



Actualities in small ruminants: Evolving old stories and new challenges

September 22th, 2020

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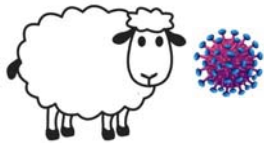
Outline

- 1. Small ruminant lentiviruses in Belgium**
2. Recent detections of prion disease in sheep
3. Sheep as sentinel for tick-borne encephalitis virus

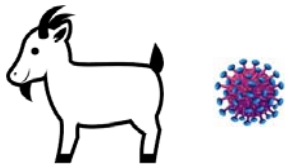
Small ruminant lentiviruses

Family: Retroviridae - Subfamily: Orthoretrovirinae - Genus: Lentivirus - ss(+) RNA virus

Until 1990



Maedi-Visna virus (MVV)

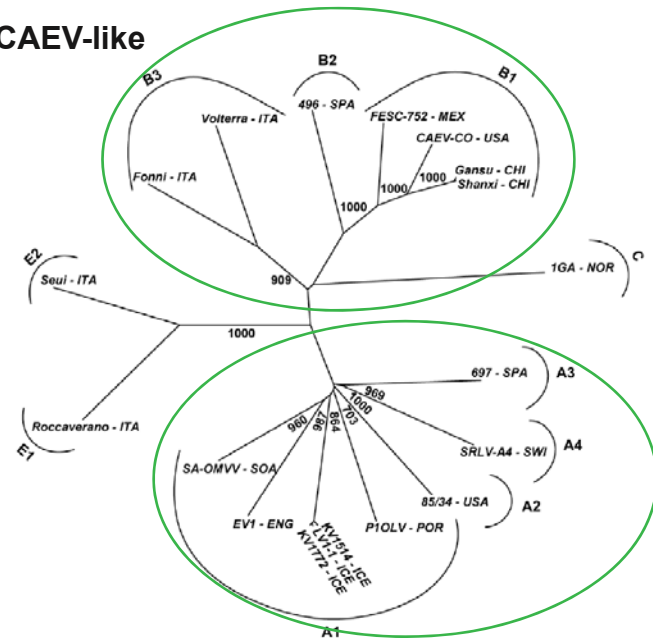


Caprine arthritis encephalitis virus (CAEV)



Present: SRLV
5 genotypes

Genotype B = CAEV-like



Genotype A = MVV-like

Cross-species transmission detected under natural and experimental conditions

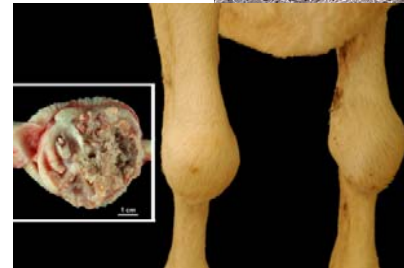
- Necessity for control program in both sheep and goats
- Impact on diagnostic tests used in control programs

Symptoms

- Clinical symptoms take years to develop
- Important part of infected animals remain asymptomatic

- repeated cycles of virus reactivation and latency, together with associated immune responses cause clinical symptoms in the long term

- **Lung: pneumonia, difficult breathing**
- **Brain: encephalitis, nervous symptoms**
- **Mammary gland: mastitis**
- **Joints: arthritis**
- **Weight loss**



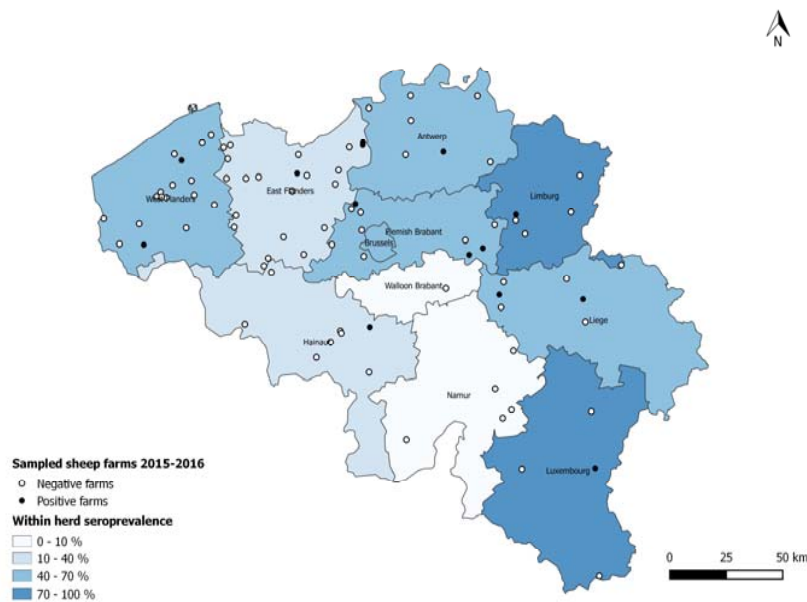
- So:
- concerns for animal welfare
 - SRLV infections cause economic losses (lower milk quality and production, reduced conception ratio, ...)
 - export limitations

⇒ Voluntary control program in Belgium

SRLV seroprevalence in Belgium

SHEEP

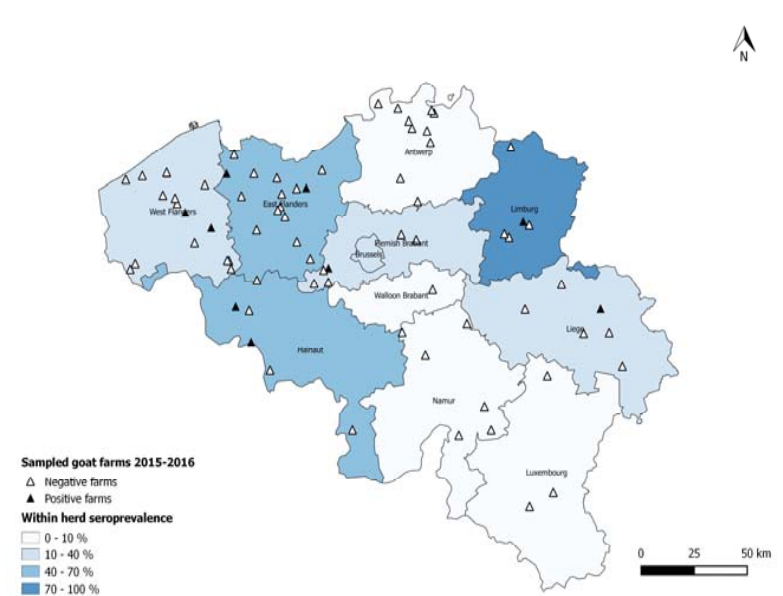
- 87 participating farms (non-certified)
- 555 serum samples



- overall seroprevalence: 9 %
- between-herd seroprevalence:17%
- positive farms all across the country

GOATS

- 76 participating farms (non-certified)
- 401 serum samples

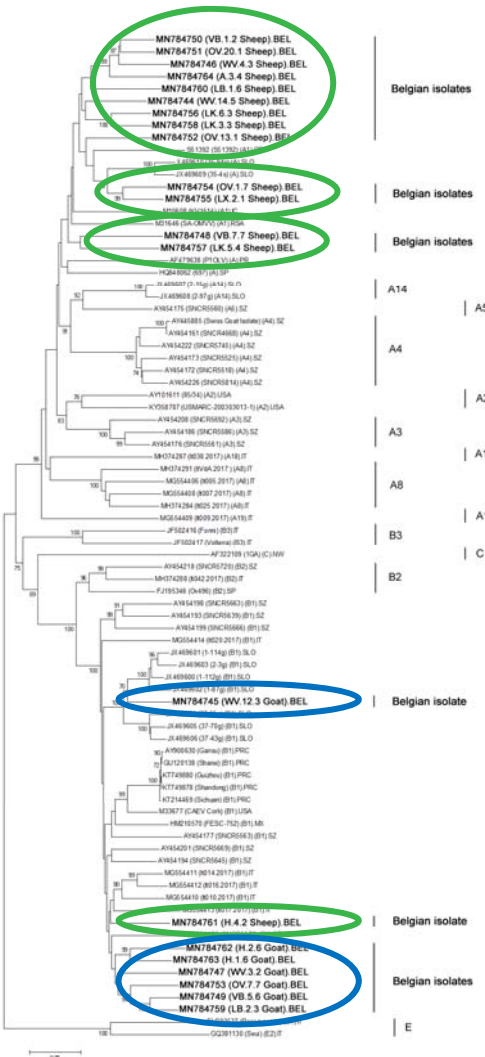


- overall seroprevalence: 6 %
- between-herd seroprevalence:13%
- positive farms all across the country

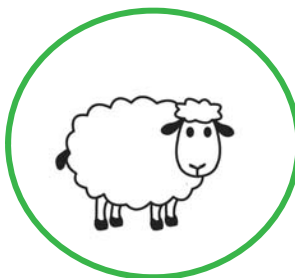
SRLV certified farms are at risk when contact with animals from non-certified farms occurs

Phylogeny of Belgian SRLV strains

Gag-Pol region (1500bp)

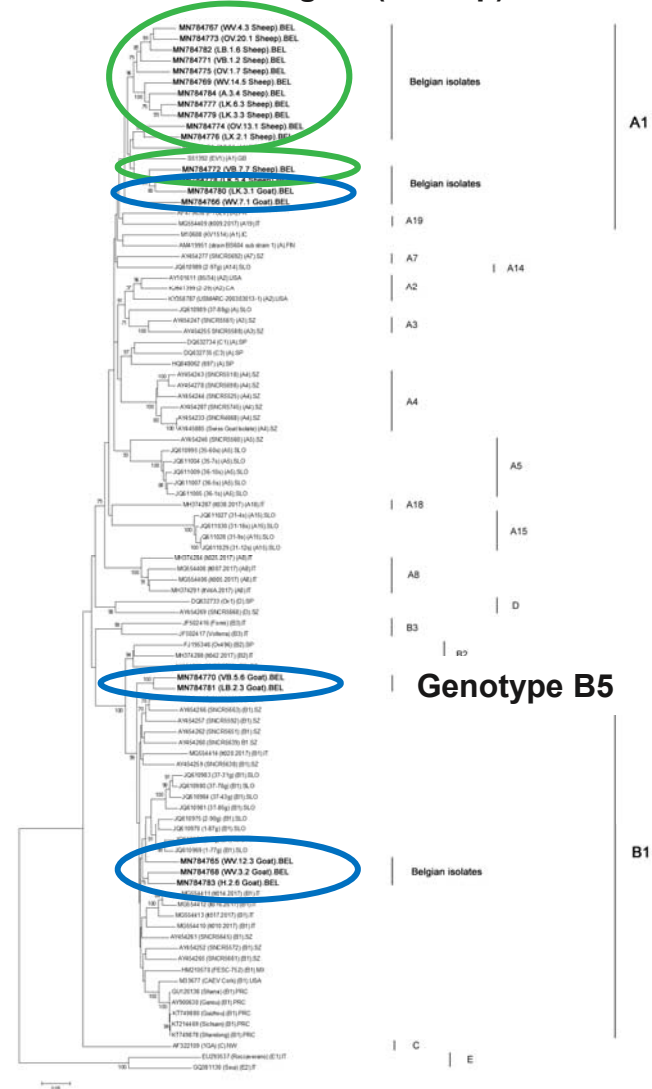


A=MVV-like



B=CAEV-like

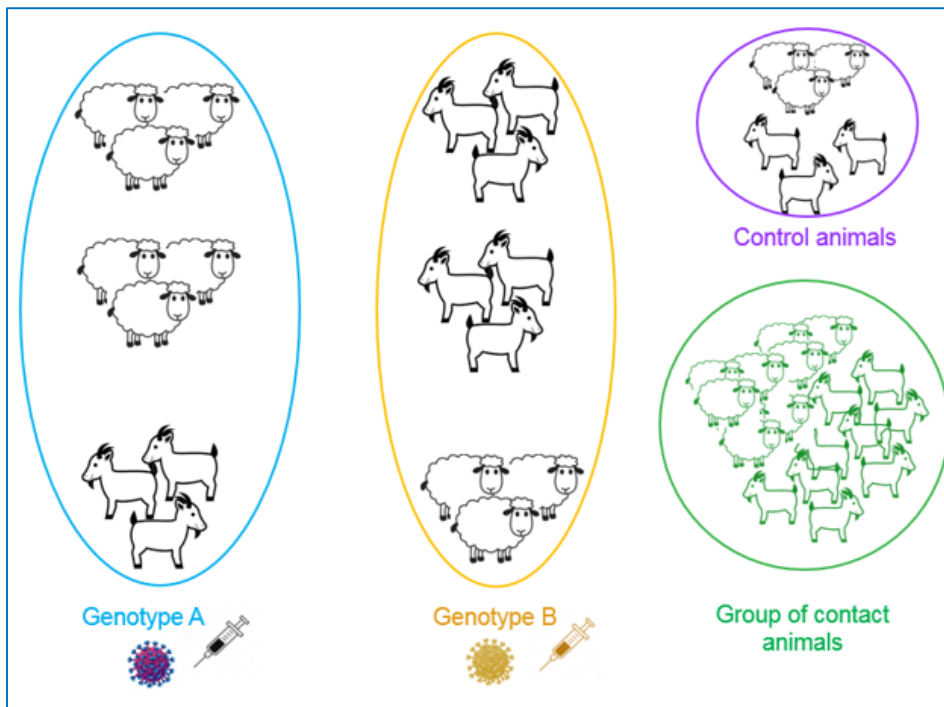
Pol region (1000bp)



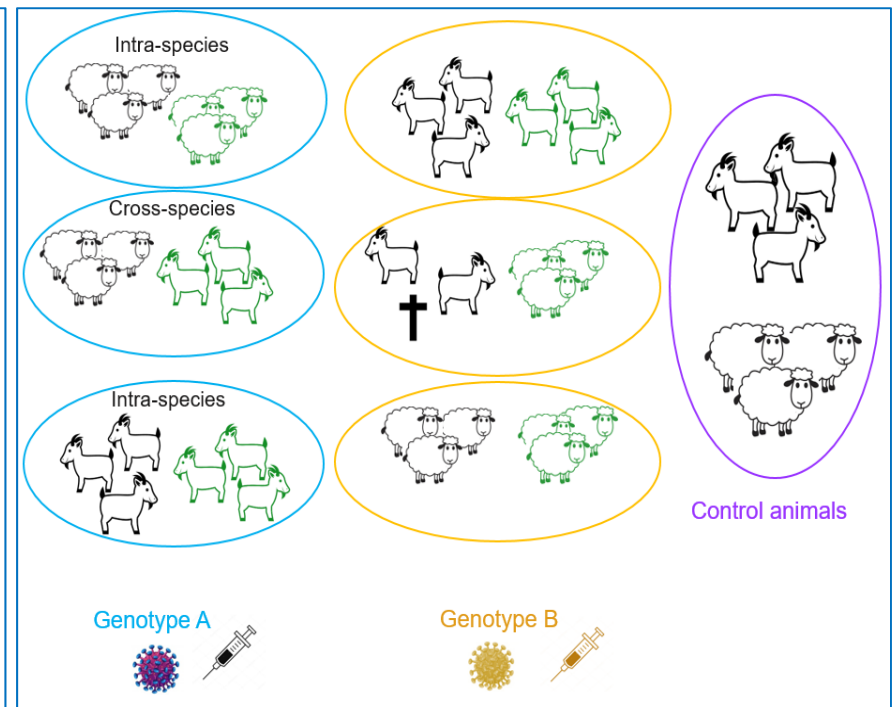
Evidence of cross species transmission in Belgium

Intra-species and cross-species transmission of Belgian SRLV strains

At start



After 1 month



↳ Serum and blood sampling for 9 months + autopsy

GOALS

- To analyse **intra-species** and **cross-species transmission** after experimental infection of sheep and goats with Belgian SRLV strains.
- To study differences in **pathogenesis** and induced **humoral** and **cellular immune responses**

Intra-species and cross-species transmission of Belgian SRLV strains

- **No SRLV transmission to any of the contact animals could be evidenced by serology and qPCR over the 9 months' time period → inefficient horizontal transmission under experimental conditions**
 - 15 out of 17 experimentally inoculated animals had successfully seroconverted
 - SRLV infection is detected earlier by qPCR than by serological methods
 - ELISA detects SRLV Abs prior to AGIDs in most of the animals
 - No differences in time to seroconversion was found between sheep and goats inoculated with a genotype A or B

Voluntary SRLV control program Belgium < 2019

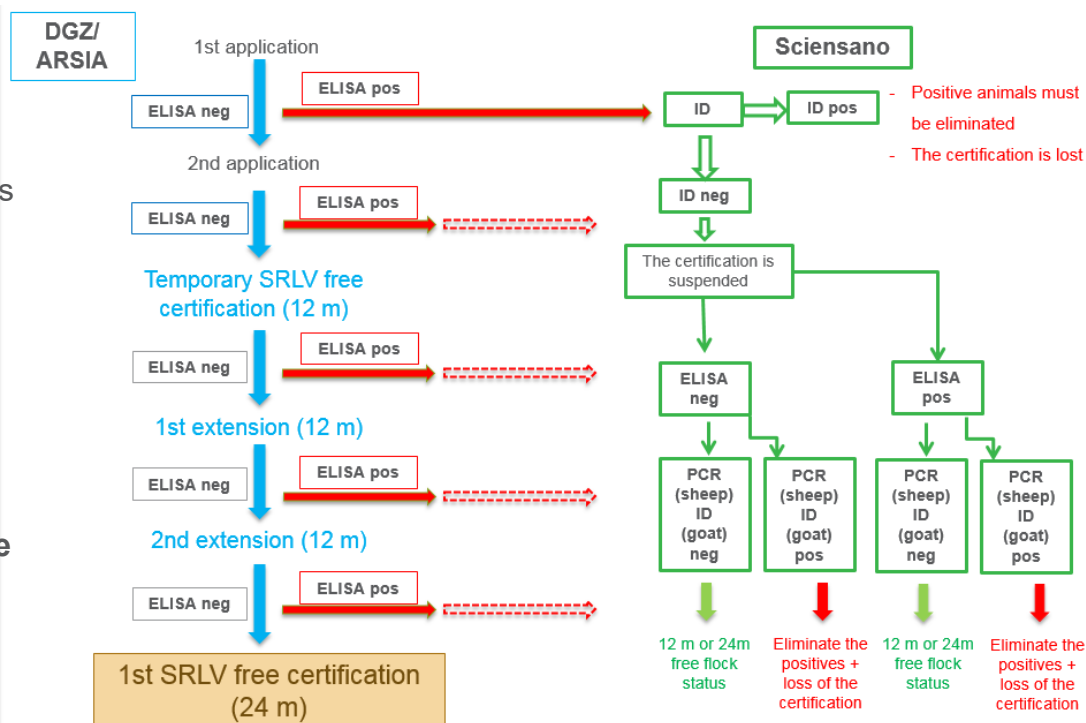
- Voluntary → less than 2% sheep/goat farmers currently participate

- 3 tests were used:

1) ELISA (Elitest)

2) AGIDs + qPCR → confirmation of positive

ELISA results



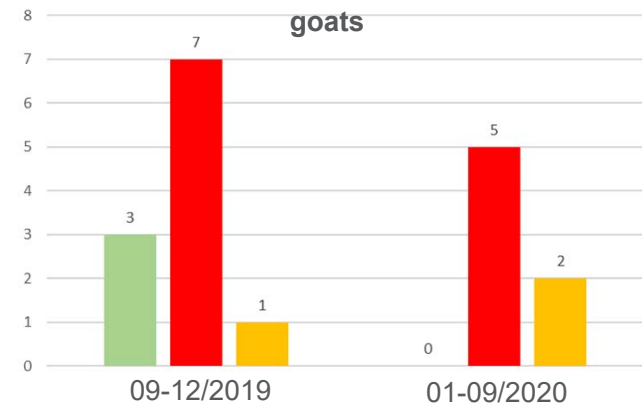
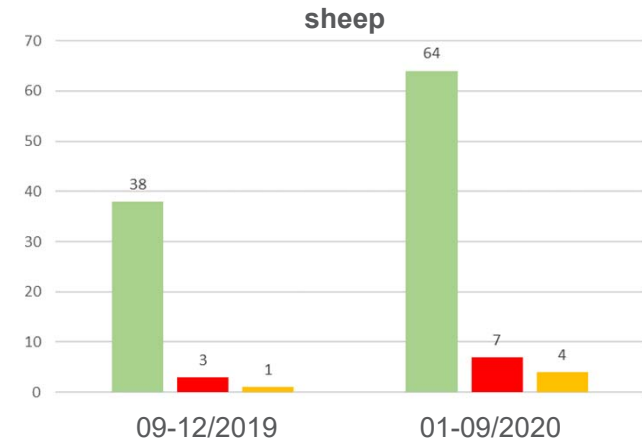
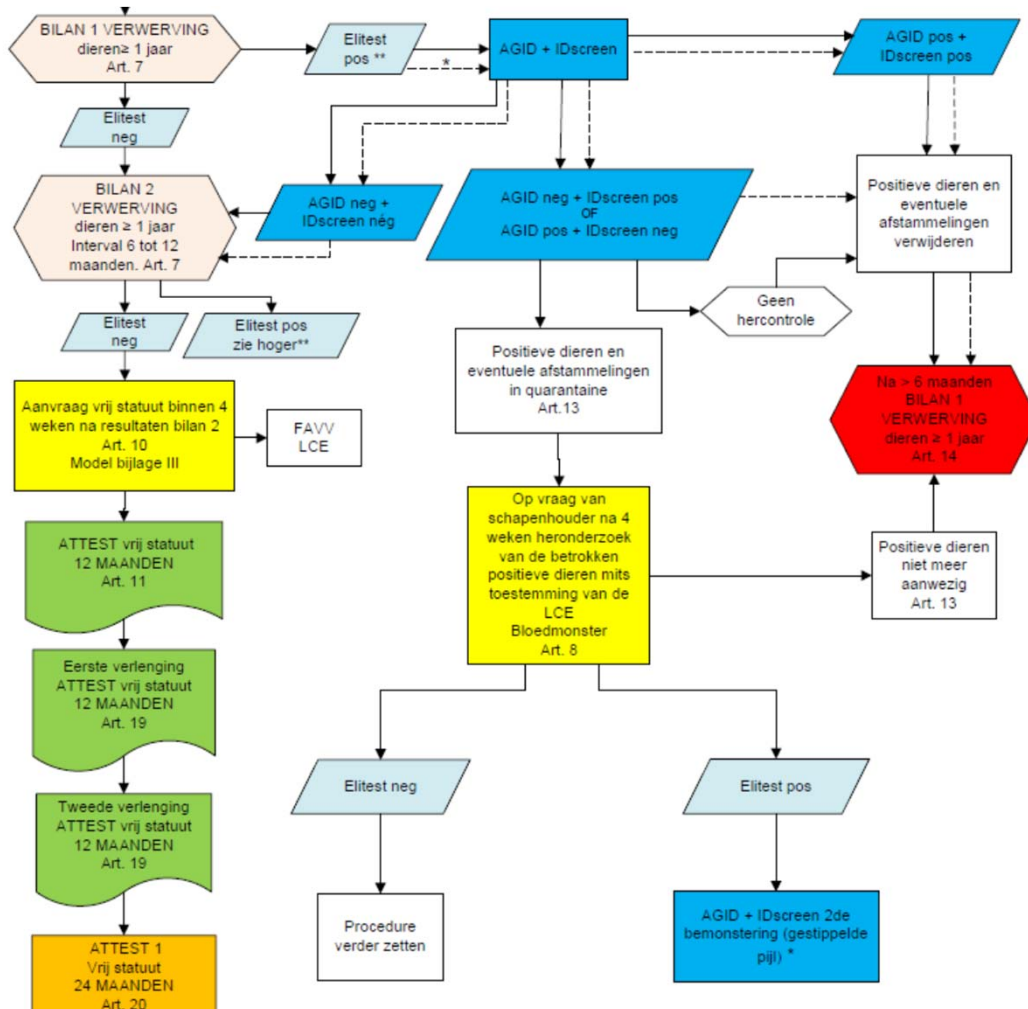
➤ Problems:

- False positive results in ELISA
- Limited sensitivity of AGID test during confirmation
- Necessity for 2th blood sampling for confirmatory PCR test (time – cost)
- Suboptimal sensitivity of PCR test

Comparative analysis of SRLV diagnostic kits

	Infection status ^a			Sheep (553)			Infection status ^a			Goats(394)			
		+	-	Total	Sensitivity	Specificity	PPV/NPV ^c	+	-	Total	Sensitivity	Specificity	PPV/NPV ^c
Agar Gel Immunodiffusion													
AGID CAEV P28 kit (Idexx)	+	18	0	18	35,3%	100,0%	100,0%	18	0	18	75,0%	100,0%	100,0%
	-	33	502	535			93,8%	6	370	376			98,4%
Maeditect kit (Apha Scientific)	+	51	0	51	100,0%	100,0%	100,0%	21	0	21	87,5%	100,0%	100,0%
	-	0	502	502			100,0%	3	370	373			99,2%
Total AGIDs ^b	+	51	0	51	100,0%	100,0%	100,0%	24	0	24	100,0%	100,0%	100,0%
	-	0	502	502			100,0%	0	370	370			100,0%
ELISAs													
Elitest MVV/ CAEV (Hyphen BioMed)	+	50	4	54	98,0%	99,2%	92,6%	23	1	24	95,8%	99,7%	95,8%
	-	1	498	499			99,8%	1	369	370			99,7%
MVV/ CAEV p28 Ab screening test (Idexx)	+	43	2	45	84,3%	99,6%	95,6%	22	0	22	91,7%	100,0%	100,0%
	-	8	500	508			98,4%	2	370	372			99,5%
ID screen [®] MVV/CAEV indirect (IDVet)	+	51	11	62	100,0%	97,8%	82,3%	24	9	33	100,0%	97,6%	72,7%
	-	0	491	491			100,0%	0	361	361			100,0%
LSIVet [™] Ruminant Maedi-Visna/CAEV serum ELISA kit (LSI)	+	46	36	82	90,2%	92,8%	56,1%	24	53	77	100,0%	85,7%	31,2%
	-	5	466	471			98,9%	0	317	317			100,0%
Eradikit [™] SRLV screening test (IN3 diagnostic)	+	49	3	52	96,1%	99,4%	94,2%	24	20	44	100,0%	94,6%	54,5%
	-	2	499	501			99,6%	0	350	350			100,0%
Total number of infected/ free animal		51	502					24	370				
PCR													
Combined genotype A and genotype B qPCRs	Sheep (536)	+	44	0	44	88,0%	100,0%	100,0%					
		-	6	486	492			98,8%					
	Goats (387)	+	20	0	20	83,3%	100,0%	100,0%					
		-	4	363	367			98,9%					

Voluntary SRLV control program Belgium >2019



■ AGID+IDscreen neg
■ AGID+IDscreen pos
■ AGID neg - IDscreen pos

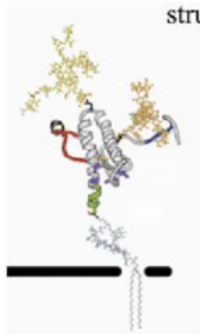
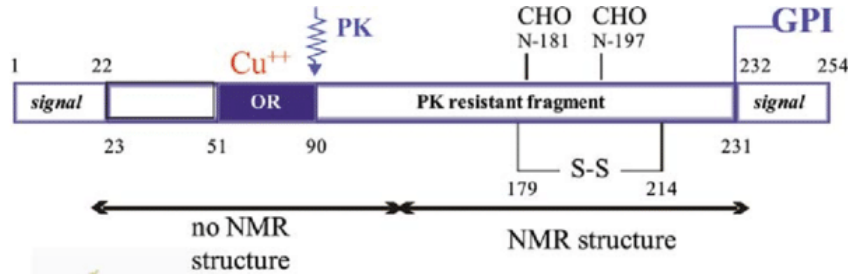
- Advantages:
 - Faster
 - Cheaper
- Remaining problems:
 - False positive results in ELISA



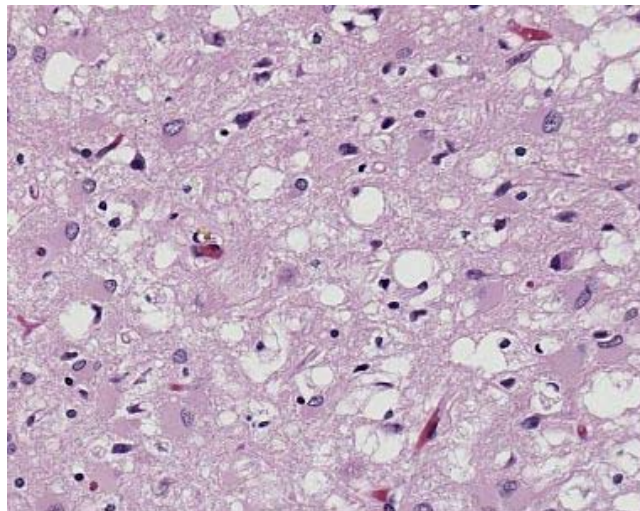
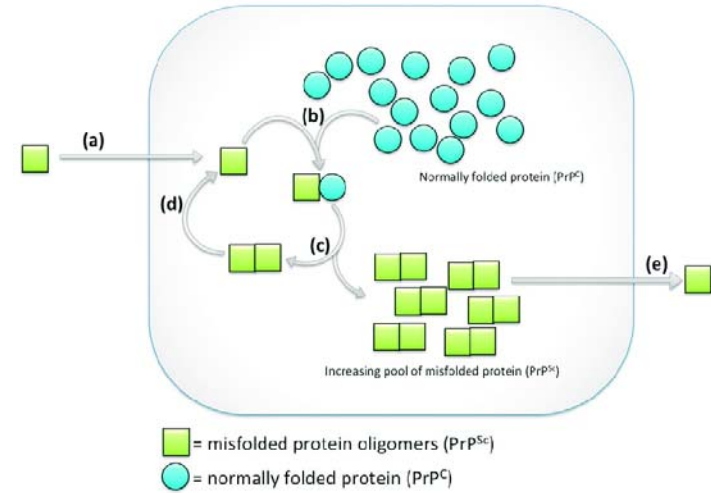
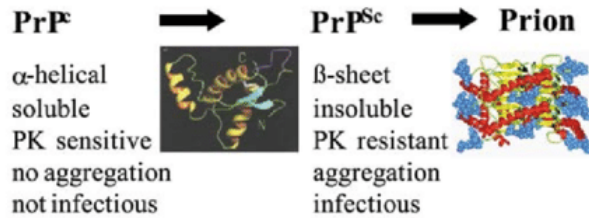
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Back-ground



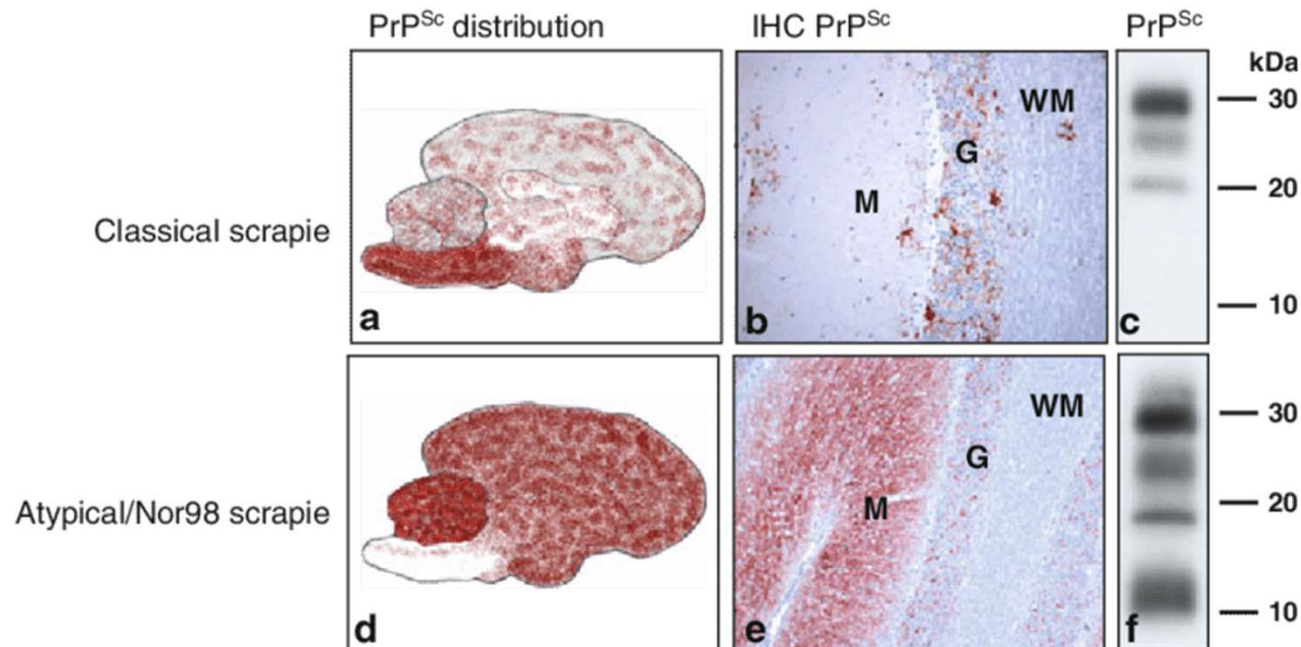
Biochemical properties



Brain damage
Spongiform encephalopathies
Fatal outcome after long incubation period

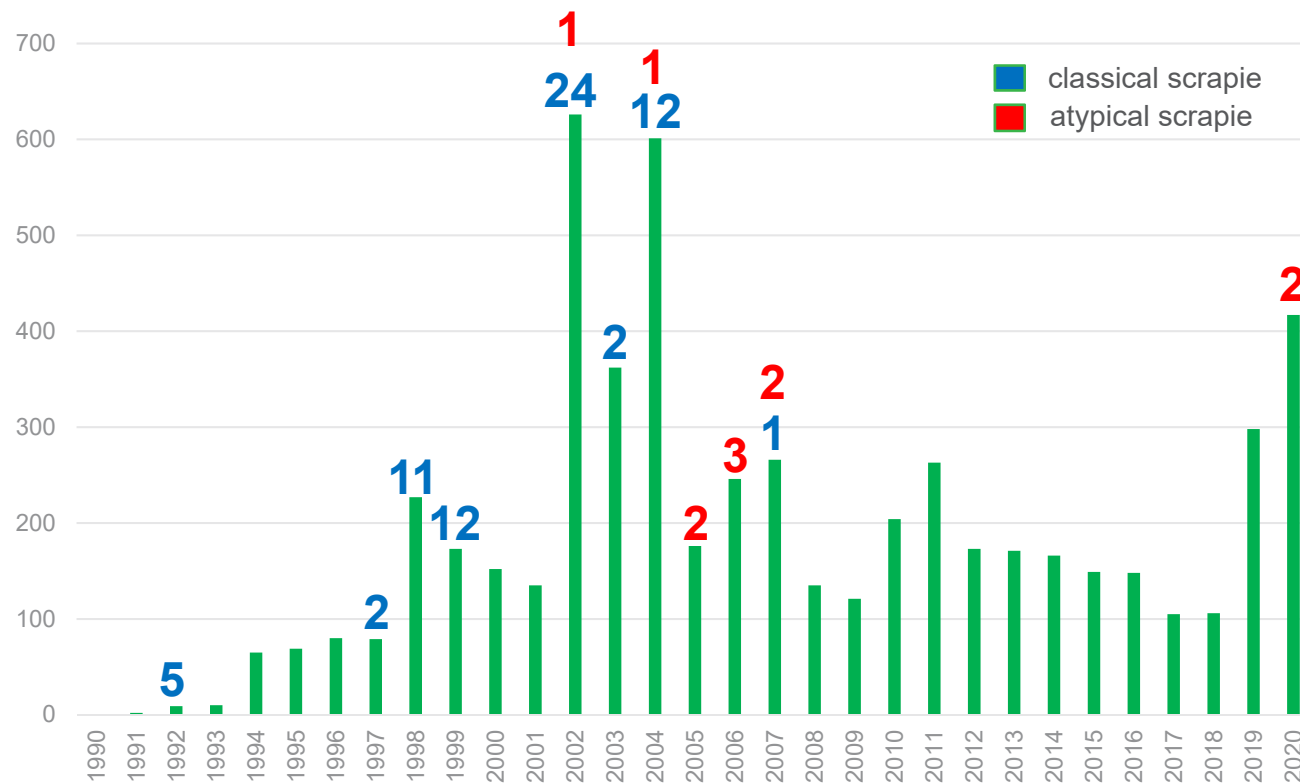
Classical scrapie vs atypical scrapie (Nor98)

Characteristics	Classical scrapie	Atypical scrapie
Age of onset natural disease	Between 2 and 5 years with average 3,5 years	On average 6,5 years old
symptoms	Behavioral change, tremor, pruritus and locomotor disorder	Weight loss, behavioral change, locomotor disorder (ataxia, circling)
Tissue distribution and infectivity	PrP ^{Sc} and infectivity occurs as constant feature Contagious	Not detected outside central nervous system or peripheral lymphoid system Non-infectious, only via artificial means



TSE analyses in small ruminants at Sciensano

- Dead animals for export certification
- Suspected animals at farm or ante-mortem at slaughterhouse
- Animals autopsied at regional labs or universities: diagnostic or suspicion
- Confirmation of fallen stock tested positive at Ecca-lab



Case studies

Case 1: February 2020, fallen stock, ewe, 5 years old, flock with 91 other ewes and 4 goats

Diagnosis: atypical scrapie

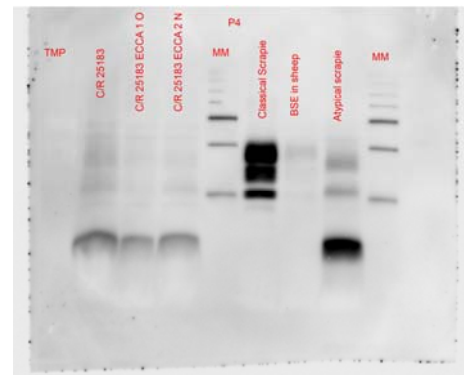
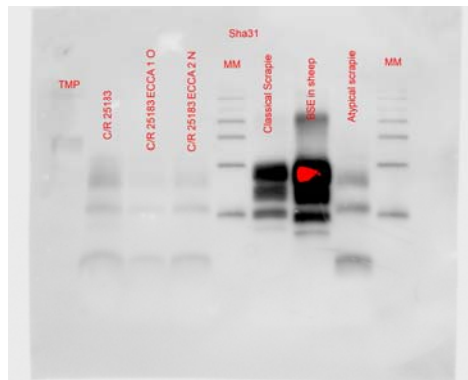
Case 2: July 2020, fallen stock, ewe, 7 years old, no neurological symptoms, unable to stand up, flock of 8 other ewes and 14 lambs

Diagnosis: atypical scrapie

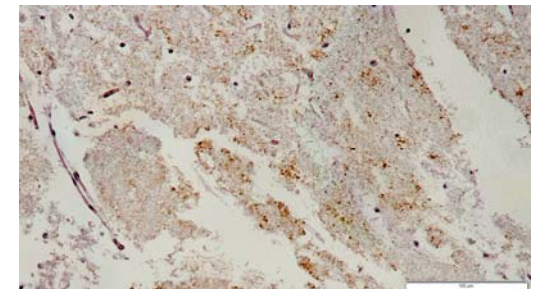
ELISA



Western blot



Immunohistochemistry



2.2.3. Bij bevestiging van atypische scrapie

Als het TSE-geval dat op een bedrijf is bevestigd een geval van atypische scrapie is, geldt voor het bedrijf gedurende twee jaar vanaf de datum waarop het laatste geval van atypische scrapie is aangetoond, het volgende protocol voor verscherpt TSE-toezicht: alle schapen en geiten die ouder zijn dan 18 maanden en voor menselijke consumptie zijn geslacht, en alle schapen en geiten die ouder zijn dan 18 maanden en op het bedrijf gestorven of gedood zijn, worden op de aanwezigheid van een TSE getest overeenkomstig de in bijlage X, hoofdstuk C, deel 3, punt 3.2, vastgestelde laboratoriumtechnieken en protocollen.

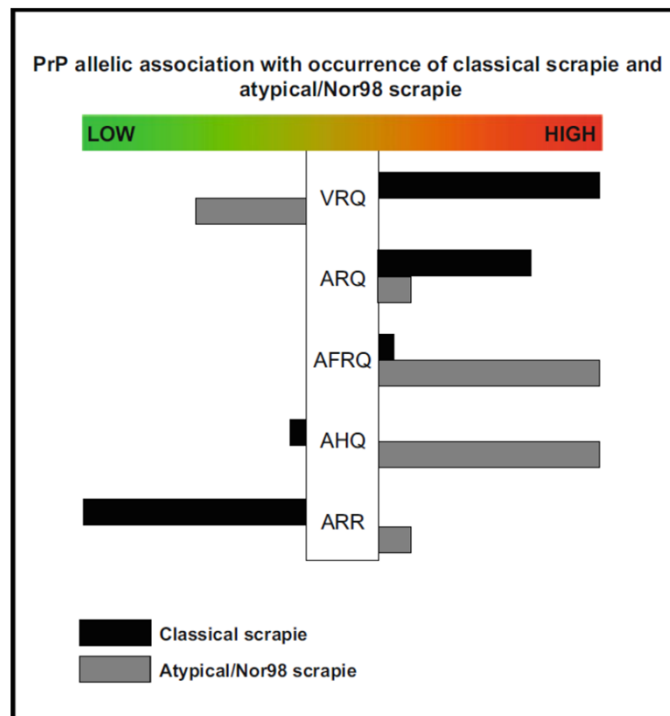
Case studies

Case 1: February 2020, fallen stock, ewe, 5 years old, flock with 91 other ewes and 4 goats

Genotyping: **AHQ/AHQ**

Case 2: July 2020, fallen stock, ewe, 7 years old, no neurological symptoms, unable to stand up, flock of 8 other ewes and 14 lambs

Genotyping: **AHQ/AHQ**





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TBEV in Belgium

Previous monitoring in animals: In Belgium, antibodies to TBEV have recently been reported in **cattle, wild boar, wild cervids** and a **dog** (Roelandt et al., 2011, 2014, 2016; Linden et al., 2012; Tavernier et al., 2015). The seroprevalence in cattle was estimated to be around 4% in the Eastern provinces of Belgium (Roelandt et al., 2014). No detection or isolation of the virus itself has been reported yet.

Human cases:

Kathleen Huys
vr 12 okt 2018 06:00



Tekenencefalitis nu ook in België?

Bij een Limburgse tuinbouwer en een jonge vrouw uit Gent is deze zomer tekenencefalitis (TBE of *tick-borne encephalitis*) vastgesteld. In ons land is nog nooit iemand besmet geraakt met het virus. Het Instituut voor Tropische Geneeskunde in Antwerpen vermoedt dat deze man en vrouw de eersten zijn, maar zeker is dat niet want allebei hebben ze van de zomer ook in het buitenland gereisd.

Tibopath project (2020-2021): Nationwide screening of ticks and ruminants for Anaplasma, Borrelia, Rickettsia, Babesia, and tick-borne encephalitis virus

TBEV in screening in sheep



The kit's cut-off

VIEU/ml	Interpretation
<63	neg
63-126	borderline
>126	Pos

The Concentration of the positive and borderline samples (VIEU/ml)

Province	Positive sample
OVLD	153,65
OVLD	145,15
LG	453,87

Province	Borderline samples
OVLD	106,88
ANT	71,45
LIM	78,04
LUX	95,74
HNT	77,23
LG	102
HNT	71,4
WBR	65,06
HNT	70,8
HNT	63,2

Results are being confirmed in virus neutralization tests

Acknowledgements

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Stefaan Ribbens (DGZ)



Service of enzootic, vector-borne and bee diseases
(Sciensano)

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Recherche contractuelle (RF19/01 Tibopath)

