

## Leptospirosis in domestic animals in France: serological results from 1988 to 2007

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### Summary

Leptospirosis is a common infection in domestic animals. The microscopic agglutination test (MAT) is used for serological diagnosis. From 1988 to 2007, the Leptospira Medical and Molecular Bacteriology Laboratory at the Nantes National College of Veterinary Medicine, Food Science and Engineering used the MAT to test serum samples from more than 40,000 cattle, 40,000 pigs, 20,000 horses and 9,500 dogs. Five *Leptospira* serogroups were prominent, with specific variations within the four animal species: Icterohaemorrhagiae, Australis, Sejroë, Grippytyphosa and Autumnalis. The prevalence and incidence of each serogroup varied for each species over the 20-year period: some serogroups were emergent during some years but disappeared later. This study reports the complex epidemiological features of leptospirosis.

### Keywords

Cattle – Dog – Domestic animal – Horse – Leptospira – Leptospirosis – Microscopic agglutination test – Pig – Serological diagnosis.

## Introduction

Pathogenic *Leptospira* species (spp.) were almost simultaneously discovered in humans in several areas of the world, including Japan (by Inada) and European countries, during World War I (1).

The first serodiagnostic tool for human leptospirosis was designed by Martin and Pettit using agglutinating antibodies directed against the lipopolysaccharide (LPS) of the infectious serovar in the sera of patients (1). The microscopic agglutination test (MAT) is currently the gold standard serological test used to detect leptospirosis in humans and animals. However, more than 250 serovars have been described to date and, therefore, use of the MAT requires the most geographically relevant panel of serovars.

Approximately ten days after the onset of the disease, the MAT begins to give positive results. Although not sensitive early in the disease process, the MAT exhibits a high specificity: the detection of agglutinating antibodies, whatever their blood concentration, is indicative of previous exposure to *Leptospira* spp. Agglutinating antibodies are evidence of an immune response, even if the animal has never exhibited clinical signs. Many domestic animals are in close contact with pathogenic *Leptospira* spp. that are present in the environment, leading to the production of an agglutinating antibody response. Therefore, a low level of agglutinating antibodies is usually found in serum samples from unvaccinated, healthy animals. The natural background of these anti-*Leptospira* antibodies is correlated with the country, area, season and animal species. For example, the serological background is lower in European countries compared with South Asia. This background has to be taken into account when the MAT is performed for diagnosis in a given geographical area.

During the 1980s, many reports highlighted the role of leptospirosis in reproductive disorders and abortions in cattle in the United Kingdom (UK) (2, 3). Using a micromethod of the MAT developed in the Central Veterinary Laboratory (CVL, Weybridge, UK), a serological survey of leptospirosis in 3,208 brucellosis-negative aborted cows was implemented in the Loire Atlantique department (France) (4, 5). Since

1987, the MAT has been used by the author's laboratory for several epidemiological surveys in domestic animals and feral species (6, 7, 8) and is routinely used for leptospirosis diagnosis in domestic animals (cattle, pigs, horses and dogs) (9).

Here, the results of the MAT performed for leptospirosis diagnoses in domestic animals from January 1988 to June 2007 are synthesised to highlight the epidemiological features of leptospirosis in four domestic animal species (horses, cattle, pigs and dogs) in France over a 20-year period. The most prevalent serogroups in each of the four species are identified and the seroprevalence of each serogroup in each year for each exposed species is reported.

## **Materials and methods**

### **Strains**

Since 1988, the panel of 21 live strains kindly provided in 1983 by the CVL has been progressively replaced by reference strains provided by the French National Reference Centre (NRC) at the Institut Pasteur. To more closely match French epidemiological conditions, field strains isolated in the author's laboratory from clinical veterinary samples were added to the reference panel after characterisation by the NRC (G. Baranton):

- NANTES 16: Isolated in 1985 from a dead dog
- NANTES 19: Isolated in 1985 from a dead dog
- NANTES 32: Isolated in 1986 from dead puppies
- NANTES 35: Isolated in 1986 from an aborted calf
- NANTES 296: Isolated in 1994 from the liver of a dog with ascites that was euthanised in a colony exhibiting leptospirosis disorders (10)
- NANTES 372: Isolated in 1995 from the kidney of another euthanised dog in a colony exhibiting leptospirosis disorders (10)

– NANTES 374: Isolated in 1995 from the kidney of a third euthanised dog in a colony exhibiting leptospirosis disorders (10).

The strains of *Leptospira* spp. used for the MAT were cultivated at 29°C in EMJH (Ellinghausen, McCullough, Johnson, Harris) medium that was enriched with an albuminous supplement (10% v/v) as previously described (11).

The strains used are shown in Table I.

### **Microscopic agglutination test**

The standard micromethod on enzyme-linked immunosorbent assay (ELISA) plates (previously developed at the CVL) was routinely used on batches of samples received for leptospirosis diagnosis. Fresh cultures were grown weekly and controlled by turbidimetric measures using a Hach turbidimeter. Prior to its use in the MAT, each fresh culture was filtered (0.8 µm) to remove the common ‘breeding nests’ of growing *Leptospira*. Cultures of each serovar were adjusted with phosphate buffered saline (PBS; pH 7.2) to approximately 10<sup>8</sup> per ml (100 Turbid Units). Serum dilutions and cultures were added to ELISA plates by hand from 1984 to 2000. Thereafter, cultures were automatically diluted and distributed by a Multiprobe 104 automate (Packard).

Twenty-five microlitres of each culture and 25 µl of each serum dilution were distributed in each well of the plates.

For each batch of analyses, several controls (different cultures and samples from different animal species) were added, as described below.

#### **Culture controls**

- Negative: 25 µl of PBS was mixed with 25 µl of each serovar
- Positive: 25 µl of a rabbit anti-serogroup specific serum was mixed with 25 µl of each serovar belonging to this serogroup.

### Animal species controls

Sometimes the sera of a given animal species exhibited a paradoxical effect by showing similar mild agglutination at each dilution, although they were negative. To control for this effect, 25 µl of two pools of serum from several animals of each species, one MAT negative and one MAT positive, were mixed with 25 µl of each culture.

### Cut-off

The serological background of each species was taken into account to define the initial dilution used in the MAT. Environmental exposure to *Leptospira* spp. is related to the lifestyle and normal behaviour of each animal species. Clinical specificity and serological reactivity were factors that were taken into account to define each specific initial dilution.

- Dogs are highly receptive and usually exhibit acute disease. In a naïve dog, agglutinating antibodies can be detected by the tenth day after the onset of the disease (11). An earlier rise in the immune response has clinical significance. To identify the start of this response, the initial dilution of the serum was 1:20.
- Horses are potent serological responders to many bacteria and are routinely exposed to environmental *Leptospira* spp. The initial dilution of the serum was 1:100.
- Cattle and pigs are weakly susceptible to acute leptospirosis but persistently exposed either at pasture or when housed. The initial dilution of the serum was 1:50.

When the analyses were performed with the above technical conditions, positive results at the cut-off level did not show clinical significance or association with the observed disease. Positivity only indicated agglutinating antibodies in the sera of the animals. The clinical significance of the MAT results (as a diagnostic aid) could only be estimated in view of several factors related to the animal species and to the results themselves:

- The animal: the age of the sampled animal, timing of the clinical disorder (or economic loss in farm animals) and vaccination status are necessary to reach a significant conclusion.
- The MAT titres: the level of antibodies can be high or low and is matched to several serovars belonging to several serogroups. The ‘pattern’ of the reacting serovars (cross-agglutination) yields significant information for the serum of a given species (11, 12).

Missing data generally reduce the usefulness of the test results as a diagnostic aid to practitioners.

However, 20 years of results from the same laboratory are of interest from an epidemiological point of view. Of course, this study was not a prospective cohort study with randomised samples, which would have allowed estimation of the prevalence and incidence of leptospirosis in domestic animals (4, 13). However, ‘seroprevalence’ and ‘seroincidence’ were estimated in this group of samples from animals suspected of having leptospirosis whose sera were sent for serodiagnosis.

## **Sera**

The blood samples sent by veterinary practitioners to the laboratory were taken from animals suspected of having leptospirosis or herds exhibiting symptoms or syndromes related to the disease.

Dogs are clinically very sensitive to acute leptospirosis, often presenting with signs of fatal liver and kidney disease, whereas infected farm animals exhibit milder symptoms but generate economic losses related to leptospirosis-associated reproductive problems. Several samples from farm animals belonging to the same herd are recommended for simultaneous analysis. For example, the author’s laboratory recommended collection of sera from aborting cows plus other cows housed under the same conditions or, from pig farms reporting reproductive disorders, sera from several sows.

For sport and companion animals (horses and dogs), sera were submitted from individual cases of clinical disease (14, 15).

## Results

### Numbers

Over the 20 years of serodiagnostic activity, 116,985 animal sera were studied, from a minimum of 1,106 samples per year to a maximum of 9,978 samples. Some 'peaks' were related to an efficient and temporary sensitisation of a regional veterinary association, for example for cattle in 2002 or pigs in 1998.

More than 42,000 sera for each farm species were tested, and approximately 22,000 horses and 9,500 dogs were individually studied. Low and irregular numbers of samples from sheep, goats and cats were analysed (data not shown).

### Seroprevalence

Positive results are reported as 'seroprevalence'. This estimation is only based on the serological results performed in animals with suspected leptospirosis.

A serum sample was defined as 'MAT positive' to one serogroup when it showed agglutinating antibodies against one or several serovars (Table I) belonging to this serogroup, at or above the recommended cut-off dilution.

A positive animal often exhibited cross-reacting antibodies to several serogroups of *Leptospira*. Consequently, the total number of positive animals ( $n = 37,764$ ) was different from the total number of positive reactions to the major serogroups ( $n = 63,942$ ), illustrating that many serum samples reacted to more than one serogroup among those used in the MAT. Most of the positive reactions were produced by just five of the 15 serogroups (Table I), namely: Icterohaemorrhagiae (IH), Grippotyphosa (GRIP), Sejroë (SEJ), Australis (AUS) and Autumnalis (AUT) (Fig. 1). Therefore, this paper reports the results for these major serogroups (with noted exceptions). High titres against IH or AUS in pigs were generally associated with lower titres against Pomona (POM) or Tarassovi, as previously shown by Plesko and Lataste-Dorolle (16).

### **Serogroup seroprevalence**

For a given serogroup, significant differences were identified among the different species. The most prevalent serogroup in domestic animals was IH, with large differences between species, for example cattle and dogs (Fig. 1). Similar differences were noticed for AUS, whereas the seroprevalence of SEJ, GRIP and AUT was similar among species. However, in the dog, it is necessary to take into account the bias induced by the usual vaccination with IH and *Canicola* (CAN) bacterins.

### **Serogroup seroincidence**

High titres indicate recent infection by pathogenic *Leptospira* spp., whereas low titres may indicate an older immune response (or an early sample). For the epidemiological statistical analysis, the highest titre shown by one of the serovars belonging to a given serogroup was attributed to this serogroup. Consequently, the field infectious serovar generating this antibody response was said to belong to this serogroup, even though this classification is not conclusive (17). Therefore, the MAT results give a rough estimation of the 'seroincidence' of each infectious serogroup. If high titres are related to the serogroup of the strain that induced the enhanced serological response, serogroup seroincidences may be compared. The most infectious serogroup in French domestic animals (between 1987 and 2007) was IH (30.2% of the high titres), followed by AUS (27.7%) and SEJ (21.9%).

### **Specific results**

#### **Cattle**

In this species, over the 20-year analysis, 9,727 out of 42,982 samples were positive. In herds suspected of having leptospirosis, the overall mean seroprevalence (cut-off 1:100) was 22.63% (95% confidence interval [CI]: 22.03–23.03) for the five studied serogroups (IH, GRIP, AUS, SEJ, AUT).

The samples were collected from at least 11,310 herds. However, data regarding the herds were missing for positive sera sampled during the



years 1988, 1990 and 1992. Therefore, the herd seroprevalence (ratio of infected herds to the total number of sampled herds) could not be estimated.

### *Serogroup seroprevalence*

For the 9,727 positive sera, the total number of positive results for the five major serogroups was 11,510, showing that a positive animal exhibited agglutinating antibodies cross-reacting with a mean of 1.18 serogroups. The most prevalent serogroup was SEJ (34.0% [95% CI: 33.4–34.6]), followed by GRIP (29.9% [95% CI: 29.3–30.5]), AUS (27.6% [95% CI: 27.0–28.2]) and, to a lesser extent, IH (17.3% [95% CI: 16.8–17.8]) and AUT (9.5% [95% CI: 9.1–9.8]).

These results only indicate exposure to the infection. Clinical significance can only be established when high serological titres are shown in animals suspected of disease.

### *Serogroup seroincidence*

High titres, 1:400 or above, suggest a current immunological response. This allows comparison of the seroincidence for each serogroup in the positive cattle (except for 1991, when details about the 281 positive cattle were missing). Among the 1,532 high serological titres exhibited by the 9,727 positive animals, SEJ was the most infectious (46% of the high titres), followed by GRIP (25%) and AUS (17%).

### *Annual dominant serogroup*

Serogroup dominance varied during the study period. The dominant serogroup in each year (with the exception of 1991) is shown in Fig. 2. (Only the dominant serogroup is shown for each year; data on the other four serogroups have not been included, except for 2000, when the incidence of IH was the same as that of GRIP.) AUS was the dominant serogroup in cattle between 1995 and 1997, again in 1999, and between 2005 and 2007. IH appeared dominant only in 2004. GRIP was the dominant serogroup between 1989 and 1993 and again in 1998, but seroincidence decreased after the year 2000 and this

serogroup was not serologically dominant in later years, in contrast to SEJ.

## Pigs

Over the 20-year analysis, 11,265 pigs were MAT positive out of 42,479 samples. The overall mean seroprevalence (cut-off 1:100) for the six studied serogroups (IH, GRIP, AUS, SEJ, AUT, POM) was 26.5% (95% CI: 26.10–26.94).

### *Serogroup seroprevalence*

Out of the 11,265 positive samples, the total number of positive results against the six major serogroups was 15,651, indicating that a positive animal exhibited agglutinating antibodies cross-reacting with a mean of 1.39 serogroups. The most prevalent serogroup in positive animals was IH (50.3% [95% CI: 49.6–51.0]), followed by AUS (42.6% [95% CI: 42.0–43.2]) and, to a lesser extent, AUT (18.1% [95% CI: 17.7–18.5]), SEJ (13.9% [95% CI: 13.4–14.4]), GRIP (11.0% [95% CI: 10.6–11.4]) and POM (5.9% [95% CI: 5.6–6.2]).

The clinical significance of agglutinating antibodies can only be correlated with an active infection when high serological titres are present.

### *Serogroup seroincidence*

High titres, 1:400 or above, indicate a current infection, allowing comparative seroincidence estimation in the positive pigs. Of the 11,265 positive animals, the total number of high titres for the six serogroups was 3,202, of which 42.3% were positive for IH, 32.4% for AUS, 8.7% for AUT, 8.1% for SEJ, 6.9% for GRIP and 1.6% for POM.

### *Annual dominant serogroup*

Serogroup dominance varied during the study period (Fig. 3). A peak of seroincidence generally was observed on pig farms from 1992 to 1999. Serogroups IH and AUS were the most prominent. The

seroincidence of AUT decreased in 1998 then increased from 2003–2007 (it only became the dominant serogroup in 2007); GRIP was poorly represented in this species, and some POM cases were found in 1997–1999 and later in 2004.

## Horses

Among the 22,018 horses analysed over the 20 years, 11,257 were MAT positive. The overall mean serological seroprevalence (cut-off 1:200) for the five studied serogroups (IH, GRIP, AUS, SEJ, AUT) was 51.9% [95% CI: 51.3–52.6].

### *Serogroup seroprevalence*

For the 11,257 positive samples, the total number of positive results for the five major serogroups was 18,719. A positive animal exhibited agglutinating antibodies cross-reacting to a mean of 1.89 serogroups. The most prevalent serogroups in positive animals were IH (52.6% [95% CI: 52.0–53.2]) and AUS (52.0% [95% CI: 51.4–52.6]) and, to a lesser extent, GRIP (24.2%, [95% CI: 23.7–24.7]), AUT (19.5%, [95% CI: 19.1–19.9]) and SEJ (18.0% [95% CI: 17.6–18.4]).

As in other species, the clinical significance of agglutinating antibodies can only be linked to an active infection if high serological titres are present.

### *Serogroup seroincidence*

High titres, 1:800 or above, indicate a current infection, allowing comparison of seroincidence in positive horses. A total of 5,485 samples had high titres to the five serogroups, among which AUS and IH were the most prominent serogroups (38.4% and 36.0%, respectively), followed by GRIP (10.5%), SEJ and AUT (7.8% and 7.3%, respectively).

### *Annual dominant serogroup*

Serogroup dominance varied during the study period (Fig. 4). Serogroups AUS and IH were the most prominent: AUS was

dominant in most years, but IH was sometimes more common (1998, 1999, and 2002–2004). The level of GRIP was generally low.

## Dogs

Leptospirosis is often an acute and fatal disease in naïve dogs. Therefore, vaccination is recommended in this species. In France, between 1970 and 2007, only bivalent vaccines were available against the virulent IH and CAN serogroups, to which dogs have been most exposed. In contrast to other species, most dogs are vaccinated. The study of the serological results in dogs had to take into account the IH and CAN antibody background induced by vaccination (11, 18). Therefore, the total seroprevalence (including IH and CAN) in 9,506 dogs clinically suspected of having leptospirosis was 73.2% (95% CI: 72.3–74.1).

### *Serogroup seroprevalence*

As expected, serological responses against the vaccine serogroups IH (80.5% [95% CI: 79.8–81.2]) and CAN (57.0% [95% CI: 56.4–57.6]) were the most prevalent (for a positive cut-off of 1:40) in the 6,956 positive dogs. These serogroups were followed by AUS (36.3% [95% CI: 35.8–36.8]), SEJ (35.9% [95% CI: 35.4–36.4]), AUT (29.9% [95% CI: 29.4–30.4]) and GRIP (24.0% [95% CI: 23.6–24.6]). These results take into account that new field strains belonging to the SEJ and AUS serogroups were added in 1994–1995 to the diagnostic panel used in the MAT in dogs and, therefore, the seroprevalence for these serogroups was only estimated for 14 years (6,535 positive dogs).

The 6,535 positive dogs exhibited 18,062 positive results, with each positive dog showing reactivity to a mean of 2.59 serogroups.

The clinical significance of agglutinating antibodies can only be linked to an acute infection when the serological titres are high, unless the infection is fatal.

### *Serogroup seroincidence*

Vaccine and non-vaccine serogroup results had to be differentiated.

Vaccine serogroups IH and CAN:

Most of the agglutinating antibodies could have been induced by current or past vaccinations. However, within six months after vaccination, the titres decrease to less than 1:320. Comparison of seroincidence between IH and CAN showed that 77.9% of the high titres found in canine sera were IH antibodies and 22.1% were against CAN.

Non-vaccine serogroups GRIP, AUT, AUS, and SEJ:

Within the non-vaccine serogroups, the highest incidence was found for AUS (42.9%), followed by SEJ (26.3%), AUT (18.5%) and GRIP (12.3%).

#### *Annual prominent serogroups*

Vaccine serogroups IH and CAN:

Despite annual variations, IH was always the most prominent, but CAN sometimes achieved close values for incidence, for example in 1995 and 2006 (Fig. 5).

Non-vaccine serogroups GRIP, AUT, AUS and SEJ:

The non-vaccine serogroups AUT and GRIP were found in canine serum at the beginning of the study period (with AUT the dominant serogroup until 1994), and decreased later (Fig. 6). The annual seroincidence of AUS and SEJ has been measured since 1994; AUS is very infectious, although SEJ was most prominent in 1998 and 2000, and AUT was most prominent in 1990.

## **Discussion**

Leptospirosis is a global infectious disease of domestic animals. The MAT is the gold standard for serological diagnosis of leptospirosis, and the novel genospecies classification of the genus *Leptospira* has not altered the efficiency of this test. The MAT detects agglutinating antibodies against the outer membrane LPS shared by *Leptospira* spp. Approximately ten days after the onset of the infection or vaccination,

antibodies reach a detectable concentration in the serum of the host. The MAT is performed with live leptospire and sometimes they auto-agglutinate in the fresh cultures. It is essential to control each culture by the addition of a negative control without serum. Moreover, it is not unusual to observe a fresh culture with a good negative control but exhibiting features of agglutination with the sera of a given species and not for others. For these reasons, two species controls were added for each batch for analysis: a negative and a positive pool of sera for each species.

Whatever the serovar, each LPS shares many epitopes. Close serovars sharing many similar epitopes are classified in the same serogroup. However, some of these epitopes are also shared by other serogroups. For a given strain, the immunological differentiation of the epitopes depends on the general kinetics of the serological response (immunoglobulin [Ig]M is less specific than IgG) and on the specificity of the immune recognition of each host. For example, the ratio of cross-reactivity between different serogroups was 1.89 in the horse and 1.18 in cattle. These data highlight the fact that the horse is a potent serological responder. The very high cross-reactivity ratio in canine serum (2.59) reflects that, in France, most dogs are vaccinated against IH and CAN. However, because protection by the usual bacterins is serogroup specific, vaccinated dogs are not protected against field infection, especially by a strain belonging to another serogroup. The newly acquired infection triggers agglutinating antibody production specific to the new LPS type but also mildly boosts antibodies to some common epitopes shared by vaccine serogroups (11). Newly infected vaccinated animals exhibit attenuated symptoms of leptospirosis and have a better prognosis (personal data).

Serogroup CAN has been present in the bacterins since the beginning of canine vaccination. In this study, the most seroprevalent serogroups in the dog were IH (80.5%) and CAN (57.0%). These seroprevalence values probably reflect the widely used vaccine. However, if these serological titres were only due to vaccination, the serological profile would have been similar for IH and CAN, and it was not. In the environment, dogs are widely exposed to field strains, boosting their

immune response and enhancing agglutinating antibodies. When only dogs with high titres are taken into account, IH exposure (78%) is significantly higher than that to CAN (22%), possibly because IH has many maintenance hosts and reservoirs, whereas CAN is only maintained in dogs. This may explain why, in a well-vaccinated canine population, exposure to IH is higher than exposure to CAN. These results are in accordance with the decrease of CAN throughout Europe (19). However, a slight increase was shown in 2006 and 2007 (20). It is possible that the reduction in CAN cases led owners to under-vaccinate their pets. Did the consequent reduction in general canine immunity allow resurgence of CAN?

It is unclear why the prevalence of IH is low in cattle compared with horses or pigs. Pigs may be more exposed to rats (the major reservoir of IH) on intensive pig farms, but cattle are bred in more natural conditions, close to those in which horses are kept. The difference may be related to the specific nature of the bovine immune response or to the prominent infecting serovar Hardjo (in serogroup SEJ), which may inhibit IH infection in cattle.

The annual seroincidence of the serogroups estimated in this study varied among the four domestic species.

The seroincidence of GRIP in cattle and dogs decreased from 1988 to 2000, but a peak was observed in 2009 in horses (A. Leon, personal communication). However, some serogroups have remained prominent over the 20-year period: IH and AUS have remained high in all animal species, especially since 2011 in cattle and dogs (21) and through 2014 in horses.

Annual fluctuations could be related either to a bias in sample submission or to variations in exposure. Climate has a major effect on wildlife reservoirs, which shed *Leptospira* spp. in their urine, contaminating fresh water. However, an excessive amount of rain in spring can cause the loss of voles and other small rodents, which may be expected to result in a low density of the reservoir species for GRIP. On the other hand, pathogenic *Leptospira* spp. are able to survive and remain virulent for more than one year in fresh water (22).

Excessive dryness can inactivate pathogenic *Leptospira* spp., but drought also modifies the behaviour of reservoir species, concentrating them near ponds or rivers.

The rapid evolution of *Leptospira* epidemiology, with decreases and resurgence of serogroups, explains the use of a wide serogroup panel in the MAT. It is necessary to modify, from time to time, the serogroups included in the conventional bacterins. However, vaccine companies may not be able to react sufficiently rapidly to these epidemiological changes.

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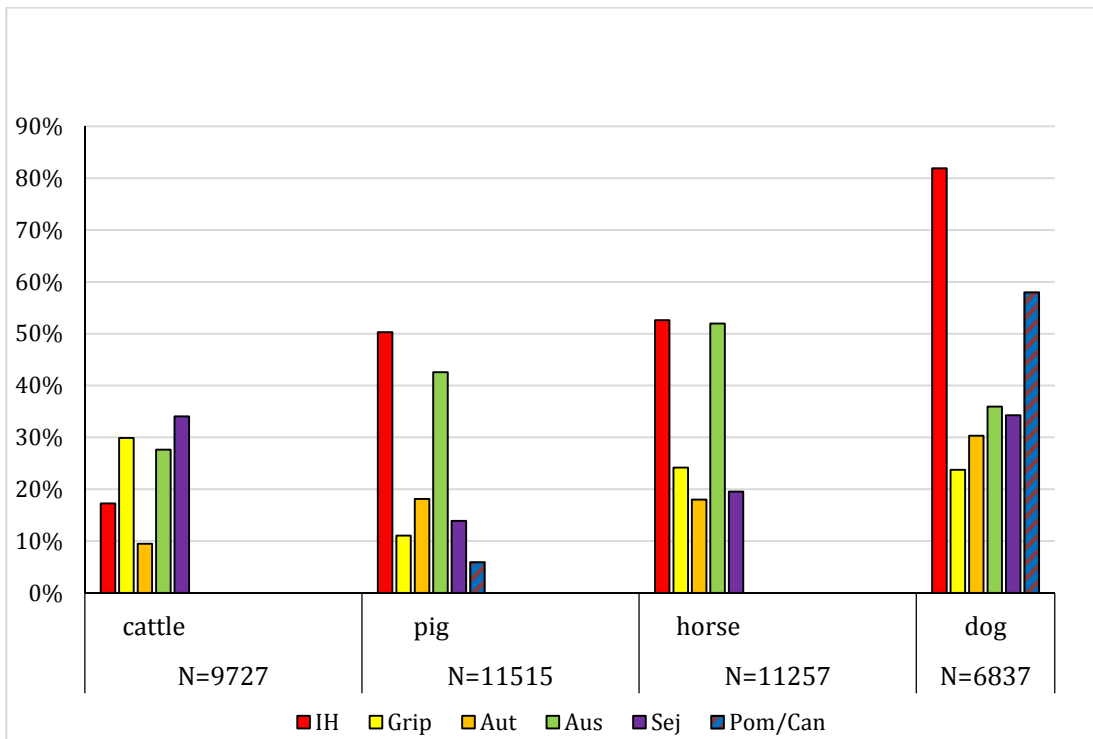
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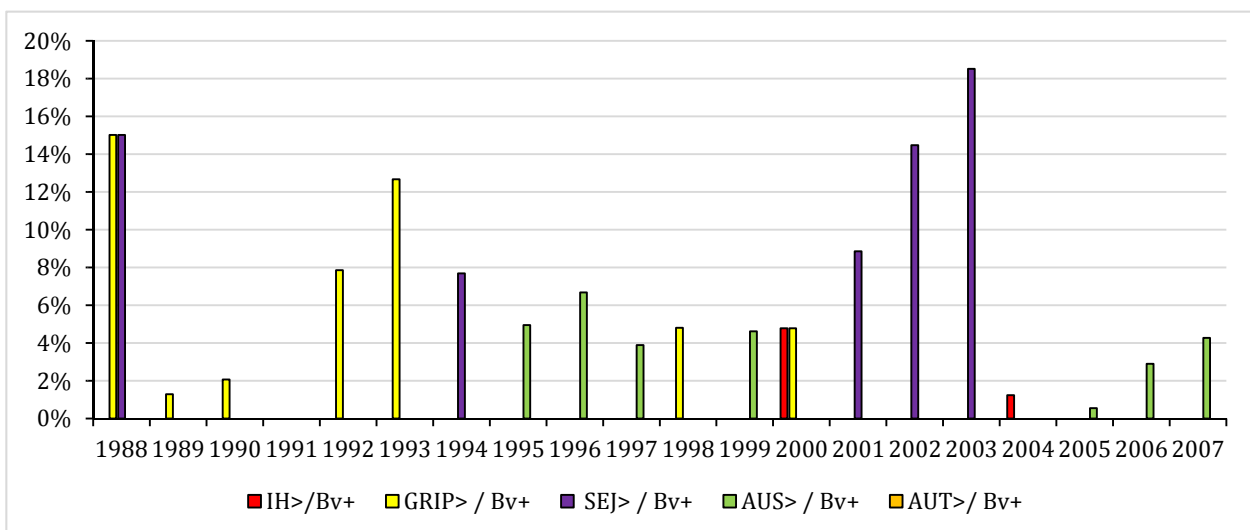
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**Table I**  
**Strains (serovars and serogroups) used in the microscopic agglutination test**

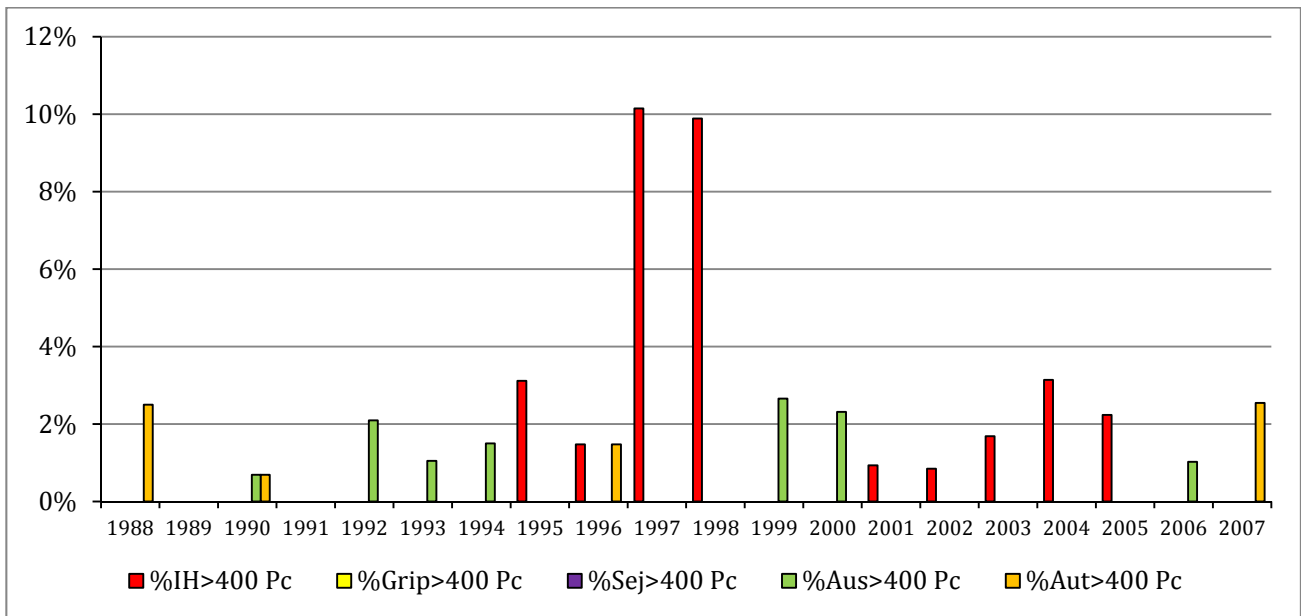
Serogroup	Serovar	Reference strain	Field isolate
Australis	Australis	Ballico	
	Bratislava	Jez Bratislava	
	Muenchen		NANTES 372
Autumnalis	Autumnalis	Akyami A	NANTES 32
Ballum	Castellonis	Castellon 3	
Bataviae	Bataviae	Van Tienen	
Canicola	Canicola	Hond Utrecht IV	
Grippotyphosa	Grippotyphosa	Moskva V	
	Vanderhoedoni		NANTES 35
Hebdomadis	Hebdomadis	Hebdomadis	
Icterohaemorrhagiae	Copenhageni	Wijnberg	
	Icterohaemorrhagiae	Verdun	NANTES 19
Javanica	Javanica	Veldrat Batavia 46	
Mini	Mini	Sari	
Panama	Panama		NANTES 374
Pomona	Pomona	Pomona	
	Mozdok	5621	
Pyrogenes	Pyrogenes	Salinem	
	Zanoni		NANTES 16
Sejroë	Sejroë	M 84	
	Saxkoebing	Mus 24	NANTES 296
	Hardjo	Hardjoprajitno	
		Bovis Sponselee	
Tarassovi	Wolffi	3705	
	Tarassovi	Mitis Johnson	



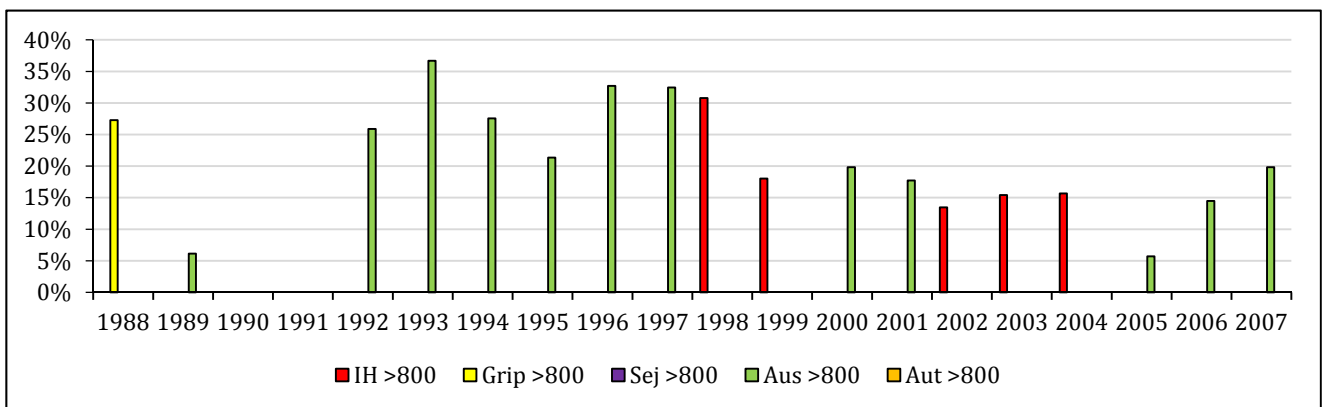
**Fig. 1**  
**Animal exposure to each serogroup (percentage of positive sera among tested sera from each species)**



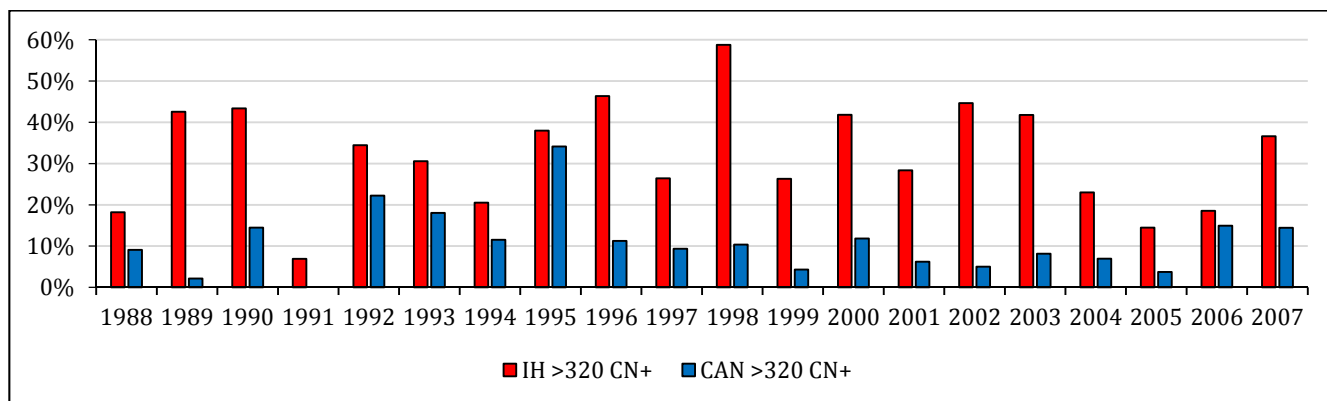
**Fig. 2**  
**Dominant serogroup in positive cattle in each year (percentage of sera with titre  $\geq 1:400$ )**



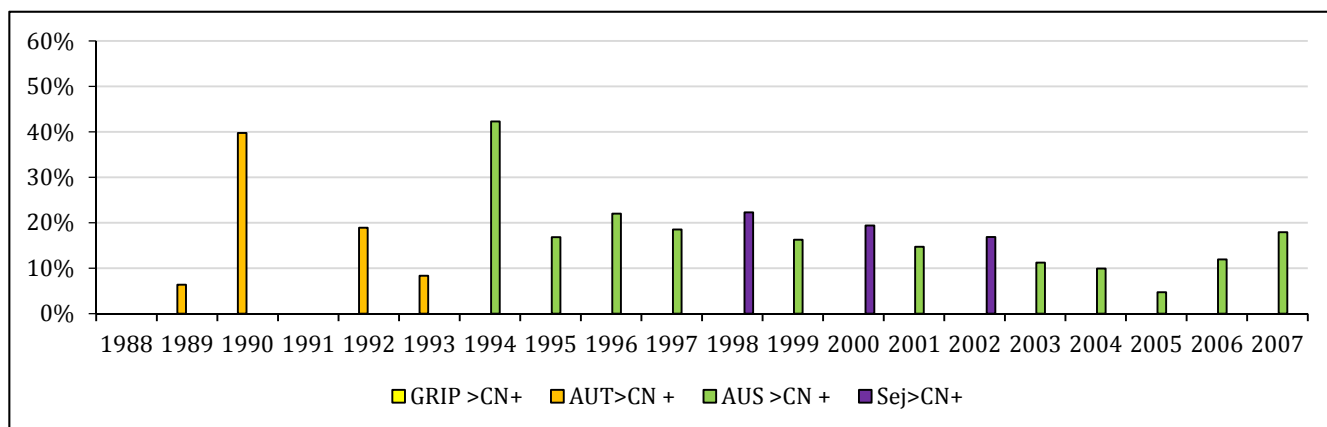
**Fig. 3**  
**Dominant serogroup in positive pigs in each year (percentage of sera with titre ≥ 1:400)**



**Fig. 4**  
**Dominant serogroup in positive horses in each year (percentage of sera with titre ≥ 1:800)**



**Fig. 5**  
**Comparative annual seroincidence of Icterohaemorrhagiae (IH)**  
**and Canicola (CAN) in positive dogs (percentage of sera with titre**  
**≥ 1:320)**



**Fig. 6**  
**Annual seroincidence of Grippotyphosa (GRIP), Autumnalis**  
**(AUT), Australis (AUS) and Sejroë (SEJ) in positive dogs**  
**(percentage of sera with titre ≥ 1:320)**