

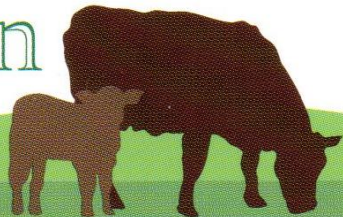


# Seroprevalence of Pestivirus infections in Belgian small ruminants

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Study Report

VetoGreen



Jean-Baptiste HANON – 22 September 2020

[jb.hanon@vetogreen.com](mailto:jb.hanon@vetogreen.com)

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- Lab tests
- Stats : seroprevalence (animal and flock level) , risk analysis

## ➤ Results

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- Tests performances
- Risk analysis : presence of cattle

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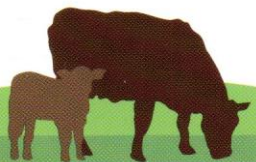
# Context

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## ➤ General context :

- Belgium small ruminants **population** (census 2016):  
86000 sheep (2500 flocks) ; 51000 goats (725 flocks)
- **Pestivirus** genus includes several virus species responsible of major animal diseases :  
→ **BVD** in cattle, **Border disease** in sheep, **Classical swine fever** (CSF) in pigs
- However : BVDV-1 and 2 and Border disease virus (BDV) can infect cattle **AND** small ruminants
- National **eradication plan** of BVDV in cattle is in the final stage
- Previous studies in other EU countries : Moderate to high seroprevalence of pestivirus in small ruminants → **Flock-level: 30-100%** ; **animal-level 5-75%**



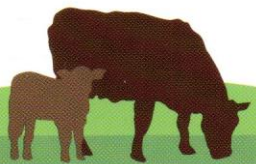
# Context

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## ➤ Objectives and design of the study :

- Estimate Pestivirus **Seroprevalence** in sheep and goats : flock level and animal level
- Evaluate **Risk** of Pestivirus transmission between small ruminants and cattle
- based on Detection of pestivirus **antibodies in serum and milk samples** + PCR test
- **Sample size** calculated on a design prevalence of 50% (flock) and 15% (within-flock)
- → study funded by **FASFC** and implemented by **NRL for pestivirus (Enzoveb-Sciensano)**



# Material and Methods (samples)

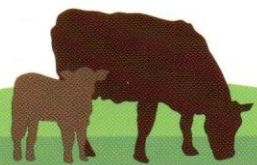
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## ➤ Serum samples

- non dairy sheep and goats flocks
- collected in 2018 for Brucellose screening (flocks in the Visna-maedi certification scheme)
- **7460** samples (animals > 1 year) from **410 flocks** (sheep : 344 ; goats 66)
- Average ~ 18 samples/flock
- sent to NRL by **ARSIA and DGZ** (50/50)
- + collection date, flock ID

## ➤ Bulk tank milk samples (BTM)

- collected in **dairy** sheep and goats flocks (Q fever surveillance program)
- **223** BTM in **2 rounds** : Nov 2019 (n= 144) + March 2020 (n=79)



# Material and Methods (Lab tests)

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## ➤ Antibody detection

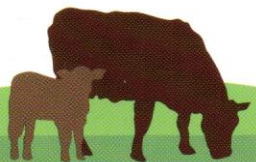
- All sera tested by BVD/BD **Ab ELISA (IDvet)** (n=7460)
- POS or NI sera tested by 2<sup>nd</sup> **Ab ELISA (Priocheck)** (n= 68)
- **Virus Neutralization Test (VNT) for BVDV-1, BVDV-2 and BDV**  
→ on POS/NI sera and on some NEG sera (n=146)
- All **BTM** tested by Ab ELISA (IDvet)

## ➤ Real-time PCR

- all Flocks with at least 2 seropositive animals (n=11) → all sera tested by PCR (n=547, pools of 10)
- all BTM positive in Ab ELISA (n=30) tested by RT-PCR

## ➤ Difficulties with milk samples

- Poor quality of milk samples at 2<sup>nd</sup> round (delay +++ collection-testing)
- Insufficient milk volume for optimal PCR extraction (100 µl instead of making cellular pellet)





# Material and Methods (Stats 1)

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## ➤ Animal seroprevalence

- All animals POS or NI in Ab ELISA (IDvet) = seropositive

## ➤ Flock seroprevalence : proportion of “exposed” flocks

- all Flocks with at least 1 seropositive animals = “exposed”

- 4 classes :

- **Neg** : no animal within the flock tested positive or doubtful in Ab ELISA
- **++** : at least 2 animals positive in Ab ELISA
- **+** : 1 animal positive in Ab ELISA
- **±** : 1 or more animals doubtful in Ab ELISA

} “**exposed**” flocks

- For dairy herds : exposed flock if BTM Pos in Ab ELISA : proportion of positive BTM

## ➤ Within-flock seroprevalence : proportion of seropositive animals in an exposed flock



# Material and Methods (Stats 2)

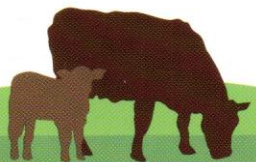
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## ➤ Risk Analysis :

- Association between **Pestivirus in small ruminants** and **presence of cattle** in the same farm
- For each flock : Presence or absence of cattle in the farm between 2013-2018 (SANITEL)
- Proportion of exposed flocks in mixed farms compared to farms holding only small ruminants
- Odds-Ratio (OR) : risk for a flock to become seropositive if cattle was present

## ➤ GIS maps : localize sampled flocks

- Geographical coordinates (Long. Lat.)
- Pestivirus status





# Results *(sera)*

## ➤ Animal Seroprevalence

	Tested	Neg	Pos	Doubt	Pos + Doubt	Prevalence	95% CI
Flanders	3810	3782	17	11	28	0.73 %	[0.51 % -1.06 %]
Wallonia	3650	3610	33	7	40	1.10 %	[0.81 % -1.49 %]
Total	7460	7392	50	18	68	<b>0.91 %</b>	[0.72 % -1.15 %]

- Belgium : ~1 %
- Wal > Vland ? No significant difference between 2 regions



# Results *(sera)*

## ➤ Flock Seroprevalence (Non dairy)

By region →

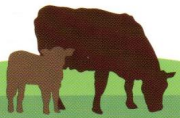
	Tested	Neg	++	+	±	Exposed (++/+/±)	Flock Prevalence	95% CI
Flanders	227	211	5	4	7	16	7.0 %	[4.4 % -11.1 %]
Wallonia	183	163	6	9	5	20	10.9 %	[7.2 % -16.3 %]
<b>Total</b>	<b>410</b>	<b>374</b>	<b>11</b>	<b>13</b>	<b>12</b>	<b>36</b>	<b>8.8 %</b>	<b>[6.4 % -11.9 %]</b>

By species →

	Tested	Neg	++	+	±	Exposed (++/+/±)	Flock Prevalence	95% CI
Goats	66	63	2	1	0	3	4.5 %	[1.6 % -12.5 %]
Sheep	344	311	9	12	12	33	9.6 %	[6.9 % -13.2 %]
<b>Total</b>	<b>410</b>	<b>374</b>	<b>11</b>	<b>13</b>	<b>12</b>	<b>36</b>	<b>8.8 %</b>	<b>[6.4 % -11.9 %]</b>

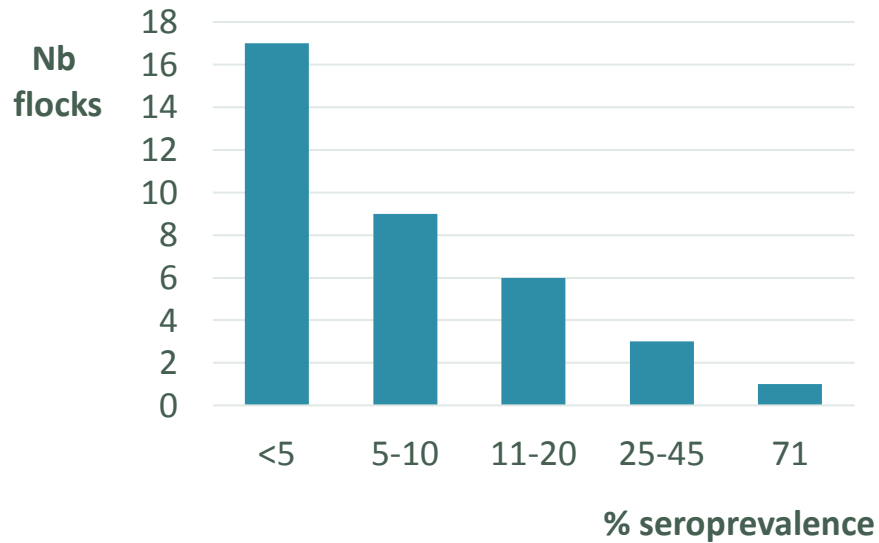
- Belgium : ~ 9 % flocks exposed to Pestivirus
- Wal > Vland ? Sheep > Goats ? → No significant difference between 2 regions and between 2 species

➤ **RT-PCR** : all samples from ++ flocks were **negative** (pools of 10)



# Results *(sera)*

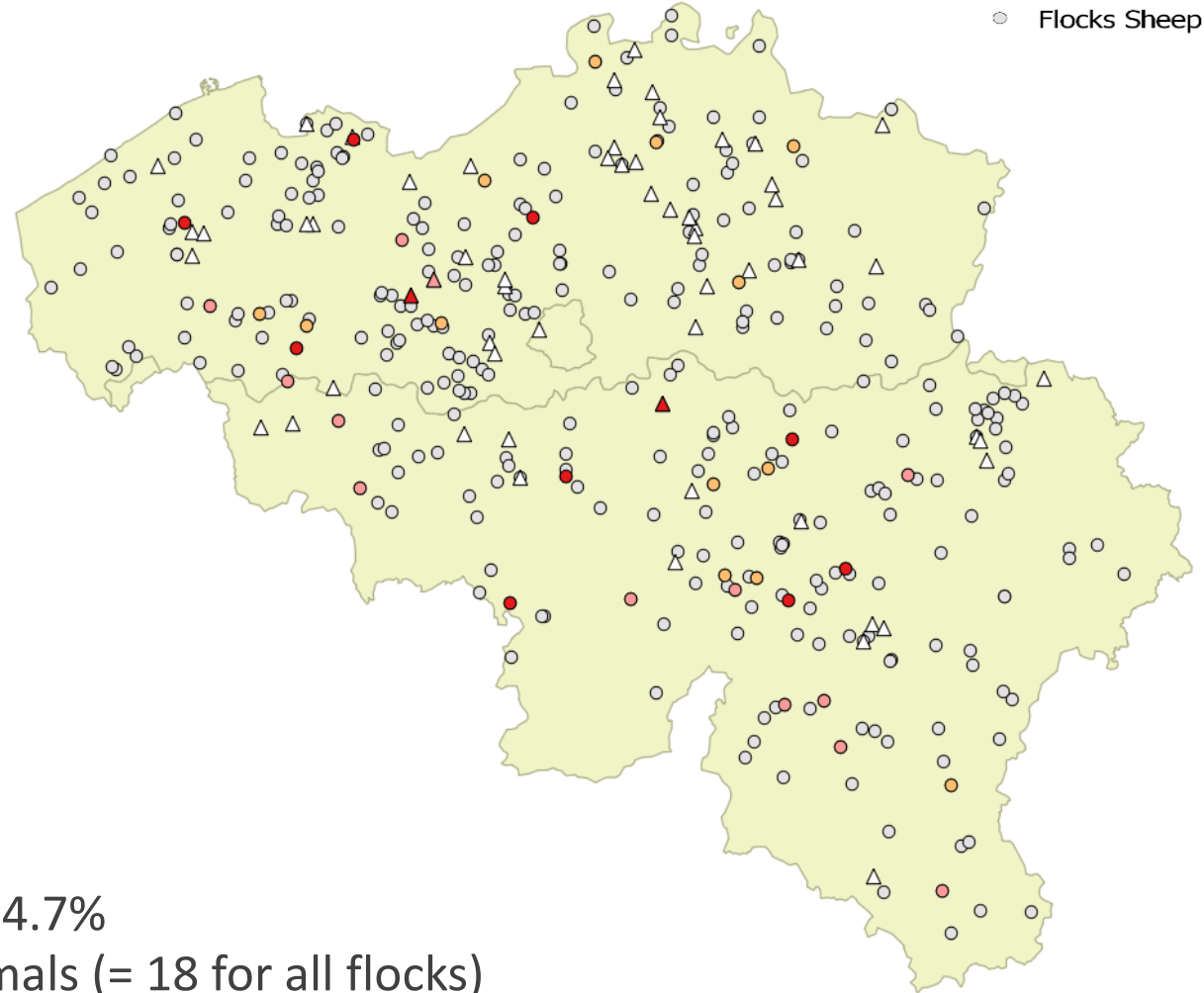
## ➤ Within-flock Seroprevalence in exposed flocks (n=36)



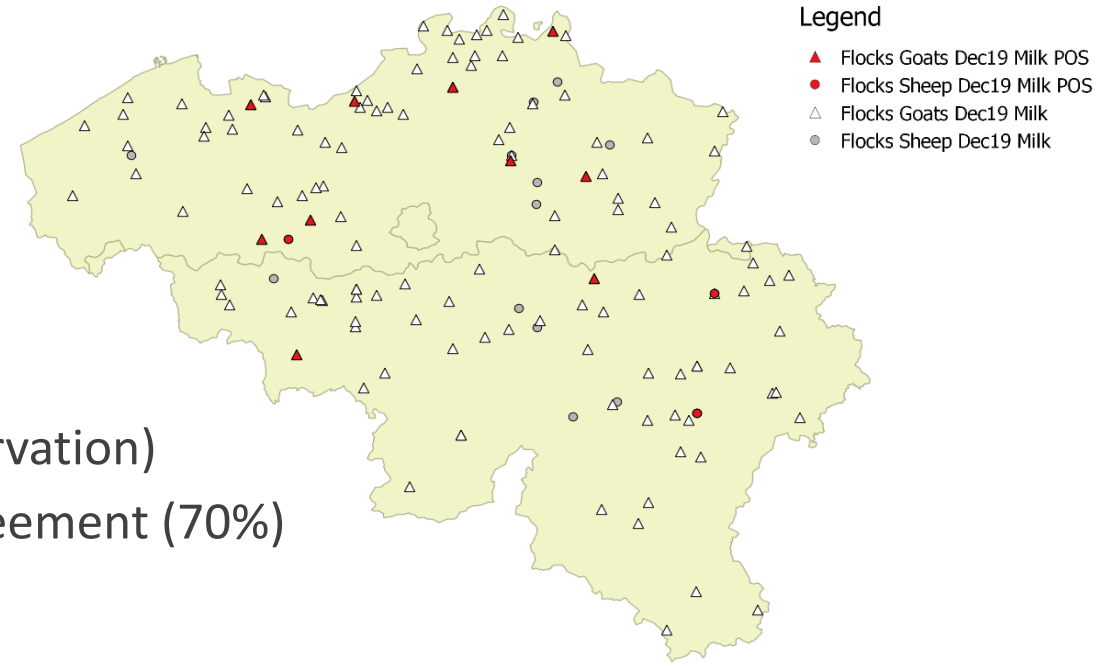
- Average prevalence= 9.9 %    Median = 4.7%
- Average size of exposed flocks = 38 animals (= 18 for all flocks)

### Legend

- ▲ Flocks Goats ++
- △ Flocks Goats +
- Flocks Sheep ++
- Flocks Sheep +
- Flocks Sheep +/-
- △ Flocks Goats 2018
- Flocks Sheep 2018



# Results *(Milk)*



## ➤ Flock prevalence *(dairy)*

- 2<sup>nd</sup> round : unreliable results (poor sample conservation)
- 54 flocks were tested at both rounds → poor agreement (70%)
- Belgium ~ 10 % Ab positive BTM

Period	N samples	NEG	POS	% POS	95% CI
November 2019	144	130	14	<b>9.7%</b>	[5.9 % -15.7 %]
March 2020	79	63	16	(20.3%)	[12.9 % -30.4 %]

## ➤ RT-PCR

- Performed on Ab positive BTM (n=30): all **negative** (but sub-optimal extraction method)



# Results *(sera)*

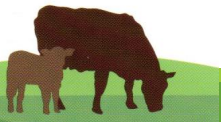
## ➤ ELISA comparison

- Poor agreement between IDvet and Prionics ELISA
- Poor agreement between Prionics ELISA and VNT (lack of Sensitivity)
- Good agreement between ID vet ELISA and VNT (n= 128/146) but VNT less sensitive

## ➤ VNT titers: Pestivirus characterization : based on VNT titer

- Samples POS/NI in AbELISA
- N= 68 → 20 characterized
- BVD-2 > BVD-1, BDV

	VNT results				
Ab ELISA (IDvet)	BDV	BVDV-1	BVDV-2	Undetermined pestivirus	VNT Negative
POS (n=50)	5	4	8	27	6
Doubt (n=18)	0	0	3	3	12
Total (n=68)	5	4	11	30	18



# Results *(Risk analysis)*

## ➤ Association between Seropositivity and Presence of cattle

		Pestivirus seropositivity		
		Pos	Neg	
Contact with Cattle herd	Yes	14 (15,2%)	78	92
	No	22 (6,9%)	296	318
		36	374	410

- More Ab positive sheep/goat flocks if cattle present in the farm in the last five years
- **OR = 2.54** (95%C.I. [1.18 - 4.94])
- For dairy flocks : no significant difference in % Ab positive BTM if cattle present (**OR = 1,03**)
- 22.4 % (92/410) of the flocks in this survey were kept in farms with cattle :  
→ extrapolation at country level : ~ 720 farms (22% x 3222) are mixed farms holding sheep/goats + cattle



# Conclusion and Recommendations

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## 1) **Low Pestivirus seroprevalence** in small ruminants in Belgium :

- Animal seroprevalence <1%
- Flock prevalence ~10% (« exposed » flocks) in dairy and non-dairy farms
- Within flock seroprevalence < 5% in 50% of exposed flocks
- → low exposure of small ruminants to pestivirus
- No pestivirus detected by RT-PCR in exposed flocks
- No significant regional differences (Wal > Vland ?)

## 2) **ELISA, VNT and RT-PCR results**

- Good performances of **IDVet Ab ELISA** (Se, Sp); low performance of **Priocheck**
- Exposure of small ruminants to several Pestivirus species (**BVDV-1, BVDV-2, BDV**)
- **Milk samples** need good conservation conditions, fast flow of samples, specific techniques (PCR)





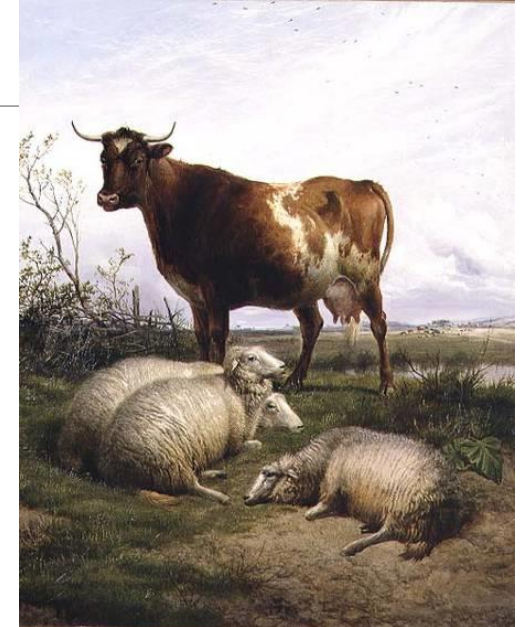
# Conclusion and Recommendations

## 3) Risk analysis : increased risk to be exposed to Pestivirus if

- larger flock size
- presence of cattle (OR 2.5) in non-dairy flocks

## 4) Low Pestivirus seroprevalence

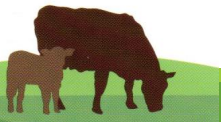
- → Low circulation of Pestivirus in small ruminants
- → Low risk of contaminating BVD-free cattle herds
  - → cross-contaminations cannot be ruled out
    - → Pestivirus surveillance in small ruminants farms where cattle is also kept (~ 700 farms) ?  
(awareness of farmers and veterinarians)



# Acknowledgements

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- ❑ **Brigitte Cay**, Head of the Service “Enzootic, vector-borne and bee diseases” (Enzoveb) at Sciensano, who promoted the study and supervised the laboratory work;
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# Questions ?

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