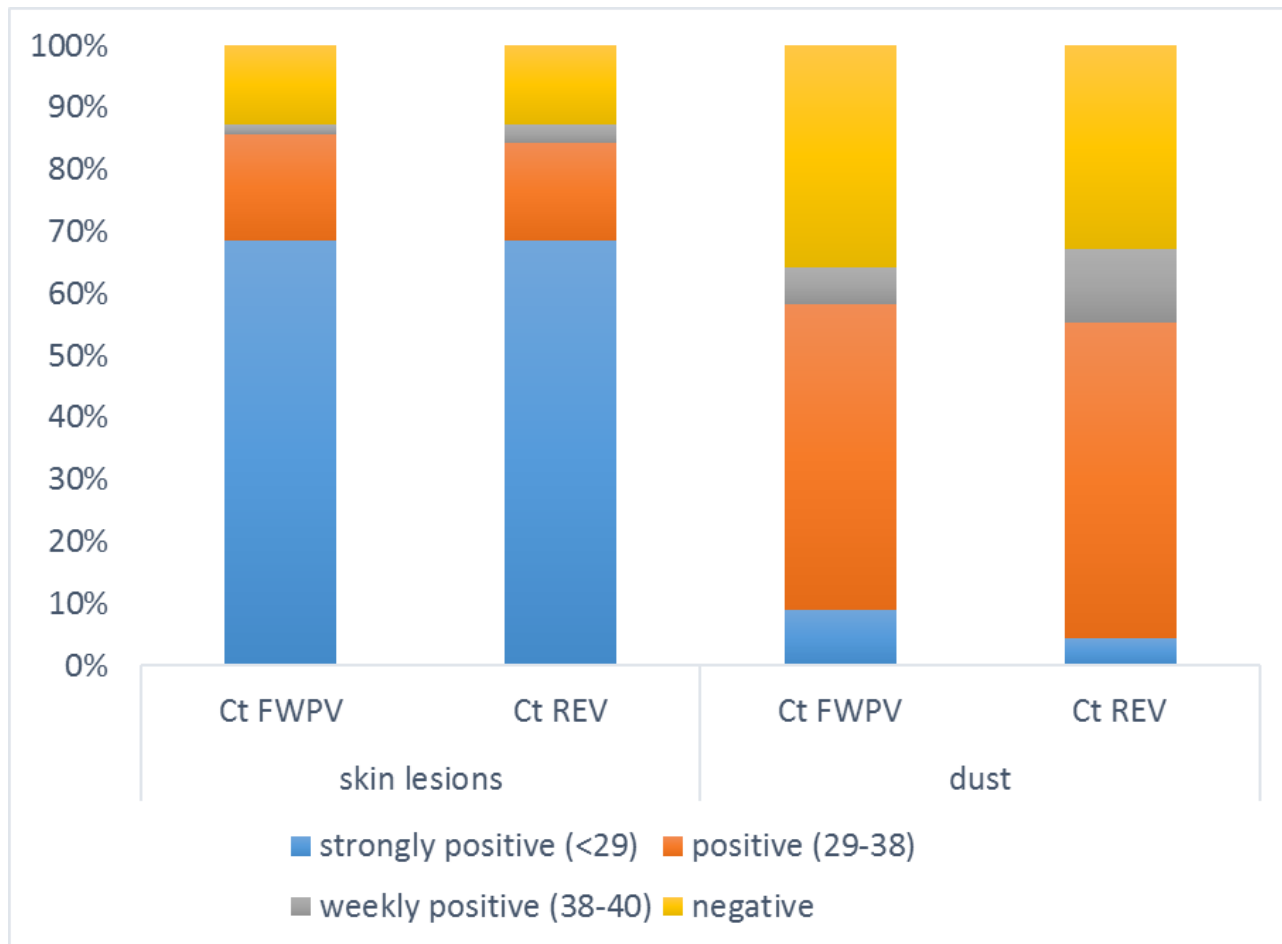
Multiplex qPCR

■ skin samples (n=73)

- ~85% positive for FWPV and REV (70% strongly positive)

■ dust (n=67, from 21 flocks)

- ~55-60% positive for FWPV and REV (5-10% strongly positive)



FWPV strains whole genome analysis

■ little is known about the *Avipoxvirus* genus

- big genome (~300kbp); very few strains fully sequenced
- huge sequence diversity within the genus
 - strains can be further subdivided in clades and sub-clades



■ Next Generation Sequence (NGS)

- assess genetic diversity of selected field strains
- significance of the REV insertions
 - pathotypic FWPV variants in the field?



Prep



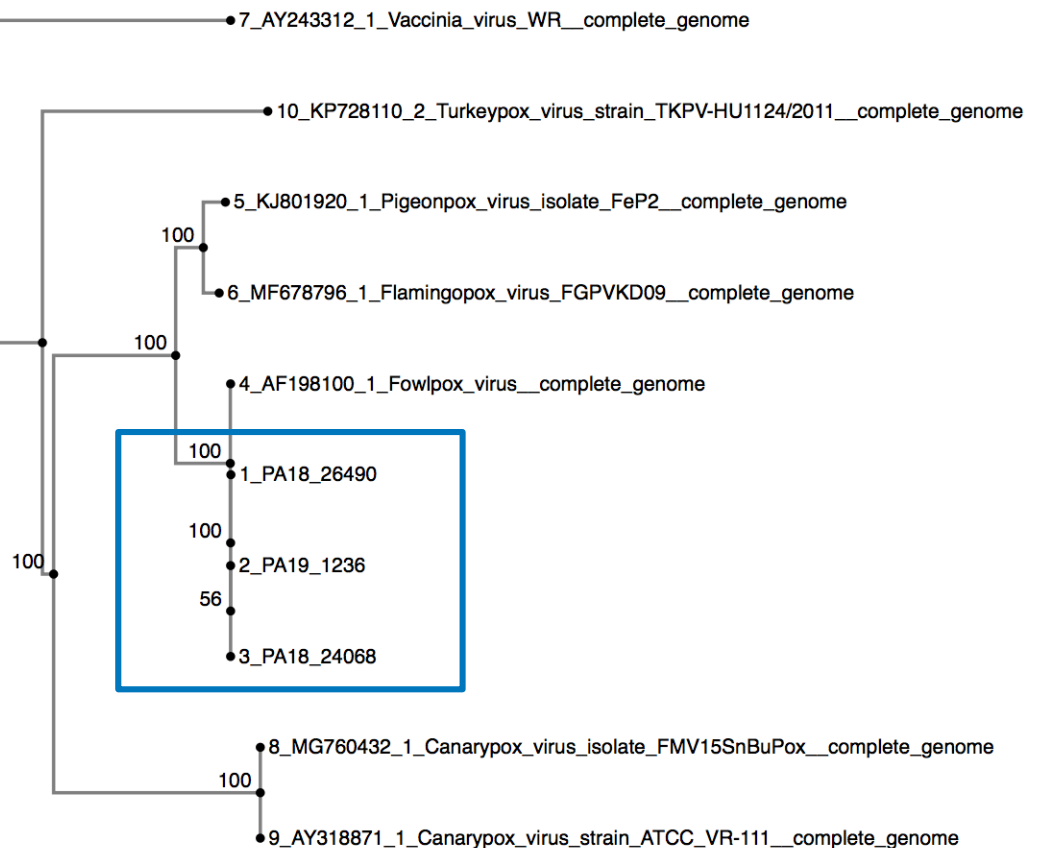
Sequence



Analyze



Poxviruses whole genome sequences: phylogenetic analysis

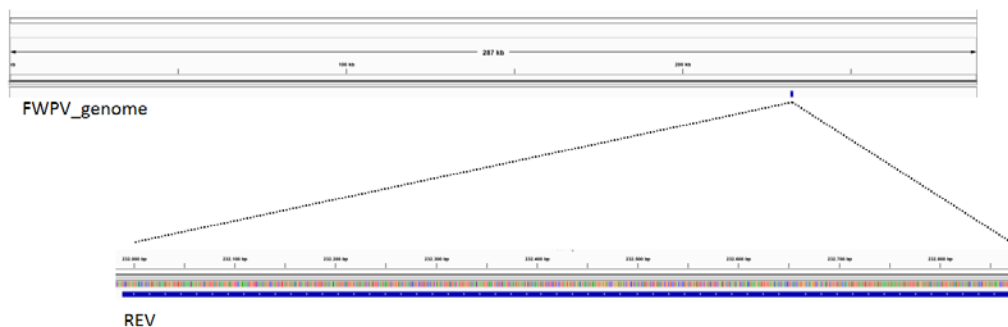


■ high similarities with previously fully sequenced FWPV pathogenic field strains



Significance of REV insertions

REV sequence within the genome of FWPV



There is anecdotal evidence that REV-containing field viruses are more problematic

- increased virulence
- resistance to vaccine-induced immunity (emergence of unrecognized antigenic variants?)
- increased virus fitness

(Giotis & Skinner, 2018)

Table 2. Characterization of the response of chickens to inoculation with either QT-35 cell lysate (control) alone or containing FWPV rMN97X or FP-VAC or MN97.^A

Days Postinoculation	Type of inoculum											
	rMN97X			FP-VAC			MN97			Control		
	Primary lesions ^B	Secondary lesions ^C	Viremia ^D	Primary lesions	Secondary lesions	Viremia	Primary lesions	Secondary lesions	Viremia	Primary lesions	Secondary lesions	Viremia
6	10	0	6 (0.8)	10	0	3 (1.2)	10	0	9 (0.8)	0	0	0
12	10	5	0	10	2	0	10	10	0	0	0	ND ^E
19	6	2	ND	0	0	ND	4	10	0	0	0	ND
26	0	0	0	0	0	0	4	8	0	10	0	6 (1.0)
33	0	0	0	0	0	0	0	4	0	10	9	0
38	0	0	ND	0	0	ND	0	4	0	10	9	ND

^AGroups of 10 chickens received cell lysates of either FWPV rMN97X-infected, FP-VAC-infected, MN97-infected, or uninfected cells and at 24 days post-primary-inoculation were challenged with FWPV NE-92.

^BNumber of birds having lesions at site of inoculation.

^CNumber of birds having lesions at locations other than sites of inoculation.

^DNumber of birds having FWPV viremia detected using PCR. The size (kb) of the resultant amplicon is in parentheses.

^END = not determined.

Singh *et al.*, 2005

Table 3. Characterization of the response of chickens to inoculation with either QT-35 cell lysate (control) alone or containing FWPV rMN97X or FP-VAC or MN97.

Anti-REV antibody positive ^a		
Post-challenge		
0/9	0/9	0/9
0/9	0/9	0/9
0/9	0/9	0/9
7/9	7/9	7/9
0/9	0/9	0/9
0/9	0/9	0/9

Chicken serum were considered positive.

Singh *et al.*, 2000

Avian Pathology (19

An outbreak of breeder vaccine co

A. M. FADLY

Fadly *et al.*



22.10.2020

Endemic outbreaks of cutaneous fowlpox in Austria

11

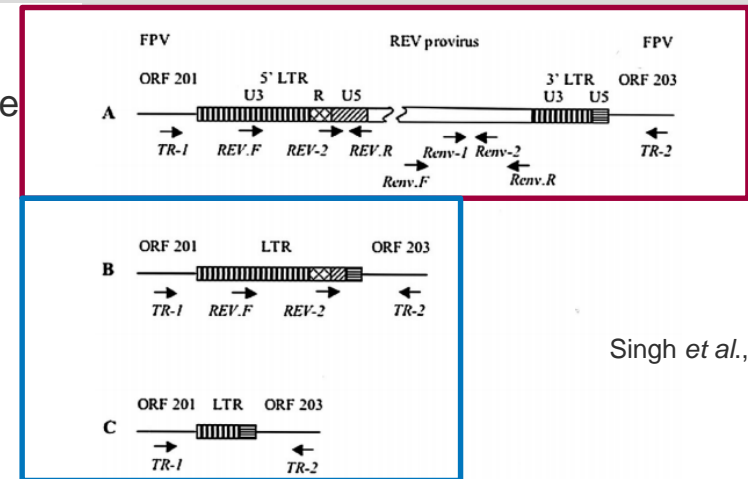
Significance of REV insertions

■ FWPV field strains

- usually carry a full copy of the RE provirus in the same locus, between ORF 201 and ORF 203

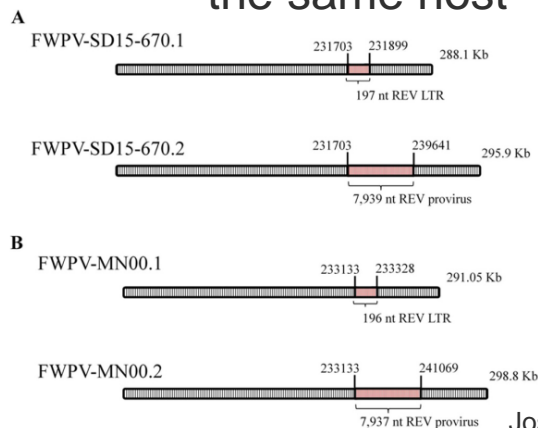
■ Strains passaged in the lab or commercial vaccine strains

- lost most of the provirus
- sometimes have just a single long terminal repeat sequence

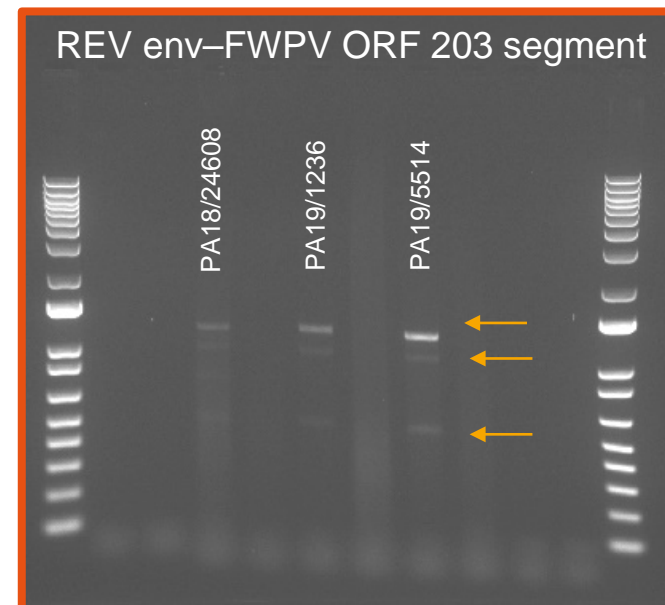


Singh *et al.*, 2003

■ Heterogeneous FWPV population in the same host



Joshi *et al.*, 2019



Significance of REV insertions

- Tendency to lose the REV sequence due to intramolecular homologous recombination between its flanking LTRs
 - nonetheless, (partial) retention of REV genetic material reflects its importance to FWPV survival in its host
 - advantage is conferred while REV is integrated?
 - or due to REV-induced immunosuppression after being released from the FWPV genome?



Summary and conclusions

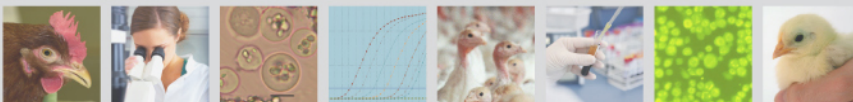
- Epizootic outbreak of fowlpox in naïve layers lasting several months
 - diagnosis in the field was made upon clinical signs and pathomorphological lesions of the skin
 - a mean drop in egg production of 6% was observed in the disease flocks
 - weekly mortality increased up to 1.2%
 - both geography and type of farms affected probably only reflect the organization of the Austrian poultry industry

- The detection of FWPV in the dust highlights its environmental persistence
 - FWPVs can survive in dried scabs for months (Tripathy and Reed, 2020)
 - movement of people, vehicles, equipment can, therefore, lead to the dissemination and perseverance of the disease in the field



Summary and conclusions

- Whole genome sequence analysis revealed high similarities with previously sequenced FWPV field strains
- The role of the RE provirus in the FWPV genome is still unresolved
 - immunosuppression induction in the host?
 - increases virus fitness?



Acknowledgements



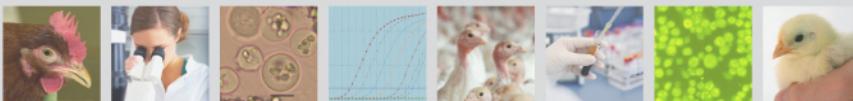
**Clinic for Poultry Medicine,
University of Veterinary Medicine
Vienna, Vienna, Austria**



Dr. Peter Mitsch

**Geflügeltierärzte
GmbH**

Dr. Franz Sommer



Thank you!

