SEROLOGIC FINDINGS IN ROE DEER IN FLANDERS (BELGIUM)

BELGIAN WILDLIFE DISEASE SOCIETY (BWDS)

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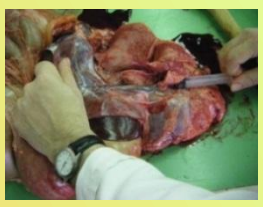
INTRODUCTION :

Roe deer are the only wild living native cervid species in Flanders. Their population number has increased steadily in the last twenty years and is estimated at 20,000 with the highest densities in the eastern provinces. Contacts between wild and domestic ruminants are facilitated by the small-scale Flemish landscape structure. In order to detect circulation of human and animal pathogens in roe deer in Flanders, a preliminary screening was carried out from May 2008 to September 2011.



MATERIALS AND METHODS :

Sampling: In 12 Flemish hunting areas, hunters collected blood samples from the v.jugularis, v.cava caudalis or hearth of roe deer, soon after the killing. Eleven samples were obtained during necropsy of culled or found-dead roe deer. One sample came from a sick roe deer calf in a rescue centre. For each animal sampled, the sex, the estimated age based on the tooth wear and the area of origin were recorded. The samples were cooled at 2-4°C, and on arrival at the lab were centrifugated for 10 min. at 4000 rpm. Decomposed samples were discarded. After dividing each serum in multiple Eppendorff tubes, the sera were kept at -20°C until analysing.

*Analysis: The sera were examined for antibodies to twelve infectious agents by means of the tests listed in **Table 1**. Statistics: Confidence intervals (CI in **Table 2**) for proportion of number positive (or suspected) versus number tested, were calculated. The log-likelihood ratio (LR in **Table 3,4,5**) test for contingency tables was used to analyse whether seropositivity was dependent on sex, age or geographical origin of the samples; p<0.05 indicates significant difference between sexes, ages or sampling regions.*

Table 1: Antibodies against	Test	Conjugate
Mycob. avium subsp. paratuberculosis	iELISA	horseradish peroxidase conjugated IgG (all species)
Brucella abortus	iELISA	horseradish peroxidase conjugated IgG (all species)
Leptospira sp.	MAT : 12 serovars (dilution 1/100)	
Coxiella burnetii	iELISA	horseradish peroxidase conjugated IgG (all species)
Anaplasma phagocytophilum	IFA	**anti-deer** fluorescein isothiocyanate conjugated IgG
Chlamydia abortus	iELISA (MOMP)	horseradish peroxidase conjugated IgG (all species)
Bovine Viral Diarrhoea (BVD) virus	VNT	
Bovine Herpes Virus 1 (BHV1, IBR)	VNT	
Bluetongue virus (BTV)	comp.ELISA	
Tick-borne Encephalitis (TBE) virus	iELISA	horseradish peroxidase conjugated IgG (all species)
Toxoplasma gondii	iELISA (SAG 1)	**anti-deer** horseradish peroxidase conjugated rabbit IgG
Neospora caninum	iELISA	horseradish peroxidase conjugated IgG (all species)

Table 2: Seroprevalences	n tested	n pos	% pos	95% CI low-high	n susp	% susp	95% CI low-high
Mycob. avium subsp paratuberculosis	115	6	5.2	1.9 - 11	3	2.6	0.5 – 7.4
Brucella abortus	73	0	0	0 - 4.9			
Coxiella burnettii	115	1	0.9	0.02 - 4.7			
Leptospira sp.	118	3	2.5	0.5 - 7.3			
Anaplasma phagocytophilum	82	46	56.1	44.7 - 67.0			
Chlamydla abortus	121	2	1.7	0.2 - 5.8	2	1.7	0.2 - 5.8
Bovine Viral Diarrhoea (BVD) virus	108	2	1.9	1.9 - 0.2			
Bovine Herpes Virus 1 (BHV1, IBR)	79	0	0	0 - 4.6			
Bluetongue virus (BTV)	115	0	0	0 - 3.2	4	3.5	1.0 - 8.7
Tick-borne Encephalitis (TBE) virus	51	0	0	0 - 7.0			
Toxoplasma gondii	120	57	47.5	38.3 - 56.8			
Neospora caninum	119	8	6.7	2.9 - 12.8			

*Comparison between sexes, between ages and between regions for the agents with the highest seroprevalences is shown in **Tables 3, 4 ,5** (sera with unknown sex, age or region were not included, explaining the different total numbers)*

RESULTS :

*One hundred and thirty samples were obtained. The seroprevalences found are shown in **Table 2**.*

Table 3 Sex	ParaTB: 6/110 (5.5 %)		*A. phagoc.* : 44/80 (55.0 %)		*T. gondii* : 53/115 (46.1%)		*N. caninum* 8/114 (6.7 %)	
	LR: p = 0.540		*LR: p = 0.769*		*LR: p = 0.762*		*LR: p = 0.493*	
M	4/54	7.4%	23/43	53.5%	25/56	44.6%	3/56	5.4 %
V	2/56	3.6%	21/37	56.8%	28/59	47.5%	5/58	8.6 %

Table 4 Age	ParaTB: 6/107 (5.6 %)		*A. phagoc.*: 44/80 (55.0 %)		*T. gondii*: 51/112 (45.5 %)		*N. caninum* 8/111 (7.2 %)	
	LR: p = 0.721		*LR: p = 0.362*		*LR: p = 0.361*		***LR: p = 0.057***	
<1	1/22	4.5%	6/11	54.5%	8/22	36.4%	0/22	0.0 %
1	2/29	6.9%	16/24	66.7%	12/30	40.0%	1/30	3.3 %
>1	3/56	5.4%	22/45	48.9%	31/60	51.7%	7/59	11.9 %

Table 5 Region	ParaTB: 6/110 (5.5%)		*A. phagoc. :* 41/76 (53.9%)		*T. gondii :* 53/111 (47.7%)		*N. caninum* 6/110 (5.4 %)	
	LR: p = 0.290		*LR: p = 0.320*		*LR: p = 0.408*		*LR: p = 0.753*	
2	0/11	0.0%	1/3	33.3%	5/11	45.5%	2/11	18.2 %
3	0/7	0.0%	3/4	75.0%	2/5	40.0%	0/5	0.0 %
4	0/2	0.0%	3/3	100.0%	2/3	66,7%	0/3	0.0 %
5	1/10	10.0%	2/3	66.7%	7/10	70.0%	1/10	10.0 %
6	0/2	0.0%	0/2	0.0%	1/2	50.0%	0/2	0.0 %
7	2/40	5.0%	16/30	53.3%	13/40	32.5%	2/40	5.0 %
8	0/6	0.0%	1/2	50.0%	3/6	50.0%	0/6	0.0 %
9	0/19	0.0%	7/16	43.8%	11/19	57.9%	0/18	0.0 %
10	3/11	27.3%	8/13	61.5%	7/13	38,9 %	1/13	7.7 %
11	0/1	0.0%			1/1	100.0%	0/1	0.0 %
12	0/1	0.0%			1/1	100.0%	0/1	0.0 %

DISCUSSION

None of the serologic tests used has been validated for roe deer. In most of the iELISA tests, a clear distinction could be made between negative and positive titers, reducing the need for cut-off values different of those in domestic ruminants. Yet for paratuberculosis, chlamydiosis and bluetongue, samples with a doubtful titer were considered "suspects" (Table 2). Results for brucellosis and TBE were confirmed by Rose Bengal Test and seroneutralisation test respectively.

Field collected samples are often hemolytic, influencing the specificity (false positives) in the IFA for A.phagocytophilum antibodies. Therefore the high seroprevalence found for anaplasmosis, yet comparable to other European studies using the same method, can be questioned.

CONCLUSIONS :

- *Like in Belgian cattle and southern Belgian cervids, paratuberculosis appears to be enzootic in Flemish roe deer. Except for A.phagocytophilum, T.gondii* and *N.caninum, exposure to the other pathogens is low or non-existent.*
- *No difference in seroprevalence was found between sexes and ages of roe deer and between regions of collection of the samples. Yet for Neospora caninum a higher seroprevalence with increasing ages (but below the threshold of significancy) is suggested by the results.*
- *Current outbreaks of Schmallenberg virus and the reemergence of brucellosis, both in domestic ruminants, illustrate the interest of continuation of serosurveillance in roe deer*